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TRDRP

Staff

SUSANNE HILDEBRAND-ZANKI, PH.D.
Director

JEROME BECK, DR. P.H.
Research Administrator,
Epidemiology and
Policy Research

JEFFREY CHEEK, PH.D.
Research Administrator,
Biomedical Sciences

PHILLIP GARDINER, DR. P.H.
Research Administrator, Social
and Behavioral Sciences

M.F. BOWEN, PH.D.
Administrative Analyst

CARLIN COLBERT
Administrative Assistant

SHARON L. DAVIS
Publications Specialist

TERESA E. JOHNSON
Administrative Coordinator

YURI MOK
Administrative Assistant

CAROLYN ROBINSON
Administrative Assistant

CHRISTINE TASTO
Administrative Analyst

AIM99

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San Francisco Airport
1 Old Bayshore Highway
Millbrae, CA 94030-3198
www.westin.com

Tobacco-Related Disease Research Program
Annual Investigator Meeting 1999

TOBACCO RSEARCH IN ACTION
A DECADE OF PROGRESS

Welcome From the Director

On behalf of the Tobacco-Related Disease Research Program staff and the University of California, I would like to extend a warm welcome to you to the 4th annual TRDRP investigator meeting. This year marks the 10th anniversary of TRDRP and this milestone is reflected in the theme of this year's conference: Tobacco Research in Action – A Decade of Progress.

California was the first state to establish a comprehensive tobacco control program in 1989, and until last year, the only one to include research as part of that plan. Thus, California has the ability to deploy its excellent research infrastructure in the fight against tobacco and to make progress on a wide variety of issues surrounding tobacco use.

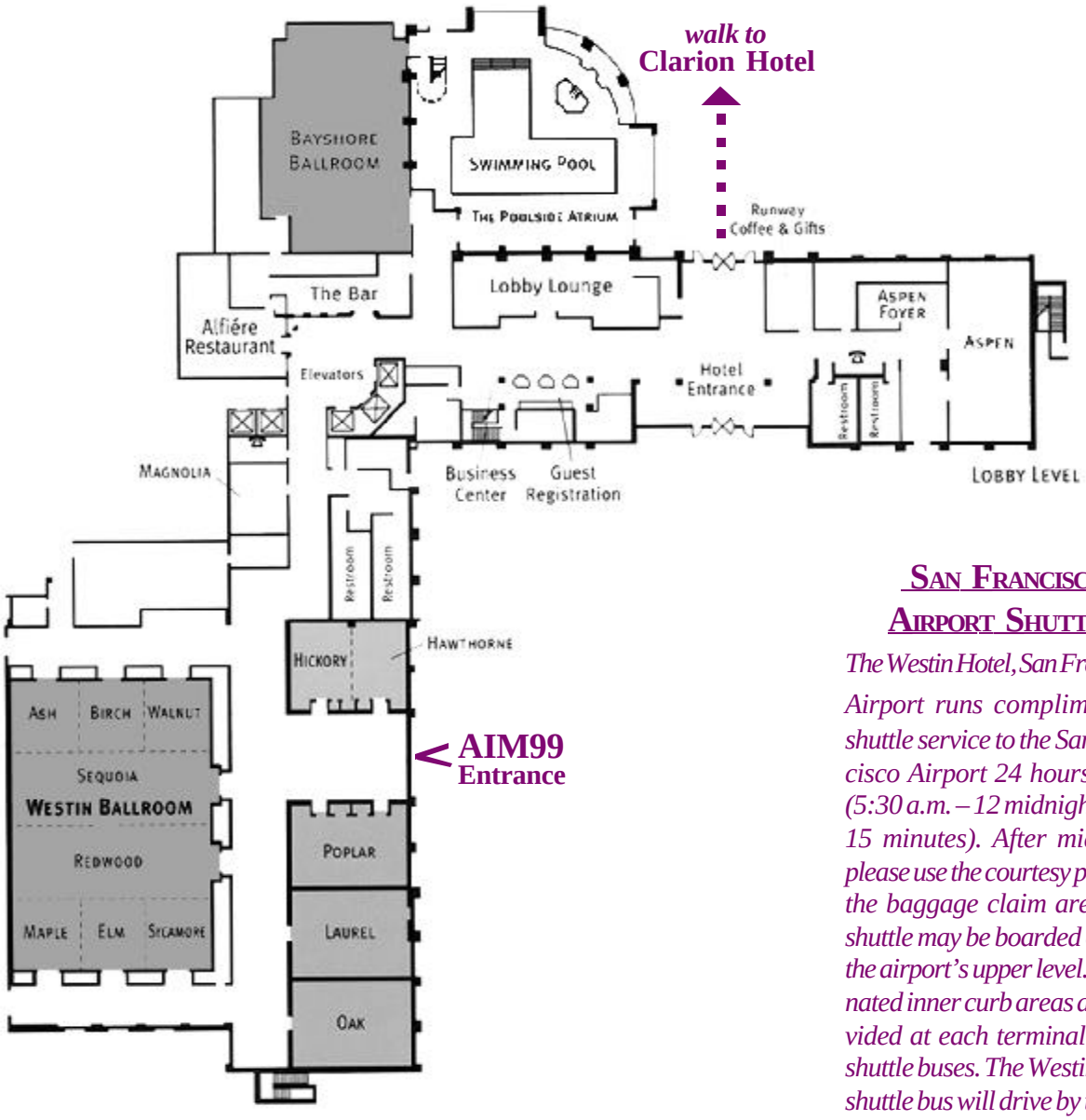
Fortunately, California has been joined by a growing list of other states, which have embarked on tobacco control efforts of their own, modeling on the California program and moving beyond. Thus, there is now the ability to learn from each other and to strengthen everyone's efforts.

California is uniquely positioned to provide models for interventions aimed at diverse populations residing within our borders. Knowledge gained through research and practice about these communities can be transferred to other parts of the country and the world.

While we have made great progress, we all know that there is still a lot of work left to be done. The tobacco control landscape is shifting continuously, and we have to stay on top of those changes and their implications for public health. I hope that this meeting will highlight just how much we have learned and achieved over the last ten years. At the same time, the workshops, plenary session, and poster presentations should stimulate discussion about the pressing research questions we need to address in the years ahead.

Susanne Hildebrand-Zanki, Ph.D.
Director. TRDRP

The Westin SF Airport Hotel



San Francisco Airport Shuttle

The Westin Hotel, San Francisco Airport runs complimentary shuttle service to the San Francisco Airport 24 hours a day (5:30 a.m. – 12 midnight every 15 minutes). After midnight, please use the courtesy phone in the baggage claim area. The shuttle may be boarded outside the airport's upper level. Designated inner curb areas are provided at each terminal for all shuttle buses. The Westin Hotel shuttle bus will drive by to pick-up guests.

Onsite Parking

Gated: 0-2 hrs. = $2
0-4 hrs. = $4
every addt. hr. = $1

Valet Parking

Overnight = $14
Daily = $12

AIM99 Schedule of Events

December 2, Thursday

12 noon - 9:00 pm

Registration

2:00 - 5:00 pm

Concurrent Workshop Sessions

American Cancer Society – California Division
"American Cancer Society - Looking to 2015"
Moderator: ***John Simmons, M.D. - Kaiser Medical Ctr., Walnut Creek, CA***

California Department of Education – Healthy Kids Office
"Innovative School-based Programs"
Moderator: ***D.J. Peterson - Healthy Kids Program***

California Department of Health Services – Tobacco Control Section
"Innovative Tobacco Control Programs"
Moderator: ***Holly Sisneros, M.P.H. - DHS Tobacco Contol Section***

California Thoracic Society – American Lung Association - California
"Tobacco and Asthma: Insights in Pathogenesis and Disease Expression"
Moderator: ***Homer A. Boushey, M.D. - Professorof Medicine, UCSF***

6:00 - 9:00 pm

Dinner & Gala Celebration

Welcome and Overview
A Celebration of the California Tobacco Control Movement Successes;
10 Years of Progress...and the Fight Continues

AIM99 Gala Celebration

The 10th Anniversary Gala Celebration will commemorate a decade of tobacco advocacy and research. The ***Tobacco-Related Disease Research Program*** *is being joined by the Tobacco Control Section of the Department of Health and Human Services, Regional Linkages, Local Lead Agencies, Ethnic Networks, American Nonsmoker's Rights Foundation, and the California Department of Education to salute the many and various tobacco control efforts in our state. This event is free to the general public and dinner is provided. The evening will feature videos, remembrances, awards and testimonies presented by people who have been on the front line of the tobacco wars.*

5:30 - No Host Bar with Music

Reception/Dinner

Welcome & Gala Overview
Phil Gardiner, TRDRP & Robin Shimizu, DHS-TCS

Opening Ceremony
Gerry Rainingbird, American Indian Tobacco Education Network

Overview of Tobacco Control Section
Tacey Buffington

Director's Message
Susanne Hildebrand-Zanki

Asian Pacific Islander Tobacco Education Network
Betty Hong & Jung Ho

California Department of Education
Gerald Kilbert

Award Ceremony - Letters of Support

African American Tobacco Education Network
Brenda Bell Caffee & Susanne Hunter

Regional Linkage Projects
Sue Heitman & Thi Pham

Local Lead Agencies

Hispanic/Latino Tobacco Education Network
Lourdes Baezconde-Garbanati

Dancing & Music

AIM99 Schedule of Events

December 3, Friday

7:00 am

Registration ongoing

7:00 - 9:00 am

Continental Breakfast

9:00 am - 12 noon

Welcome

Keynote Address - ***Barry S. Levy***

"Tobacco and Public Health: Challenges and Opportunities"

Plenary Session - ***Anti-Smoking Ad Campaigns***

Moderator: Lori Dorfman, Dr.P.H.

Panelists

Dileep Bal, M.D., M.S., M.P.H.

Valerie Graves

David Hill, Ph.D.

Peter Mitchell

Melanie Wallendorf, Ph.D.

12 noon - 1:30 pm

Luncheon

Clarion Hotel

401 Millbrae Avenue

(The Clarion Hotel is located adjacent to the Westin Hotel)

1:30 pm - 5:15 pm

Poster Sessions

1:30 - 2:45 p.m.

Cancer

Epidemiology

Nicotine Dependance

2:45 - 4:00 p.m.

Heart Disease

Prevention

Secondhand Smoke

4:00 - 5:15 p.m.

Lung Disease

Health Effects on Women & Infants

Policy

AMERICAN CANCER SOCIETY – *California Division*

"American Cancer Society - Looking to 2015"

Moderator

John Simmons, M.D. - Past President, ACS-California Division;
Senior Physician & Medical Oncologist, Kaiser Medical Ctr., Walnut Creek, CA

Participants

Tim Byers, Ph.D. - Professor of Preventative Medicine, University of Colorado, Denver, CO
Eugenia Calle, Ph.D. - Director, Analytic Epidemiology, American Cancer Society, Atlanta, GA
Tom Fogel, M.D. - Immediate Past President, ACS-California Division;
Cabrillo Radiation Oncology Center, Ventura, CA

The panel will look at the epidemiology, research & programs needed to achieve these goals by 2015.

2:00 - 5:00 p.m.

- ***Reducing Cancer Incidence by 25%***
- ***Reducing Cancer Mortality by 50%***
- ***Increasing the Quality of Life of Cancer Patients in Relation to Tobacco Control Initatives.***
- ***Roundtable Discussion***

CALIFORNIA DEPARTMENT OF EDUCATION – Healthy Kids Office

"Innovative School-based Programs"

Moderator

D.J. Peterson - Healthy Kids Program

Participants

Gus T. Dalis, Ed.D. - Los Angeles County Office of Education
L.D. Hirschklau, Project Director - Smokeless Schooldays Project
Meyla Ruwin, M.P.H.,CHES - School Health Programs Dept., SFUSD

2:00 - 2:50 p.m. ***"The Missing Link in Prevention Education"***
Gus T. Dalis

2:50 - 3:35 p.m. ***"Smokless Schooldays: Cessation Awareness"***
L.D. Hirschklau

3:35 - 3:45 p.m. **Break**

3:45 - 4:30 p.m. ***"Basement Burns: Adventures in Life Skills"***
Meyla Ruwin

4:30 - 5:00 p.m. ***Roundtable Discussion***

CALIFORNIA DEPARTMENT OF HEALTH SERVICES
Tobacco Control Section

"Innovative Tobacco Control Programs"

Moderator

Holly Sisneros, M.P.H. - DHS Tobacco Control Section

Participants

Monica Cervantes - Contra Costa County Health Services
Denice Dennis, M.P.H. - Contra Costa County Health Services
Dimitre Karadais - Contra Costa County Health Services
Pat Spratlen Etem - L.A. LINK, Smoke Free Zoo Project
Brian L. Mimura, M.P.H. - California Health Collaborative

2:00 - 2:50 p.m. ***Working with Youth to Change Tobacco Policy: TIGHT Experience***
Denise Dennis, Monica Cervantes & Dimitre Karadais

2:50 - 3:35 p.m. ***Tobacco -free Cultural Events: Altering Norms Among the Hmong Communities in the CentralValley***
Brian L. Mimura

3:35 - 3:45 p.m. **Break**

3:45 - 4:30 p.m. ***Said on Arrival: "I'm So Happy to be Back in California Because I Can Breathe Everyplace I Go" WE DID THAT!***
Pat Spratlen Etem

4:30 - 5:00 p.m. ***Roundtable Discussion***

CALIFORNIA THORACIC SOCIETY
American Lung Association, California

"Tobacco and Asthma: Insights in Pathogenesis and Disease Expression"

Moderator

Homer A.Boushey, M.D. - Professor of Medicine, UCSF

Participants

Carol Basbaum, Ph.D. - Professor of Anatomy, UCSF
Wendy Cozen, D.O., M.P.H. - Assistant Professor of Preventive Medicine, USC
John Fahy, M.D. - Associate Professor of Medicine, UCSF
Laurel Gershwin, D.V.M., Ph.D. - Professor of Immunology, UCD
Kenneth Serio, M.D. - Clinical Instructor of Medicine, UCSD

2:00 - 2:45 p.m.	***The Pathogenesis of Asthma: Potential Relationship to Injury Induced by Tobacco Smoke*** John Fahy
2:45 - 3:05 p.m.	***The Role of Tobacco in Epidemiology of Asthma*** Wendy Cozen
3:10 - 3:30 p.m.	***Effects of Environmental Tobacco Smoke on Pulmonary Allergy*** Laurel Gershwin
3:35 - 3:45 p.m.	**Break**
3:45 - 4:05 p.m.	***Regulation of Lung Inflammation by LTC-4 Synthetase Pathway*** Kenneth Serio
4:10 - 4:30 p.m.	***How Does Tobacco Smoke Produce Mucous Secretion in Lung Cells?*** Carol Basbaum
4:30 - 5:00 p.m.	***Roundtable Discussion***

Tobacco and Public Health: Challenges and Opportunities

BARRY S. LEVY, M.D., M.P.H.

Barry S. Levy, M.D., M.P.H., is an internationally recognized author and speaker on health and environment issues, an independent consultant in environmental and occupational health, and an Adjunct Professor of Community Health at Tufts University School of Medicine. In 1997, he served as president of the American Public Health Association.

Dr. Levy has devoted his entire career to public health — as a practitioner, educator, scientist, consultant, program director, and organizational leader. He has been a medical epidemiologist for the Centers for Disease Control; a professor at the University of Massachusetts Medical School, where he founded and directed its Occupational Health Program; and an international health program director. He has worked in more than 20 countries, primarily developing countries of Africa and Asia and countries in Central and Eastern Europe.

Dr. Levy has written more than 100 published article and book chapters and edited 15 books and monographs, including four editions of *Occupational Health*, a textbook on work-related disease and its prevention; the book, *International Perspectives on Environment, Development, and Health;* and the book, *War and Public Health.*

He co-wrote and performs in ***Damaged Care: A Cabaret-Style Musical Comedy about Health Care in the '90s***, which has been presented at many conferences and on Capitol Hill.

Anti-Smoking Ad Campaigns Panelists

Lori Dorfman, Dr.P.H. - Panel Moderator

Lori Dorfman is Director of the Berkeley Media Studies Group, a project of he Public Health Institute, where she directs BMSG's work with community groups, journalists and public health professionals. The Berkeley Media Studies Group operates out of the belief that the mass media, especially the news, have a significant influence on people's beliefs and actions regarding public health and social issues and that the news media can be a powerful force for advancing healthy public policy. BMSG studies the news and news gathering to support professional education for journalists and media advocacy training for grass roots and public health leadership groups. Dr. Dorfman's current research examines how local television news and newspapers portray youth and violence. She edited *Reporting on Violence*, a handbook for journalists illustrating how to include a public health perspective in violence reporting, published by BMSG. Based on this work, she is part of an interdisciplinary team that is conducting workshops on violence reporting for newspapers and local TV news stations. Dr. Dorfman taught a course for masters students on mass communication and public health at the School of Public Health at the University of California, Berkeley, has published articles on public health and mass communication issues, coauthored *Public Health and Media Advocacy: Power for Prevention*, (Sage Publications, 1993) and *News for a Change: An Advocates' Guide to Working with the Media*, (Sage Publications, 1999) and is completing a book on how television reports on health. Dr. Dorfman has consulted for government agencies and community programs across the U.S. and internationally on a variety of public health issues, including violence prevention and injury control, alcohol control, tobacco control, children's health, child care, childhood lead poisoning, affirmative action, nutrition and exercise, and women and HIV/AIDS.

Dileep G. Bal, M.D., M.S., M.P.H.

Dr. Bal is the Chief of the Cancer Control Branch of the California Department of Health Services. His responsibilities include cancer prevention and control and the statewide tumor registry. This includes California's acclaimed Tobacco Use Prevention and Cessation Program, the Breast and Cervix Cancer Screening Program, the Nutrition and Cancer Program, and the new Cancer Research Program.

Dr. Bal also has an appointment as a Clinical Professor at the medical school of the University of California at Davis. Prior to coming to California in 1981, Dr. Bal was in Tucson, Arizona for ten years, where he was the Director of the Pima County Health Department and on the full-time faculty of the University of Arizona, College of Medicine. Dr. Bal was born and educated mainly in New Delhi, India. He also has graduate degrees in Public Health from Columbia and Harvard Universities.

Dr. Bal is very active with the American Cancer Society at the local, state, and national levels. He is a member of the National Board of Directors and a National Officer, in addition to being an Honorary Life Member, and Past President of both the Sacramento Unit and the California Division of the American Cancer Society.

ANTI-SMOKING AD CAMPAIGNS PANELISTS

VALERIE J. GRAVES

Valerie Graves is currently Senior Vice President and Chief Creative Officer for the UniWorld Group in New York City, a large African American-targeted advertising agency with a roster of Fortune 500 companies. Her long and productive career in the advertising and communications field has included employment in a similar executive capacity at Motown Records as well as more recent service as Creative Director for ethnic initiatives for a billion-dollar health communication corporation. In this latter capacity, she developed a multi-media program for World AIDS Day 1999 program and served as a consultant for a teen-focused nationwide HIV testing campaign. Ms. Graves has received a number of awards from the advertising industry for her efforts, which has included being honored by Advertising Age as "One of the 100 Best and Brightest" in the field. Of particular relevance to tobacco control has been her ongoing participation on a Columbia University School of Public Health expert panel established to develop innovative anti-tobacco youth countermarketing campaigns. She has also been instrumental in the hit male singing group Boyz II Men emerging as national spokespersons against teen smoking.

DAVID HILL, Ph.D.

David Hill is a behavioural scientist and Director of the Centre for Behavioural Research in Cancer at the Anti-Cancer Council of Victoria, Australia. He has authored or co-authored over 150 scientific articles and reports in the medical, public health and psychological literature. His published works include research on the prevalence of adolescent and adult smoking, strategies for smoking cessation, reduction of smoking uptake, smoking regulation, behavioural aspects of screening mammography, management of primary operable breast cancer, efficacy of breast self-examination, monitoring trends in skin cancer prevention and exploring determinants of behaviours related to skin cancer prevention.

Dr. Hill has served on a number of national and international committees and task forces in cancer prevention and formerly headed the UICC's project on Doctor Involvement in Public Education About Cancer. He advises the UICC on behavioura1 science applications in cancer control. In 1996, under the auspices of the UICC, he organized the inaugural World Conference for Cancer Organizations. The conference was designed for professional staff and volunteers involved in community approaches to cancer control and was attended by representatives from over fifty countries. Later that year, the Federal Minister for Health invited him to chair the Ministerial Tobacco Advisory Group to establish the first comprehensive national anti-smoking campaign ever launched in Australia. He is now chairman of the National Expert Advisory Committee on Tobacco which is responsible for the National Tobacco Strategy to which all jurisdictions committed in June 1999.

Anti-Smoking Ad Campaigns Panelists

Peter Mitchell

Peter Mitchell is a senior program officer at the Academy for Educational Development (AED), provides training and technical assistance in social marketing to a variety of federal agencies, including the Centers for Disease Control and Prevention, the National Highway Traffic Safety Administration, the Environmental Protection Agency and the Health Care Finance Administration. Mitchell joined the AED, a Washington-based nonprofit, in April after serving as the original marketing director of Florida's groundbreaking teen-oriented anti-tobacco program. In Florida, Mitchell worked with teens to develop the irreverent "truth" advertising campaign aimed at repositioning tobacco in the youth culture as well as the communications and advocacy strategies that surrounded it. Research showed dramatic results in a year: not only changes in attitudes, but also a drop in the teen smoking rate. At AED, Mitchell has continued his specialization in the areas of youth and tobacco control and is currently overseeing a CDC-funded project to assist parents in preventing their children from using tobacco. Before being appointed by the late Florida Gov. Lawton Chiles to work on Florida's anti-tobacco campaign, Mitchell served as the policy coordinator for the Florida Senate. Mitchell came to the Senate after a career in journalism, which included covering government and health care in Florida for *The Wall Street Journal* and serving as the senior political reporter for *The Orlando Sentinel*. Mitchell holds a BA in political science from Colgate University.

Melanie Wallendorf, Ph.D

Melanie Wallendorf is Professor of Marketing, and of Comparative Cultural and Literary Studies at the University of Arizona. She received an M.A. in sociology in 1977 and a Ph.D. in Marketing in 1979, both from the University of Pittsburgh. Her research focuses on the sociological aspects of consumer behavior. Currently, she has received funding from the Arizona Disease Control Research Commission to conduct a longitudinal study of young children's understandings of tobacco advertising. This study addresses two central research questions: Which children understand what from tobacco advertising, and when do they understand it? Furthermore, how do these understandings contribute to the onset of tobacco use in later adolescence? Children in grades 2 through 7 are being interviewed using a projective method to overcome social desirability bias in reporting, and followed for three years to track longitudinal change in beliefs and behavior.

A01

The uncommon phenotype of poor inducibility of CYP1A1 in human lung is not ascribable to polymorphisms in the AHR, ARNT, or CYP1A1 genes

Lei, Xiang-Dong; Anttila, Sisko; **Hankinson, Oliver**
University of California, Los Angeles

Polycyclic aromatic hydrocarbons (PAHs) are one of the most important carcinogens in cigarette smoke. However, they must be first metabolized to electrophilic derivatives to have their carcinogenic effects. This conversion is principally catalyzed by CYP1A1, the major cytochrome P450 in the lung. Importantly, it is well known that CYP1A1 is induced by cigarette smoke. Therefore, the expression of CYP1A1 has been of major concern regarding cigarette smoke-induced lung cancer. The induction of CYP1A1 by PAH involves two transcription factors, the aryl hydrocarbon receptor (AHR) and the aryl hydrocarbon receptor nuclear translocator (ARNT). Thus, AHR, ARNT and the promoter of CYP1A1 gene constitute the key players with the respect of CYP1A1 inducibility by PAH.

Although the CYP1A1 gene is induced by cigarette smoke in most people, we observed that in nine cases out of sixty-five, CYP1A1 protein was not detectable either by enzyme activity assay or by immunohistochemistry staining even though these people were active smokers. RT-PCR analysis of the CYP1A1 mRNA revealed that eight of nine of these CYP1A1 protein-negative cases express much lower levels of the mRNA compared with the controls. Further analysis showed that no significant differences were observed in the mRNA levels of AHR and ARNT in the lungs of the nine cases relative to the controls. We hypothesized that functional polymorphisms or mutations exist in the coding regions of the AHR or ARNT gene or in the 5' regulatory region of the CYP1A1 gene in the nine cases with poorly inducible CYP1A1. We developed a strategy for screening for unknown polymorphisms, and optimized the conditions under which heterozygous polymorphisms can be detected. The risk of missing very rare polymorphisms was minimized. We then searched for polymorphisms or mutations in the coding regions of the AHR and ARNT genes, and the CYP1A1 promoter region. The full-length cDNAs of AHR and ARNT were amplified by RT-PCR and sequenced. A known codon 554 mutation of AHR was found as homozygous in one and as heterozygous in one of the nine individuals. In addition, silent mutations of AHR (codon 44) and ARNT (codon 189) were detected. A 1554 bp of genomic DNA corresponding to the promoter of CYP1A1 was also amplified and sequenced. In one individual a heterozygous C to T mutation at - 455 upstream of CYP1A1 was observed. One of the cases that were negative for CYP1A1 protein expressed a moderate level of CYP1A1 mRNA. In this case, full-length CYP1A1 coding region, amplified and sequenced, turned out to be of wild type. Our results demonstrate that a low inducibility phenotype of CYP1A1 in human lung is not due to mutations in the coding regions of AHR or ARNT, or in the promoter of CYP1A1. We propose that polymorphisms in other (perhaps currently unknown) genes may be responsible for the lack of expression of CYP1A1 in the nine cases. We are continuing to analyse CYP1A1 non-inducible placenta and lymphocyte samples.

Acknowledgement: This work is supported by TRDRP grant 7RT-0061.

A02

Dissecting signaling pathways to cancer-killing agents

Chen, Yi
University of California, San Diego

Tumor cells can be killed by drugs through programmed cell death (PCD) called apoptosis. Many of these drugs, however, can also activate mechanism that prevents cells from apoptosis. Therefore, those drugs are less effective in killing tumor cells than otherwise expected. Recently, this reduced effectiveness has been suggested to result from the anticancer agent-induced activation of transcription factor of the NF-κB family, which form key components of a primary signal transduction pathway. The goal of this project is to characterize a kinase complex IKK which controls NF-κB activation.

IKK is a large protein complex and contains at least three IKK polypeptides. During past three years, this laboratory has purified and cloned all three polypeptides. Two of these polypeptides, IKKα and IKKB are serine-threonine kinases. To determine these two kinases are direct regulator of NF-κB, we expressed recombinant IKKα and IKKB individually in insect cells using baculovirus expression system. Both of them are catalytic active and able to phosphorylate directly inhibitors of NF-κB. IKKα and IKKB forms homodime as well as heterodime in vitro. We have also obtained a large amount of pure recombinant IKKB from baculovirus expression system and are doing crystal structure analysis.

Characterization of IKK may provide important information for designing or screening agents that can reduce IKK activity. Using purified recombinant wild type or mutated IKK molecules, we have demonstrated that anti-inflammatory cyclopentenons prostaglandins can directly inhibit IKKα and IKKB activation. Studies from other laboratory also showed that another anti-inflammatory aspirin binds and inhibits IKKB activation. Therefore, results of this study should be directly applicable to drug-designing or searching program and will help cancer-killing agents to treat many kind of cancers including lung cancer.

A03

A retroviral link to human tobacco-related lung cancer?

Barsky, Sanford H.
University of California, Los Angeles

Peripheral adenocarcinoma (PAC) and bronchioloalveolar carcinoma (BAC) are forms of lung cancer whose etiology and pathogenesis are controversial and whose link to either main stream tobacco smoking or second hand smoking unproven. While squamous cell carcinomas and small cell carcinomas have shown an overall decrease in incidence during the past decade, peripheral adenocarcinomas (PACs) and bronchioloalveolar lung cancers (BACs) have shown exponential increases. These increases have been observed equally in both smokers as well as non-smokers. These epidemiological observations suggest that either different etiological factors exist (other than main stream or second hand smoke) that cause PACs and BACs or that different etiological co-factors that are synergistic with main stream or second hand smoke play a role in the genesis of PAC/BAC. Some of the distinguishing pathological, biological, epidemiological, and perhaps etiological features of PAC/BAC include its peripheral location, its association with desmoplasia (scarring), its significant occurrence in non-smokers, its comparatively high female/male ratio, and its high incidence of multifocality (especially BAC) which reflects its multiclonal origins. Unlike other forms of lung cancer, PAC/BAC naturally occurs in cats and sheep, the latter occurring as ovine pulmonary adenomatosis or jaagsiekte, a disease caused by an exogenous retrovirus (JSRV).

Antibodies made to a recombinant JSRV major capsid protein of this virus (derived from gag) in our initial immunocytochemical studies surprisingly were able to recognize an immunologically related protein in a significant number of human PAC/BAC cases but not in other types of lung cancer nor in normal lung. More surprisingly, in our subsequent studies, RT-PCR performed on these PAC/BAC cases revealed expression of JSRV gag transcripts which were 95-100% identical to both the endogenous and exogenous gag transcripts (distinguished by a ScaI site) expressed in sheep jaagsiekte.

These findings raise several intriguing possibilities: 1) an exogenous retrovirus is involved in either the initiation and/or promotion of human PAC/BAC; 2) activation and expression of an endogenous retrovirus and the gene products of this retrovirus, eg., gag in human PAC/BAC is an important step in the pathway of tumorigenesis analogous to the activation of oncogenes; 3) the mechanism of oncogenesis in human PAC/BAC reactivates retroviral transcripts as downstream events of the neoplasia. These downstream events have nothing to do with transformation or progression per se but may reflect the end point of a common pathway present in both PAC/BAC and sheep jaagsiekte (since in jaagsiekte there is also reactivation of endogenous gag).

A04

Development of a blood test for pancreatic cancer

Hao, Ying; **Lowe, Anson**
Stanford University

Pancreatic cancer is the most lethal human cancer. The median survival is 2-3 months after diagnosis. It is the fourth leading cause of cancer-related death among men and women in California and the United States and accounts for 28,000 deaths per year.

Numerous scientific studies designed to reveal the causes of pancreatic cancer have consistently identified cigarette smoking as a significant risk factor. In fact, cigarette smoking remains the only well-established risk factor for pancreatic cancer. The risk of pancreatic cancer correlates with the amount of cigarette smoking. These findings have been supported by experiments in the laboratory where rats fed chemicals derived from tobacco develop cancers of the lung and pancreas. Thus cigarette smoking is a cause of pancreatic cancers.

An effective blood test for human pancreatic cancer will result in earlier surgical intervention, which currently represents the only hope for cure. As new therapies are developed in the future, a blood test for pancreatic cancer will also be used to assess the effectiveness of treatment.

The project's goal is to develop a test to measure blood levels of a pancreatic protein known as GP2. Previous studies performed in rats demonstrated that GP2 blood levels rise in pancreatic disease. The generation of anti-human-GP2 antibodies represents the first step in the development of a blood test for patients. For this purpose, we previously cloned and characterized the human GP2 gene. The gene was then expressed in mammalian cell lines to produce sufficient protein for the production of monoclonal antibodies. We have had recent success in generating anti-human GP2 antibodies that can be used for the development of a clinical assay. A prototype assay has been made for which its sensitivity and specificity are currently being assessed. The determination of GP2 levels in patients with pancreatic cancer will be possible in the very near future.

Thus far all of the pancreatic cancer victims from whom samples have been collected possess a history of cigarette smoking. Because smoking serves as the only well-substantiated risk factor for pancreatic cancer, this project strives to reduce the human cost of this disease through earlier detection. The test will also provide a means to measure the progress of the cancer as new therapies are developed.

A05

Novel imaging methods for diagnosing tobacco related cancers

Conolly, Steven; **Macovski, Albert**
Stanford University

The objective of this research project is to demonstrate the technical feasibility and low cost of a novel form of magnetic resonance imaging (MRI) for diagnosing and staging of tobacco-related cancers in the head and neck.

Conventional MRI systems cost between $1M and $3M. We propose a novel Prepolarized MRI system that uses two inexpensive electromagnets rather than one expensive superconducting magnet. We aim to demonstrate that a complete dedicated head and neck Prepolarized MRI scanner imaging can be constructed for less than $50,000 while maintaining excellent image quality.

During the first two years of this grant, we have made excellent progress towards these goals. We have invented three new methods for designing electromagnets that show great promise for improving patient comfort, physician access, and for reducing system cost. We also designed and constructed three new magnet power supply circuits, all of which which will improve image quality for reduced cost. We also constructed several transceiver circuits which are necessary for sensitive low-frequency MRI detection, a key engineering challenge for the PMRI concept. We recently accepted a bid from a local magnet contractor (Alpha Magnetics in Hayward, CA) to build our latest PMRI system for just $20,000. This new system incorporates all of latest magnet design inventions, and it is the first we have designed with bore wide enough (30-cm free bore) to image a human head and neck. The low price clearly indicates some significant breakthroughs in low-cost magnet technology.

Cancers of the head and neck account for about 1.5% of all cancer deaths. Studies have shown that non-drinking smokers have a 4- to 13-fold increase in risk for this cancer. MRI is currently the key clinical tool to indicate surgery over chemotherapy. Our PMRI system could reduce the cost of treatment staging. Ultimately, we hope that a PMRI scanner could be inexpensive enough that society could justify screening high-risk patients. Screening is now routine for diagnosing colon cancer, and early diagnosis is the best predictor of survival. Hence, PMRI could improve the survival rate and reduce the total societal costs of treating tobacco-related tumors of the head and neck.

A06

Early detection of oral cancer

Wilder-Smith, Petra; Ebihara, Arata
University of California, Irvine

Chewing or smoking tobacco is the main cause of oral cancer, a condition which claims the lives of almost 10,000 people each year. Because of the difficulty in detecting oral cancer early, it has one of the worst survival rates of all major cancers. This project investigated use of fluorescence for non-invasive (non-surgical), early diagnosis of potential precancers (leukoplakias) and cancers (squamous cell carcinoma) in hamster cheek pouches, which best simulate conditions in the human mouth. Both autofluorescence (the tissue's own fluorescence under laser light) and fluorescence under laser light after the application of the photosensitizer 5-aminolevulinic acid (ALA) are being studied.

These investigations were performed using the standard hamster cheek pouch model for oral cancer, and low light level fluorescence microscopy as well as an in vivo fluorescence detection system which could be used directly in the hamster mouth. Fluorescence under laser light of ALA-treated healthy tissues did not differ significantly between animals or in different locations in the mouth. Fluorescence in tissues pre-treated with ALA was significantly different in healthy tissues than in cancerous lesions. Both time-point of the fluorescence maximum and intensity of fluorescence differed. In healthy tissues, the strongest fluorescence occurred approximately 4-5 hours after topical ALA application; in cancerous tissues the peak occurred after 1.5-3 hours. The fluorescence maximum in cancerous tissues was up to 10x more intense than in healthy tissues. In premalignancy, the fluorescence maximum averaged 3-5x that found in healthy tissues.

The next step in our work will be to determine to what extent fluorescence correlates with the exact pathological status of the tissues. We will determine whether each stage in the progression of healthy tissue first to precancerous lesions, then to early, established and advanced cancer, correlates with a specific fluorescence signature. From this data we will be able to determine exactly how precise a diagnostic tool this modality can be. In summary, to date we have shown that non-invasive fluorescence measurements can be used for the early detection of premalignant or malignant change in the mouth. This information brings us one step closer to developing a sensitive and accurate tool for the early detection, diagnosis and monitoring of oral lesions. Clinical impact will be enormous, as currently >2/3 of all oral carcinomas are detected at the advanced stage, when 5 year survival is <16%. If detected early, 5 year survival is 75%. By the early detection of malignancies, and effective monitoring of suspect lesions, much pain and suffering will be prevented, and many treatment costs avoided.

A07

Developing new vaccines for lung cancer

Basak, Saroj; **Roth, Michael D**
University of California, Los Angeles

The goal of this study is to boost a patient's immune system so that it can react against lung cancer. The first specific aim is to test two immune stimulating drugs, GM-CSF and IL-4, for their ability to increase the number and function of special antigen presenting cells called dendritic cells. Dendritic cells are essential for stimulating the immune system and appear to be turned off in patients with cancer. The second aim is to determine whether these dendritic cells can enhance the immune response to genetically-engineered vaccines. The third and final specific aim is to combine GM-CSF and IL-4 therapy with genetically-engineered vaccines in a manner that boosts the immune response against lung cancer.

Research to date has concentrated on the first specific aim. Different combinations of the two immune stimulating hormones, GM-CSF and IL-4, were given to mice by a special continuous infusion pump. Spleens removed from mice treated with GM-CSF alone demonstrated a 15.9-fold increase in the number of lymphoid-type dendritic cells (CD11c+/CD8a+/MHCII+) and a 34.5-fold increase in the number of myeloid-type dendritic cells (CD11c+/CD11b+/MHCII+). In addition to generating dendritic cells, the combination of GM-CSF and IL-4 also produced a 5-6-fold increase in the number of spleen macrophages and natural killer cells - an effect that was not observed with GM-CSF alone. These studies suggest that GM-CSF and IL-4 can be used to enhance the number of dendritic cells, macrophages and natural killer cells. The dendritic cells stimulated in response to GM-CSF, or GM-CSF and IL-4, appear to function normally and are capable of stimulating T cells in a mixed-leukocyte reaction. In recent experiments we have examined the lymph nodes and spleens of treated mice under the microscope to determine the effects of our treatment on their lymphoid organs.

The next step will be to test whether this increase in antigen presenting cells can be used to enhance the immune response to a tumor vaccine. A common cold virus (adenovirus) has been modified in the laboratory so that it carries a gene encoding for a tumor antigen. When injected into tumor-bearing mice (or people), this virus should infect their dendritic cells, cause them to express the tumor antigen, and result in an immune response against their tumors If successful, mice treated in this way will destroy their tumor.

The administration of GM-CSF and IL-4 is now being tested in patients with cancer. We have observed up to a 300-fold increase in the number of circulating dendritic cells, and a restoration of immune function, in several patients. When finished, it is hoped that these studies will lead to a vaccine treatment which will stimulate the patients own immune system to cure lung cancer.

A08

Tobacco-related cancer prevention by vitamin A derivatives

Dawson, Marcia I.; Hobbs, Peter D.; Jong, Ling; Waleh, Nahid; Fontana, Joseph A.; Leid, Mark; and Zhang, Xiao-kun
Molecular Medicine Research Institute; SRI International, Wayne State University; Oregon State University; The Burnham Institute

In spite of recent advances in treating cancer, lung cancer continues to be the second most common cancer and the highest cause of cancer death in California. Although prostate cancer and breast cancer are only slightly more common, lung cancer kills about three times as many victims. Therefore, there is an urgent need for more effective methods to prevent and treat lung cancer. Tobacco use and exposure are the main causes of lung cancer. Because more women and young people are smoking cigarettes than in the past, these groups are at increased risk.

The carcinogens in tobacco smoke cause mutations in genes that regulate the cell cycle, with a resulting loss of the normal controls that allow the cell to repair itself or begin the process of natural cell death (apoptosis). Drugs that reintroduce cell-cycle control points permit abnormal (transformed) cells to either undergo repair or die rather than to continue their uncontrolled growth.

Recent studies indicate that apoptosis induction in damaged cells is one method to prevent cancer. We discovered that the vitamin A derivative or retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl)]-2-naphthalenecarboxylic acid (AHPN) inhibits lung cancer cell growth and induces lung cancer cell apoptosis. AHPN is far more effective (20- to 100-fold) than other retinoids, including the natural retinoid trans-retinoic acid, in inhibiting the growth of lung cancer cells in the laboratory, including those cells that have mutations in genes for the tumor suppressor protein p53 and the retinoid receptors. These genes, which have important roles in cancer cell growth inhibition, are commonly mutated in lung cancer cells so that they no longer function. Our studies indicate that AHPN exerts its anticancer activity without interacting with the retinoid receptors, an advantage because toxic effects caused by use of standard retinoids are less likely. Eliminating retinoidal activity in an analog of AHPN would reduce side effects due to interaction with retinoid receptors but retain lung cancer preventive effects.

We identified one variant of AHPN that causes cancer cell apoptosis but does not activate the retinoid receptors. Therefore, side effects caused by activation of retinoid receptors would be reduced. Moreover, this novel compound inhibited the growth of new blood vessel cells, which only grow during normal wound repair or reproduction, or when stimulated to do so by cancerous tumors. Formation of new blood vessels permits tumors to invade neighboring normal tissue and to migrate to other sites in the body to form second tumors. In the final year of this grant, we plan to evaluate the inhibitory activity of this novel compound and its analogs against lung cancer cell growth, including cancer cell lines whose growth is not inhibited by standard retinoids.

A09

Nicotine suppresses vitamin A activity by inhibiting RARß expression

Zhang, Xiao-kun; Chen, Guo-quan; and Lin, Bingzhen
The Burnham Institute

Epidemiological and animal studies have demonstrated that vitamin A and its natural and synthetic derivatives (retinoids) are effective agents in preventing the development of lung cancer. Unfortunately, clinical trials of retinoids on cigarette smokers have found lack of efficacy in preventing lung cancer, suggesting that cigarette smoking might impair retinoid activities. The overall objective of our project is to study the mechanism by which nicotine inhibits the anti-cancer activities of vitamin A.

Previously, we showed that nicotine could suppress anti-cancer activities of retinoids in lung cancer cells. We also found that the suppressive effect of nicotine is likely due to induction of nur77 by nicotine. In this study, we analyzed whether nur77 could regulate expression of retinoic acid receptor-beta (RARß), which is known to mediate the anti-cancer effect of retinoids.

Expression of RARß is highly induced by retinoic acid (RA) through a RA response element (ßRARE) that is activated by heterodimers of RARs and retinoid X receptors (RXRs). However, RARß induction is often lost in cancer cells despite expression of RARs and RXRs. In the present study, we observed that orphan receptor COUP-TF is required for RA to induce RARß expression, growth inhibition, and apoptosis in cancer cells. Expression of COUP-TF correlates with RARß induction in a variety of cancer cell lines. In addition, stable expression of COUP-TF in COUP-TF-negative cancer cells restores induction of RARß expression, growth inhibition, and apoptosis by RA, whereas inhibition of COUP-TF by expression of COUP-TF anti-sense RNA represses the RA effects. In transient transfection assay, COUP-TF strongly induced transcriptional activity of the RARß promoter in a RA- and RARß -dependent manner. The effect of COUP-TF requires both a DR-8 element that binds strongly with COUP-TF and the ßRARE in the RARß promoter. Mutations that either abolished COUP-TF binding to the DR-8 element or RARß binding to the ßRARE impaired RA- and RARß - dependent transactivation function of COUP-TF. By GST-pull-down assay, we observed that COUP-TF, through its interaction with RARß, strongly enhanced interaction of RARß with its co-activator CBP, suggesting that COUP-TF functions as an accessory protein for RARß to induce RARß promoter transcription. These results demonstrate that COUP-TF, by serving as an accessory protein for RARß to induce RARß expression, plays a critical role in regulating anti-cancer activities of retinoids. We previously showed that nur77 could physically interact with COUP-TF. We, therefore, investigated effect of nur77 on transactivation function of COUP-TF in the RARß promoter. Our results demonstrated that nur77 could strongly interfere with COUP-TF activity in various cell types examined. Together, our results demonstrate that nur77 could inhibit expression of RARß through its interaction with COUP-TF and suggest that nicotine may suppress anti-cancer effect of retinoids in part through its ability to interfere with RARß expression. (supported by a funding from TRDRP, University of California)

B01

Brachial vasoreactivity in young adult smokers

Barth, Jacques; Zhang, Ling; Zonjee, Maud
Southern California Prevention and Research Center, Los Angeles

Introduction: Smoking has been shown to be an independent cardiovascular risk factor. Complications of smoking can include the development and progression of atherosclerosis, myocardial infarction and stroke. The current view of the development of atherosclerosis indicates the following steps prior to an acute event. Initially, exposure to a risk factor inhibits endothelial function. If the cardiovascular risk factors persist, plaque formation and other complications can occur. The ability to detect the early changes in endothelial function (in particular in young adults who smoke) would be a valuable prevention tool because its reversal could prevent future complications later in life. Our group has experience with this type of quantitative assessment of the level of endothelial function. It is done using ultrasound images of the brachial artery, testing the response to hyperemia in the underarm, distal to the antecubital crease. We also have the capability of assessing peak velocity (using Doppler techniques) to assess the blood flow response to hyperemic challenge.

Hypothesis: To assess and compare the extent, severity and persistence of endothelial dysfunction between young adult smokers and nonsmokers using a sonographic flow-mediated dilation procedure.

Methods/Procedures: The method of choice to assess endothelial dysfunction is quantitative assessment of the vessel diameter using ultrasound images during a period of induced hyperemia. One hundred and fifty young adults (aged 18-25 years of age) were recruited and underwent this procedure. As designed, 75 were smokers and 75 had never smoked before. The images and vessel diameters were measured off-line after the series of images were acquired using a 7.5 MHz linear array B-mode/Doppler transducer. The vessel diameter was measured at baseline and at 30-second intervals for 5 minutes post-hyperemia. The peak velocity of flow as measurement of recuperation capacity after hyperemia was also analyzed.

Results: To date, all 150 individuals have been analyzed and 43 individuals have returned for a 1-year assessment. The cohort description is as follows: Smokers: 39% were female (n=29) with a mean age of 21 years. 61% male (n=46) with a mean age of 21.8 years. Also, 72% were Asian (n=54), 24% Caucasian (n=18), 4% African American (n=3), and 0% Hispanic (n= 0). Nonsmokers: 52% were female (n=39) with a mean age of 21.9 years. 48% male (n=46) with a mean age of 23.7 years. Also, 47% were Asian (n=35), 28% Caucasian (n=21), 14% African American (n=11), and 11% Hispanic (n=8).

Conclusion: In these cases we have seen that cigarette smoking had a deleterious effect on the normal level of vessel responsiveness to an oxygen-deprived situation, hyperemia (as measured by vessel elasticity). This suggests the beginning of atherosclerosis. The normal would have been a dramatic vascular dilation, which did not occur in the smokers group. Furthermore, the endothelial dysfunction was greater than expected and could lead to further complications. Analysis of the one-year follow-up data will provide additional information on the persistence of endothelial dysfunction. A sub-analysis on ethnic response patterns will be performed. These findings suggest the need for smoking prevention efforts in young adults, tailored to the specific ethnic origin.

B02

Trends in cigarette smoking among adolescents by ethnicity

Burns, David M; Anderson, Christy M; Shanks, Thomas G.
University of California, San Diego

Since smoking initiation varies between ethnic groups, we compared adolescent initiation rates among different ethnicities. For each gender, overall adolescent initiation rates and initiation rates among non-Hispanic White, Hispanic, and African-American adolescents were compared.

Adolescent initiation rates have been reconstructed from cross-sectional Tobacco Use Supplements to the Current Population Surveys (CPS) administered in 1992, 1993, 1995, and 1996. Initiation rates were reconstructed for each calendar year based on respondents who were 12 to17 years old during that calendar year. Rates were compared from 1942until 1990.

During this period, White male adolescents generally initiated cigarette smoking at higher rates than Hispanic or African-American male adolescents. Overall, initiation rates among male adolescents of all three ethnicities declined during this period. Initiation rates among White and Hispanic male adolescents followed a similar pattern, both rising beginning in 1983. Unlike initiation rates among Hispanic male adolescents, initiation rates among White male adolescents continued to rise until the end of the study, 1990. Initiation rates among Hispanic male adolescents declined from 1986 to 1990.

Although the overall patterns of initiation among Hispanic and African-American male adolescents look different, their initiation rates for almost every individual year were not significantly different. The initiation rates among African-American male adolescents have been declining at a greater rate than Hispanic or White male adolescents since 1972. Unlike initiation rates among Hispanic male adolescents, but similar to White male adolescents, initiation among African-American male adolescents increased from 1985 until 1990.Initiation rates among White and Hispanic female adolescents generally increased during this period.

Initiation rates among female adolescents of all three ethnic groups followed similar patterns with an increase in rates until the late 1950s and another sharper increase in themid-1960s, lasting until the mid-1970s. Relative to initiation rates among White female adolescents, initiation rates among Hispanic and African-American female adolescents were relatively flat for this period, and were rarely significantly different from each other for this period.

Initiation rates among African-American female adolescents have decreased more than initiation rates among White and Hispanic female adolescents since the mid-1970s. Whereas initiation rates among White and Hispanic female adolescents increased from 1984 to the end of the study, initiation rates among African-American female adolescents continued to decrease.

Different patterns of increasing and decreasing smoking initiation among gender and ethnic adolescent groups suggest the effect of varying social and cultural influences. These findings support the importance of including ethnic factors in studies of smoking behavior.

B03

The influence of advertising on adolescent initiation

Burns, David M; Anderson, Christy M; Vaughn, Jerry
University of California, San Diego

Cigarette manufacturers have claimed that advertising encourages smokers to switch cigarette brands without causing non-smoking youth to take up cigarettes. To evaluate this assertion, we compared sales and advertising of cigarettes with male and female adolescent initiation rates. In particular, the sales and advertisements of Marlboro and Camel were each compared to male adolescent initiation, and the sales and advertisements of the combined brands Virginia Slims, Eve and Silva Thins were compared to female adolescent initiation.

We collected quantitative estimates of the number of cigarette advertisements appearing in eleven popular magazines available from the beginning of the century to 1996, and these data were summed to present the number of advertisements per year for each primary brand of cigarette. Adolescent initiation rates were reconstructed from the cross-sectional Tobacco Use Supplements to the Current Population Surveys (CPS) administered in 1992, 1993, 1995, and 1995. Initiation rates were reconstructed for each calendar year and based upon respondents who were 12 to 17 years old during that calendar year.

In 1954, Philip Morris started a new advertisement campaign featuring a Marlboro Man who was either a cowboy, a sailor, etc. This introduction was accompanied by an immediate rise in the number of advertisements for Marlboro. This rise in advertising activity was quickly followed by arise in cigarette consumption but not a corresponding rise in male adolescent initiation, possibly indicating the switching of brands. In1963, Philip Morris concentrated solely on the cowboy as the Marlboro Man and in 1964 launched the now familiar Marlboro Country ad campaign. This introduction coincided with a renewed increase in advertisements and with the continued rise in consumption of Marlboro cigarettes. The initial rise in the number of advertisements for this new campaign occurred from 1963 to 1970, followed by a rise in male adolescent initiation from 1970 to 1975.

Like previous studies, we show that in the late 1960s a dramatic rise in cigarette sales among brands marketed to women, coincided with a corresponding increase in female adolescent initiation. This rise also matched a notable rise in the number of advertisements placed by these brands, in particular by Virginia Slims.

In 1987, R.J. Reynolds introduced Joe Camel to U.S. consumers at a time when male adolescent initiation had been increasing. In contrast to the rise in adolescent initiation coincident with the introduction of the Marlboro Country campaign and of Virginia Slims, male adolescent initiation rates did not change with introduction of Joe Camel. The number of advertisements for Camel cigarettes has increased since 1993,but it was not possible to evaluate the effect on adolescent initiation since the data only provided estimates for adolescent initiation rates up to 1990.

Adolescent initiation rates have coincided with increases in advertisements placed by some manufacturers, in particular the advent of the Marlboro Country ad campaign and the introduction of Virginia Slims.

B04

Global determinants of tobacco use onset in diverse youth

Carvajal, Scott C.
ETR Associates

In order to develop more potent adolescent tobacco-prevention projects, the factors most predictive of smoking in diverse youth need to be identified. This three-year study will test a model of adolescents' smoking using a broader array of theory-derived determinants than typically addressed in prevention programs. The determinants of interest include tobacco-specific and more general psychosocial factors of importance.

This poster reports on the predictiveness of our hypothesized model variables using our first year data. The tobacco-specific factors include respondents' confidence (self-efficacy) in their ability to avoid smoking, their attitudes towards smoking, the degree to which others smoke around them (smoking norms), perceived risks of smoking, barriers to smoking, and whether they intend to smoke in the next year. The general determinants of interest include the respondents' academic success, sense of future, level of depression, coping styles, involvement by their parents, and school involvement. The participants (N = 2004) were surveyed in seven middle schools in Northern California (we oversampled 6th graders). The participants completed paper and pencil questionnaires asking about their levels of tobacco use, background and cultural factors, and the psychosocial determinants of interest. Only enrolled students with positive parental (active) consent were surveyed (over 60% of the targeted sample participated). Ethnic grouping of the sample was as follows: 44% Latino (65% Mexican American, 8% Central American), 27% Euro American, 17% Asian American (35% Vietnamese, 28% Chinese, 20% Filipino), 4% African American, and 9% "mixed ethnicity" or another ethnicity not listed above. Twenty-three percent of the respondents had smoked at least one cigarette, and 9% had smoked in the last month. The findings suggest all of the determinants significantly predicted the smoking outcomes, and in the expected direction. The more stringent statistical tests suggest intentions, attitudes, self-efficacy, and academic success, in that order, were the strongest predictors of smoking in the last month. Because many students may be at risk for future smoking, yet had not ever tried smoking at the time of the survey, we used our general determinants to predict susceptibility in students who had not yet tried a cigarette. Having less parental involvement, more maladaptive coping strategies, exhibiting more depression, and not wanting to go to college were the strongest determinants of smoking susceptibility.

Our work is related to the prevention of cigarette-related diseases. By helping identify the key predictors of adolescent smoking, we can help target prevention efforts toward the most important factors related to youths' onset and escalation of smoking. Though preliminary, our findings suggest that programs combining cognitive & behavioral skills-based smoking curricula with youth development approaches may be most effective in reducing smoking in youth. Over the next 2 years we will further test our model using more rigorous scientific tests made possible by following these students over time and explore whether the findings hold for adolescents of various cultural backgrounds, especially for Latinos.

B05

Does age affect self-reported age of smoking initiation among adolescents? An analysis of adolescent data collected through the computer assisted telephone interviewing technique in California

Chen, Xinguang; Unger, Jennifer B.
University of Southern California

Age of smoking initiation is an important indicator of the success of tobacco control efforts. Such information is usually collected through self-reported recall data. Both the California Tobacco Survey (CTS) and the California Youth Tobacco Survey (CYTS) have collected data on the age of smoking initiation using the computer-assisted telephone interviewing (CATI) technique. Studies with data from in-classroom surveys and face-to-face interviewing have indicated that these data may be biased; self-reported age of smoking initiation tends to increase as respondents grow older (Labouvie et al., 1997, Newman et al., 1976). This study examines if age of the respondents at the time of the survey affects their self-reported age of smoking initiation using the CATI data collection mechanism.

Methods: Data were analyzed from 20,327 adolescents 12 through 17 years of age (50.3% males). 13,978 were from CTS in 1990-1991, 1992, and 1993; 6,349 were from CYTS in 1994, 1995 and 1996. 49.8% were Caucasian, 29.5% were Latino/Hispanic, 7.4% were African American, and 8.6% were Asian Americans, and 4.7% were from other ethnic groups. The mean age of the subjects by gender, data source and ethnicity ranged from 14.38 to 14.52 years with no significant differences by data source, gender, and ethnicity. 4,715 respondents (23%) reported an age of smoking initiation. The reported mean age of smoking initiation ranged from 12.34 to 12.97 years with no significant differences by data resource, gender and ethnicity.

The data on age of smoking initiation were analyzed according to the respondents' age at the time of the survey. If self-reported age of smoking initiation indeed increases as the respondents grow older, the older respondents in the sample would be expected to show an older age of smoking initiation than the younger respondents would.

Results: A cross-tabulation of age at survey by the self-reported age of smoking onset indicated no significant increasing trend in self-reported age of smoking initiation along with the increase of the subjects' age at survey. For example, the proportion who reported they started cigarette smoking at 12 years of age was 17%, 21%, 18%, 20% and 19% for subjects with age at survey of 13, 14, 15, 16 and 17 respectively. A similar trend was observed for both male and female subjects, for subjects from both CTS and CYTS data, and for the four ethnic groups of White, Latino/Hispanic, Black, and Asian. Regression analysis indicated that the age at survey did not have significant impact on the self-reported age of smoking initiation in general (beta = -0.068, p = 0.084, r square = 0.002). However, such impact was significant for females (beta = -0.100, p = 0.049, r square = 0.06), for non-Hispanic Whites (beta = -0.129, p=0.008, r square = 0.009), and for Asian Americans (beta = 0.530, p = 0.009, r square = 0.108).

Conclusions: The data on age of smoking initiation reported through CATI mechanism does not show a significant upward trend with the respondent's age at the time of the survey. However, there was a slight upward trend for Asian American adolescents and a slight downward trend for females and for Caucasian adolescents. Although these trends were statistically significant in this large sample, they accounted for a very small proportion of the variance in self-reported age of smoking initiation. In addition, these trends appear not to exert significant bias on smoking initiation data, because a one-year difference in the age of the subject at survey will result in only a 1-6 month difference in the reported age of smoking initiation according to the regression analysis. These results indicate that retrospective self-reports of age of smoking initiation may be more accurate than previous studies have suggested. Further longitudinal population-based studies are necessary to determine the causes and extent of bias in self-reported age of smoking initiation among adolescents.

B06

A multivariate, multigene approach to the genetics of complex traits

Comings, David
Beckman Research Center, City of Hope, Duarte, CA

The identification of the genes involved in complex, polygenic traits has proven to be a difficult challenge. Polygenic disorders are due to the interactive effect of multiple genes, each with a small effect. Our studies of over 60 genes in many different behavioral disorders have shown that on average each gene contributes to less than 2% of the variance of the phenotype. Family based linkage techniques do not have the power to identify genes with such small effects. Population based association studies do have the power to detect small effect. However, random genome screens would require over 500,000 single base pair polymorphisms (SNPs). More suitable techniques for studies of complex traits must be developed before we can hope to clarify the role of individual genes as risk factors in smoking.

We propose that the best way to identify the genes used in disorders that are due to the interactive effect of multiple genes is to examine the additive effect of multiple candidate genes. Most of the first line candidate genes for behavioral and addictive behaviors have been identified. SNPs are available for half of these and SNPs for the remaining half are rapidly being identified. We have developed a technique for studies of complex traits called Multivariate Analysis of Associations (MAA). One or more quantitative phenotypic traits are chosen for study in a sample of 200 or more subjects. The genotypes of each of 5 to 100+ genes are assigned a score of 0 to 2 depending upon whether they are associated with low, intermediate or high mean for each phenotypic score. Multivariate regression analysis is utilized with the phenotypic trait as the dependent variable and gene scores as independent variables. With pin set to .1 and pout to .2, with backward elimination to select the genes included in the equation, this identifies those genes that contribute to the phenotype in the presence of all the other genes being tested.

The MAA technique provides six pieces of information about the genes involved in a complex trait. 1. Which genes are included in the equation, 2. The fraction of the variance attributed to each included gene, 3. The level of significance for each included gene, 4. The relative involvement of the different genotypes of each involved gene, for each trait, 5. The sum of the variance of functional groups of genes, for each trait, and 6. The ratio of the variance for each gene group to all the other gene groups, for each trait. To demonstrate the effectiveness of the MAA technique, we present the results of an examination of the role of 59 genes in the seven personality traits of the Temperament and Character Inventory.

The MAA technique can also be adapted to dichotomous traits such as smokers vs non-smokers. These studies are in progress and will provide information on the relative involvement of dopamine, serotonin, norepinephrine, GABA, neuropeptide, hormone, and other functional groups of genes in tobacco dependence. This can lead to the identification of new treatments for nicotine addiction.

B07

Cigarette smoking, arterial wall thickness, and systolic distention of the common carotid artery

Dwyer, James H
University of Southern California

Smoking is associated with increased risk of cardiovascular disease events. This relation may be achieved via promotion of atherosis (fatty lesions in the arterial intima), sclerosis (stiffening of the artery wall), plaque rupture, thrombosis (formation of clots in the arterial lumen), or some combination of these pathological pathways. The current study is investigating the relations between cigarette smoking and an indicator of early atherosis (intima-media thickness, IMT) and sclerosis (systolic disention) in the common carotid arteries.

Our laboratory has developed highly reproducible procedures for the measurement of intima-media thickness (IMT) and systolic distention in the common carotid arteries using high resolution B-mode ultrasound at 7.5 MHz. IMT is a measure of arterial wall thickness and the extent of early atherosis. Systolic distention measures the change in diameter between diastole and systole of the cardiac cycle and is thus related to stiffness of the artery wall.

The data for this study are generated from a cohort of 573 employees of a large utility company who were free of symptomatic cardiovascular disease and aged 40-60 years at recruitment. Members of the cohort are examined at 18-month intervals for arterial wall thickness, arterial stiffness and risk factors (such as smoking).

Preliminary results from partial data at the baseline examination suggest that cigarette smoking has a greater impact on arterial wall thickness than on arterial stiffness. If this tendency is confirmed by complete and longitudinal observation, this will suggest that the pathological pathway for promotion of cardiovascular disease by smoking is via more rapid progression of fatty lesion development in the arterial wall, rather than promotion of stiffness and ensuing vasomotion disorders. If confirmed by further observation, this finding would have implications both for the treatment of coronary artery disease among smokers and for the use of early detection of progressing IMT among smokers as a motivator for smoking cessation.

B08

Racial/Ethnic patterns of adolescent smoking over six years

Ellickson, Phyllis L.
RAND, Santa Monica, CA

The adverse health effects associated with cigarette smoking are extensive and well documented (US Department of Health and Human Services, 1994). Yet the public health gains in youth smoking prevention that were experienced during the 1980's have eroded in recent years (Johnston et al., 1997). To improve prevention efforts, we need to better understand the natural history of youth smoking initiation and cessation and the factors that place young people at risk for cigarette use. To address these goals, we are using data from a longitudinal study of youth recruited from 30 California and Oregon schools and followed from Grade 7 (N=6527) to Grade 12 (N=4390).

This analysis examined whether patterns of smoking established during early adolescence remain stable across racial/ethnic groups throughout the middle and high school years. We examined lifetime and annual prevalence rates, weekly smoking, and daily smoking for four groups—Whites, Mexican Americans, African Americans and Asian Americans. All analyses were adjusted for attrition over time. The obtained sample at grade 12 included about 10% who had dropped out of school before high school graduation.

Results for Grades 7-10 and 12 show substantial crossover in relative rankings across groups. Although a large proportion of African American youth started smoking during or before early adolescence, most did not make the transition to regular use. Quit rates for African Americans rose substantially between grades 8 and 9 and continued to rise thereafter. Correspondingly, African Americans had the lowest rates of weekly smoking from grade 9 to grade 12 (between 7 and 12%), while daily smoking for this group never rose higher than four percent. In contrast, relatively few Asian American youth started smoking as young adolescents (35%). However, they began to catch up with other groups after entry into high school (grade 9). By grade 12, Asian American rates of daily smoking approached those for Mexican Americans.

Although Mexican American youth had higher early initiation rates than whites, they did not progress as quickly to regular and daily use as did whites. By grade 12, non-Hispanic white youth actually had the highest rates of weekly and daily smoking. Mexican American youth had the next highest rates, but daily smoking in this group was only two thirds that for whites. Because the sample included a substantial proportion of dropouts and was weighted to adjust for attrition over time, these findings are unlikely to be an artifact of differential dropout rates.

These results indicate the need to continue prevention efforts throughout the high school years, when substantial changes occur in smoking behavior across racial/ethnic groups. They also underscore the importance of identifying what factors help protect African American from progressing to regular smoking once they start, what factors increase the risk for white and Mexican American adolescents, and what factors accelerate use for Asian American teenagers during high school. Such information would help program developers adapt prevention efforts to better meet the needs of different youth populations. Subsequent analyses for this study will seek to identify common and unique risk and protective factors for continued smoking across these four racial/ ethnic groups.

B09

Natural history of tobacco addiction in polydrug users over 3 years

Hser, Yih-Ing; McCarthy William J.; Zhou, Yun
University of California, Los Angeles

Insight into the natural history of tobacco addiction was sought by observing tobacco use annually in 254 polydrug users over a 3-year period. Two studies are reported here.

Study 1: The pattern of stability/change in smoking status was examined annually. Respondents' smoking status was classified as non-smoker, intermittent smoker, or regular smoker. Smoking was defined as "intermittent" if respondents reported smoking for less than 15 days of the last month. Results showed that the typical probability of retaining the same smoking status across any two consecutive assessments was: 0.77 for non-smokers, 0.82 for regular smokers, and 0.16 for intermittent smokers. Fifty-five percent of intermittent smokers converted to regular smoking within a year but 29% converted to former smoker status in the same period. No other transition exceeded 12%. Considerable flux in individual smoking status, particularly among intermittent smokers, was observed despite stable prevalence of smoking status in this population. Intermittent smoking status appeared to be a temporary "way station" between the two more stable regular smoker and non-smoker classifications, affecting 26% of the entire cohort over a 3-year period. These results challenge past characterizations of intermittent smoking as a rare personality trait. Intermittent smoking may be more justifiably described as a temporary but predictable behavioral state potentially affecting more than a quarter of all smokers at some point during their smoking careers.

Study 2: Two popular measures of addiction were compared for their ability to predict future smoking status. An adaptation of the Fagerstom Nicotine Tolerance Questionnaire (FNTQ) explained 38% and 30% of the variance respectively, in Time 2 and Time 3 smoking status. When previous smoking status was included as a covariate, however, the variance in Time 2 and Time 3 smoking status was reduced to 0.3% ($p > 0.20$). A subset of measures from the Addiction Severity Index (ASI) were also used as predictors. The ASI explained 2.5% and 4.7% of the variance in prospective Time 2 and Time 3 smoking status, respectively, but when previous smoking status was included as a covariate, their relation to future smoking status was only slightly reduced (1.4% and 4.0% of the variance, respectively; both $p < .05$).

Results highlight the epiphenomenality of self-report tobacco addiction severity and suggest that more clinical benefit may be obtained by focusing on changing a respondent's relationship to his community (his legal status) and changing other lifestyle habits associated with tobacco addiction, particularly alcohol use, even though the variance explained by these factors in prospective smoking status is initially small.

Immediate future research will focus on estimating the impact of tobacco use status on other drug use and on physical health outcomes among polydrug users followed for 3 years. Longer-term future research will examine novel non-pharmacological approaches to helping smokers live more healthfully. Novel approaches are needed to help the 80% of smokers who want to quit, but who have thus far not been able to sustain long-term abstinence, even with nicotine adjuncts.

B10

The popularity of cigars: Continuing phenomenon or fading fad?

Reimann, Joachim O.F.; Liles, Sandy; Hostetter, C. Richard; Chu, Sandy; Angulo, Ofmara Y.; Hovell, Melbourne F.
San Diego State University Foundation

Over the last two years, trends in cigar use have become increasingly unclear. From 1992 to 1996, the popularity of cigars increased steadily, with the number of estimated regular U.S. smokers jumping from 8 to 10 million. A growing number of social events, magazines, internet sites, and clubs celebrated cigars. While research linked cigar use to serious health risks including laryngeal and esophageal cancer, the misperception that this form of smoking was safe remained common.

Some more recent indicators, however, suggest that cigar use is decreasing. For example, one major cigar company reported that its net income fell by nearly half during the latter part of 1998. Subsequently, industry analysts predicted that general cigar sales will drop at least ten percent in 1999, and that the sale of premium brands will fall by as much as 25%. These figures have lead media sources to respond with headlines such as "Ashes to Ashes: Stogies' Trendiness Takes a Tumble." In actuality, it is difficult to deduce if these figures reflect a real decline in cigar use. It might, for example, be speculated that an overestimation of projected demand has resulted in overstocked retail outlets. Thus no automatic conclusions about the trend's demise can be made.

As part of a multi-faceted research effort, our epidemiological study determined current cigar smoker prevalence rates and use patterns. A total of 1,137 San Diego County residents ranging in age from 18 to 93 (Mean = 44.2) participated in a random digit dial telephone survey. Forty-six percent of the total sample indicated having smoked cigars at some point, and 10.5% reported themselves as current smokers. For males, the current use rate fell at 13.3% while the corresponding figure for females was 3.8%. Among ethnic groups, Latinos reported the highest percentage of current cigar smokers (13%), followed by Whites (10.8%), African Americans (8%) and Asians (4.8%). None of those identifying themselves as Middle Eastern or American Indians described current cigar use. Male smokers reported having consumed an average of 10.5 cigars, while females reported having smoked an average of 8.7 cigars in the last 30 days. Age of initiation ranged from 12 to 63 with a mean of 25. Thirty-four percent of smoking respondents said they planned to use cigars for the rest of their lives.

Approximately two percent of those who had smoked cigars at some point, and 7.3% of current smokers attributed health problems to their cigar use. Reported difficulties included the aggravation of asthma and allergy symptoms, chronic cough, and bleeding gums.

A comparison of current results to 1996 cigar smoking prevalence rates among California adults (8.8% for men and 1.1% for women) suggests a recent increase rather than decrease in cigar use. While the metropolitan characteristics of San Diego County may contribute to higher than state-wide prevalence rates, overall results suggest that, at best, cigar smoking has leveled off. In conclusion, it appears that cigar use is not fading by itself. Given associated health risks, continued efforts to counteract this tobacco's popularity appear important.

B11

Gay male smokers: Prevalence of current smoking

Stall, Ronald D; Greenwood, Greg; Paul, Jay
University of California, San Francisco - Center for AIDS Prevention Studies

Research objective: To measure the prevalence of current smoking among men who have sex with men (MSM) among a population-based sample of MSM in four large American cities.

Background and significance: Although results from a small historical literature tend to conclude that MSM smoke at higher rates than do men drawn from comparison samples of the general population, the opportunistic sampling methods used in these studies may have oversampled gay male smokers (i.e. through recruitment at gay bars). In addition, little attention has been paid to identifying the social and behavioral characteristics of gay male smokers. Estimating the prevalence of current smoking with data drawn from probabilistic sampling methods should be attempted before claims of higher current smoking rates among MSM populations are accepted.

Methods: From a previously recruited population-based sample of MSM (n=2881) in four urban areas (Chicago, Los Angeles, New York and San Francisco), respondents who were willing to participate in a follow-up interview (89% of the original sample) were re-contacted beginning in December 1998. So far, 1551 MSM have participated in a brief telephone interview on history of tobacco use and methods used to quit.

Findings: Prevalence rates of smoking (with 95% C.I.'s) for gay men were compared to published rates in 1996 of smoking from men drawn from general population samples at the county (San Francisco and Los Angeles) and state level (New York and Chicago):

	UMHS	Men in Gen. Population
San Francisco	**29.1%** (25.1-33.1) (N=498)	**23.7%** (21.4-26.0) (N=1690)
New York	**30.7%** (26.4-35.1) (N= 433)	**23.3%** (21.0-25.5) (N= 1744)
Los Angeles	**26.1%** (21.8-30.5) (N=391)	**21.6%** (20.5-22.8) (N= 7582)
Chicago	**37.8%** (31.5-44.1) (N=225)	**26.2%** (23.4-29.1) (N=1151)

Discussion: Although the prevalence estimates for MSM interviewed to date from the UMHS were always higher than for men in the general population, in two cities (San Francisco and Los Angeles) confidence intervals overlapped. Although from the preliminary data it cannot yet be concluded with a high degree of certainty that gay men smoke at higher rates than other men, it should also be recognized that rates of smoking are too high among all men. Advocates for tobacco prevention efforts among gay men should not have to claim that MSM populations have the highest rates of smoking of all men in order to justify need for prevention campaigns in their community. Analyses of the final, completed sample as well as the associations of current smoking will be presented.

B12

Susceptibility to smoking among California adolescents

Unger, Jennifer B. - *University of Southern California*

Susceptibility to smoking is the absence of a firm commitment not to smoke. It is a risk factor for adolescent smoking initiation. This study examines the demographic, attitudinal, psychosocial, and environmental correlates of susceptibility among California adolescents. We have analyzed data from Wave 1 of the Independent Evaluation of the California Tobacco Control, Prevention, and Education Program, which included school-based surveys of 15,834 California adolescents.

Using data from 5870 8th-grade students, we compared susceptible youth with non-susceptible never smokers, experimenters, and addicted smokers on tobacco-related attitudes, beliefs, and behaviors. Two main patterns of results emerged. In the first pattern, susceptible never smokers showed a level of risk that was in-between that of the nonsusceptible never smokers and the experimenters. This supports the view that susceptibility is an intermediate stage of smoking initiation that precedes experimentation. In the second pattern, however, susceptible adolescents showed especially high levels of risk, higher even than those of experimenters and addicted smokers. This pattern was evident for cigarette refusal self-efficacy and tobacco-related knowledge.

Among adolescents who had not yet tried smoking, logistic regression analyses were used to identify the risk factors for susceptibility. Among the respondents who had not yet smoked, the risk factors for susceptibility were similar to the risk factors for smoking initiation found in previous studies. These included older age, Latino ethnicity, acculturation, low smoking-related knowledge, low perceived negative consequences of smoking, high perceived positive consequences of smoking, low cigarette refusal self-efficacy, friends’ smoking, friends’ approval of smoking, perceived access to cigarettes, cigarette offers, and use of other forms of tobacco.

Next, we analyzed data from 6887 10th-grade California adolescents to examine attitudes toward anti-tobacco policy among susceptible adolescents, including awareness of anti-tobacco policies and support for anti-tobacco policies. Awareness of anti-tobacco policies was especially low among susceptible never smokers, lower than all other stages of smoking initiation. Support for anti-tobacco policies among susceptible never smokers was lower than that among nonsusceptible never smokers, but it was higher than that among experimenters, addicted smokers, and former smokers. Policy awareness and support were significantly associated with psychosocial tobacco-related variables, including perceived consequences of smoking, friends’ smoking, perceived access to cigarettes, prevalence estimates of smoking among peers, cigarette offers, and cigarette refusal self-efficacy. Policy awareness and support were associated with the probability of performing advocacy actions against tobacco use.

These results suggest that susceptibility to smoking is a stage of heightened vulnerability to tobacco-related influences. Susceptible adolescents are characterized by a low ability to refuse cigarette offers, low knowledge about the dangers of tobacco, and low awareness of anti-tobacco policies. This information may be useful for the development of effective tobacco control programs to prevent susceptibility among never-smokers and to prevent smoking among adolescents who already are susceptible to smoking. A better understanding of the conditions associated with susceptibility may make it possible to design tobacco control programs that prevent or counteract susceptibility and thereby prevent adolescents from progressing to the experimentation stage.

POSTER SESSIONS
Session C: Nicotine Dependence

C01

Discrete sites of action for spinal epibatidine and a-85380 in eliciting analgesic responses in rats

Khan, I.; Stanislaus, S.; Yaksh, T. and **Taylor, P.**
University of California, San Diego

Previously, we demonstrated that intrathecal (i.t.) administration of the nicotinic agonist. epibatidine (EPI), elicits a short-lasting but profound analgesic response in rats. However, the analgesic response is accompanied by cardiovascular and irritation responses. Our data suggest that a different subtype of spinal nicotinic receptor mediates the analgesic response, as opposed to the irritation response. Recently, others have demonstrated that a newer nicotinic agonist, A-85380, upon s.c. injection can elicit an analgesic response with minimal cardiovascular or other behavioral responses.

In the present study, we examined the spinal action of A-85380 to determine if it could selectively elicit analgesic response at the spinal level. Sprague-Dawley rats were implanted with i.t. catheters and the analgesic response to A-85380 was determined by using the Hot Box apparatus. Intrathecal A-85380 elicits an analgesic response in a dose-dependent fashion. However, similar to other nicotinic agonists, it also elicits profound pressor and heart rate responses as well as an irritation response. In addition, A-85380 also displaced [3H]-epibatidine binding from spinal cord membrane in a dose-dependent fashion. The effects of i.t. nicotinic receptor antagonists on A-85380 elicited analgesic and irritation responses were examined. Mecamylamine blocked both of the behavioral responses to A-85380. DbE blocked the analgesic response but not the irritation response elicited by the nicotinic agonist. Methyllycaconitine significantly blocked both of the behavioral responses. In contrast, neither competitive nicotinic receptor antagonist blocked the analgesic response to i.t. EPI. Prior administration of A-85380 desensitized the analgesic response to subsequent i.t. A-85380. However, repeated administration of A-85380 did not desensitize subsequent EPI elicited behavioral responses. In contrast, repeated prior administration of EPI desensitized the A-85380 elicited analgesic response. The irritation response to A-85380 was significantly blocked by pretreatment with AP-5, a NMDA receptor antagonist. This suggests that, similar to other nicotinic agonists, A-85380 elicits the spinal release of EAA to produce the irritation response. Interestingly, pretreatment with i.t. phentolamine almost completely abolished A-85380 elicited analgesic response. In contrast, phentolamine had no effect on EPI elicited analgesic response.

The data suggest that analgesic response elicited by A-85380 may be produced by stimulation of nicotinic receptors localized on adrenergic descending bulbospinal terminals. The data also argue that A-85380 when compared to EPI, acts on separate spinal sites as well as different icotinic receptor subtypes to elicit an analgesic response at the spinal level. (Supported by HL-35018 and TRDRP).

C02

Functional neuronal nicotinic receptors on primary afferent terminals

Khan, I., Stanislaus, S., Calvo, R., Osaka, H.,
Yaksh, T. and **Taylor, P.**
University of California, San Diego

Intrathecal (i.t.) nicotinic agonists including epibatidine (EPI) elicit dose-dependent increases in pressor, heart rate and pain responses in rats. [3H]-EPI reveals two types of binding sites in spinal cord membranes, which differ in both affinity and number of sites. Higher affinity sites are present in the superficial dorsal horn layer, which contains c-fiber primary afferents.

To correlate function with location of nicotinic receptors in the spinal cord, we examined the nicotinic receptor subunits on c-fibers and their role in eliciting pain responses to i.t. nicotinic agonists. Neo-natal rats were treated with the neurotoxin capsaicin (50 mg/kg, s.c.) to degenerate c-fiber neurons. At 12-14 weeks of age, the capsaicin-treated rats were implanted with i.t. catheters and responses to nicotinic agonists examined. Cytisine or EPI did not show differences in magnitude of pressor, tachycardia or pain responses between capsaicin-treated and control rats. However, in the EPI treated rat, the duration of the pain response was significantly shortened in capsaicin-treated rats as compared to control rats. In control rats, repeated cytisine dosing partially desensitized the pain response to subsequent EPI dosing; however, in capsaicin-treated rats, i.t. cytisine pretreatment almost completely desensitized the pain response to subsequent i.t. EPI. Autoradiography also revealed reduction in [3H]-EPI binding in the superficial dorsal horn in capsaicin-treated rats. Immunostains for $\alpha 3$, $\alpha 5$ and $\beta 2$, but not $\alpha 4$ nicotinic receptor subunits could be detected in the presynaptic terminal in superficial layer of the dorsal horn. Moreover, there was significant loss of staining for $\alpha 3$, $\alpha 5$ and $\beta 2$ in capsaicin-treated vs. control rats in this region. The data reveal that nicotinic receptors on the primary afferent terminals play a role in mediating pain response to i.t. nicotinic agonists and they also show different desensitization properties to specific nicotinic agonists. ***(Supported by HL-35018 and TRDRP).***

C03

Augmented responses to spinal nicotinic agonists in a genetic model of rat hypertension

Khan, I., Stanislaus, S., Yaksh, T., Printz, M. and **Taylor, P.**
University of California, San Diego

Abnormalities in central cholinergic system have been implicated in the pathogenesis of high blood pressure in different models of rat hypertension including the Spontaneously Hypertensive Rats (SHR). Nicotine and other nicotinic agonists, when administered intrathecally (i.t.) elicit dose-dependent increases in blood pressure, heart rate and nociceptive responses in rats. These cardiovascular and behavioral responses to spinal nicotinic agonists are augmented in SHR as compared to normotensive WKY or Sprague-Dawley rats. Moreover, the genetically hypertensive SHR shows a slower rate of desensitization to the cardiovascular and behavioral responses to repeated administration of i.t. cytisine. Interestingly, in the presence of heightened responses to spinal nicotinic agonists, a paradoxical decrease in spinal nicotinic receptor is observed in adult SHR versus age-matched WKY rats.

In this study, we sought to determine if the hyper-responsiveness to spinal nicotinic agonist in SHR as compared to normotensive rats is a determinant, but not the consequence, of higher blood pressure in the genetic model of rat hypertension. Similar to those observed in adult rats, pressor, heart rate and pain responses to i.t. cytisine were ugmented in the 6 week old prehypertensive SHR vs. age-matched WKY rats. In SHR and WKY rats, hydralazine (25 mg/kg/day, p.o.) treatment from 5 to 12 weeks of age significantly lowered the blood pressure in both strains of rats. Interestingly, at the end of the treatment period, the mean arterial pressure between hydralazine-treated SHR and tap water drinking WKY rats did not differ. However, the responses to i.t. cytisine were still augmented in hydralazine-treated SHR versus hydralazine-treated WKY rats. In a separate set of experiments, captopril (200 µg/kg/day, p.o.) treatment from in utero to 12 weeks of age resulted in significantly lowered blood pressure in both strains of rats. However, similar to untreated rats, cardiovascular and pain responses to spinal nicotinic agonists were still augmented in captopril-treated SHR versus captopril-treated WKY rats. Finally, seven-week-old WKY rats were uninephrectomized and then treated with DOCA and oral saline for 5 weeks. The DOCA-salt treatment resulted in hypertension in these WKY rats as compared to sham-operated vehicle-treated WKY control rats. However, the DOCA-salt treated WKY rats with a chronically elevated blood pressure did not exhibit augmented cardiovascular or behavioral responses to spinal cytisine as compared to control WKY or untreated WKY rats. Overall, our data suggest that the hyper-responsiveness to spinal nicotinic agonists in the SHR compared to normotensive rats is a determinant, but not the consequence, of the higher blood pressure in SHR, a genetic model of rat hypertension. (Supported by HL-35018 and TRDRP).

C04

Analgesia induced by exposure to tobacco smoke and hyperalgesia during withdrawal

Anderson, Kenton L.; Pinkerton, Kent E.; Carstens, Mirela Iodi and **Carstens, E.**
University of California, Davis

The primary goal of our research project is to investigate the analgesic (pain-reducing) and irritant effects of nicotine in tobacco smoke. While nicotine is known to produce analgesia, the effect of tobacco smoke on pain-sensitivity has not been well studied.

Eight adult rats were exposed to controlled conditions of cigarette smoke in an environmental chamber over 4 successive 5-day blocks (6 hr/day), with 2 smoke-free days between blocks. The concentrations of total suspended particulate matter (TSP), nicotine and carbon monoxide (CO) in the exposure chambers averaged 87.3 +/- 8.8 mg/mm^3, 3.67 +/- 0.59 mg/mm^3 and 210.1 +/-24.6 (SD) ppm, respectively. On days 1, 3 and 5 of each smoke exposure block, and on the first or second smoke-free day between smoke exposure blocks, the following behavioral tests were conducted: tail-flick latency (TFL), thermal paw withdrawal latency (TPW), von Frey threshold (VF), and hot plate (HP) latency. For TPW, rats stood on a glass surface and the time to paw movement after heating the foot, from below, was measured. For VF, rats stood on wire mesh and the force required to elicit touch-evoked paw withdrawal was measured. For HP, the rat was placed on a pre-heated surface and the time measured until it jumped or licked a hindpaw. Behavioral measures during smoke exposure were compared to pre-exposure baselines in the same rats, as well as to behavioral data taken from control rats (N=8) not exposed to cigarette smoke.

There was a significant loss in body weight during smoke exposure, with small weight gains during the smoke-free interludes and a rapid weight gain after cessation of smoke exposure. During each block of smoke exposure, TFL increased significantly. The magnitude of the TFL change progressively decreased across smoke exposure blocks, indicative of tolerance. During smoke-free intervals, TFL increased significantly within 1 day, indicating short-term reversal of analgesia and possible development of hyperalgesia. Similar results were obtained with TWP and HP. VF, which measures tactile sensitivity, was not consistently affected during smoke exposure. However, during smoke-free intervals, the VF threshold decreased, reflecting an increase in mechanosensitivity.

The data obtained using several different tests consistently show a reduction in pain-like behavioral reactions, i.e. analgesia, during exposure to cigarette smoke. These changes recovered rapidly during smoke-free periods, indicating that the analgesic effect wears off quickly and/or that increased sensitivity to pain (hyperalgesia) develops during acute withdrawal from smoke exposure. Future pharmacological studies are planned to test the hypothesis that nicotine in cigarette smoke is the primary factor involved in analgesia. The findings are significant in terms of nicotine addiction and withdrawal. The analgesic effect may provide positive physiological reinforcement for smoking, while the increase in pain-sensitivity during withdrawal is likely to be a negatively reinforcing factor which makes it more difficult to quit smoking. ***Supported by TRDRP # 6RT-0231***

C05

Structural determinants of ?-toxin: nicotinic acetylcholine receptor interactions

Malany S, Taylor P - *University of California, San Diego*

The selective α-neurotoxin, *Naja mossambica mossambica* (NmmI) inhibits agonist binding to the nicotinic acetylcholine receptor (nAChR). The nAChR from muscle, which is composed of four homologous subunits with composition $\alpha_2\beta\delta\gamma$, binds agonists and competitive antagonists at the boundary between the αδ and αγ interfaces. NmmI, a three-loop polypeptide, binds with high interfacial contact and high affinity to the subunit interfaces and thus serves as an excellent probe for delineating key side chain interactions within the nAChR:NmmI complex. By mutating specific positions in both the nAChR α-subunit and the NmmI, we have examined pairwise interactions between residues on the αδ and αγ interfaces of the receptor and the α-neurotoxin loops.

Seven positions on the α-toxin have been mutated and assayed relative to wild-type receptor These mutations include: two residues on loop I, three cationic side chains on the tip of loop II, and two adjacent cationic side chains on loop III. Within the nAChR extracellular region of the α-subunit, we have constructed a total of eleven mutations at conserved positions in the three domains identified as contributing to agonist binding and specificity.

Initial binding experiments were conducted for single mutant combinations (mutant NmmI:wild-type nAChR and mutant nAChR:wild-type NmmI). Equilibrium dissociation constants (K_D values) were measured by binding NmmI, expressed from recombinant DNA bacterial systems, with mouse muscle nAChR, expressed transiently from transfected cDNAs on the surface of human embryonic kidney cells, by competition against initial rates of ^{125}I-labeled α-bungarotoxin. Mutant NmmI binds with different affinities to the two receptor binding interfaces. By blocking the αδ site with another neurotoxin, α-conotoxin MI, we have confirmed the αδ site as the high affinity site and αγ as the low affinity site. In addition, greater losses of affinity occur at the αγ site relative to the αδ site suggesting that more of the stabilization energy for binding the nAChR:NmmI complex is contributed from the αδ site. These studies have not only confirmed the ligand affinity distinctions between the αδ and αγ sites, but establish that the α-toxin interacts at a multipoint attachment with both α-subunit and δ/γ-subunit determinants.

Presently we have conducted ~ 80 double mutant combinations (mutant NmmI:mutant nAChR) and have analyzed interaction energies for the mutant pairs by thermodynamic mutant cycle analysis. In a majority of the binding assays, we have linked the toxin mutations, which all involve charge reversal, with mutations in the α-subunit of the receptor also involving charge reversal. For example, position 8 in NmmI is a serine and position 198 in the α-subunit of the receptor is a tyrosine. We constructed two mutations at each position to both a negative and positive residue and calculated the overall binding energy for the double mutant combination, Lys8Glu : Glu198Arg. Using this approach we obtained pairwise energies highlighting possible electrostatic attractive and repulsive forces between NmmI and nAChR. From the Columbic interactions, we can approximate relative distances between residue positions in the NmmI:nAChR complex, based on published x-ray crystal structure models.

C06

Long-term nicotinic effects on acetylcholine receptors containing a7 subunits

Kawai, Hideki, and **Berg, Darwin K.**
University of California, San Diego

The initial biological step in establishing tobacco addiction is that of nicotine acting on nicotinic acetylcholine receptors (nAChRs) in the nervous system. A variety of nAChRs are expressed by neurons in the brain and they are thought to be pentameric transmembrane proteins that act as ionotropic receptors, meaning that in response to the naturally-occurring neurotransmitter, acetylcholine (ACh), the receptors open and allow ions to enter the neuron, producing an electrical signal. Nicotine also activates the receptors but, like ACh, can desensitize the receptors such that they stop signaling in the continued presence of either compound. One of the most abundant and interesting nAChRs in the nervous system is a species that contains subunits encoded by the α7 gene (α7-nAChRs). The receptors are unusually effective at allowing calcium to enter neurons and thereby influence a diverse array of calcium-dependent events in the nervous system. This makes α7-nAChRs potentially one of the most important sites of nicotine action.

We have examined the effects of nicotine exposure on **a**7-nAChRs expressed by rat cortical neurons grown in cell culture. Using radioiodinated alpha-bungarotoxin (a snake venom protein that binds to the receptors) to quantify **a**7-nAChRs, we find that cultures prepared with embryonic neurons achieve a steady developmental increase in receptor number for at least three weeks. Whole-cell patch clamp recording from rounded, phase-bright tripolar neurons yields ACh-induced currents that average about 0.3 nA and rapidly decay in a manner characteristic of **a**7-nAChR responses. Pharmacological manipulations confirm the currents as being **a**7-nAChR responses: they are reversibly blocked by 1 nM methyllycaconitine and pseudo-irreversibly blocked by 50 μM alpha-bungarotoxin. Application of 100 μM nicotine quickly desensitizes the receptors, causing almost complete loss of the ACh-inducible current. Rinsing away the nicotine reverses the desensitization and allows almost full recovery within minutes. Long-term exposure to 100 μM nicotine for periods up to 4 days has no long-lasting effects. Full recovery can still be obtained within minutes after removing the nicotine.

These results are different from those reported for other classes of nAChRs which are thought to undergo long-lasting, if not irreversible, inactivation after chronic nicotine exposure. Long-lasting nicotine-induced inactivation was also thought to explain changes in al **a**7-nAChR responses previously seen in Xenopus oocytes expressing an artificial **a**7 gene construct. The present results show, however, that rat cortical neurons can sustain competent **a**7-nAChRs under such conditions. Thus **a**7-nAChRs in the mammalian brain are likely to remain available, shifting in and out of the desensitized state, depending on fluctuations in systemic levels of nicotine produced by smoking. As a consequence, the many cellular processes subject to **a**7-nAChR control are likely to be subject to the same fluctuations. ***(Supported by TRDRP 6RT-0050)***

C07

Differences between native brain and α4β2 nicotinic receptors

Cohen, Bruce N. - *University of California, Riverside*

Brain nicotinic receptors initiate the train of events that produce the psychologically rewarding effects of nicotine. Gene knockout experiments suggest that nicotinic receptors containing the α4 and β2 subunits form the high-affinity nicotine binding site in the brain and are responsible for the reinforcing effects of the drug. However, there have been relatively few comparisons between the pharmacological properties of native brain and artificially expressed α4β2 nicotinic receptors.

To compare the pharmacological properties of a4-containing native rat brain, recombinant rat α4β2, and recombinant rat α4β4 nicotinic receptors, we immunoprecipitated these receptors onto a fixed substrate with the anti-α4 antibody mAb 299 and measured their [^{3}H]epibatidine dissociation rate constants, their [^{3}H]epibatidine affinities, and the ability of three competitive antagonists (dihydro-β-erythroidine (DHβE), N-n-decylnicotinium iodide (NDNI), (trimethaphan) to displace [^{3}H]epibatidine binding from the receptors. Our experiments show that there are significant differences between the kinetic and pharmacological properties of the native brain and recombinant α4β2 nicotinic receptors. The [^{3}H]epibatidine dissociation rate constant for the native receptors (0.005 ± 0.002 min^{-1}, mean ± standard error) was 2-3 times less than that of the recombinant α4β2 receptors expressed in *Xenopus* oocytes (0.015 ± 0.001 min^{-1}) or CV-l cells (0.011 ± 0.001 min^{-1}) at 20-23 °C. However, it was similar to that of recombinant a4b4 receptors expressed in *Xenopus* oocytes (0.006 ± 0.001 min^{-1}). In contrast to the dissociation rate constants, the native brain and recombinant α4β2 receptors expressed in oocytes displayed similar [^{3}H]epibatidine affinities. The [^{3}H]epibatidine equilibrium dissociation constant (K_D) for the native receptors was 13 ± 1 pM (Hill coefficient of 0.9) at 20-23 °C and that for the α4β2 receptors expressed in oocytes was 17 ± 2 pM (Hill coefficient of 1.0). Recombinant α4β4 receptors expressed in *Xenopus* oocytes (K_D 73 ± 8 pM, Hill coefficient = 1.1) and recombinant α4β2 receptors expressed in CV-1 cells (K_D = 32 ± 4 pM, Hill coefficient = 0.7) displayed slightly lower [^{3}H]epibatidine affinities. The antagonists DHβE and NLNI inhibited 30 pM [^{3}H]epibatidine binding to the native and recombinant α4β2 receptors in a similar fashion. However, trimethaphan inhibited 30 pM [^{3}H]epibatidine binding to the α4β2 receptors expressed in CV-1 cells (IC50 = 55 ± 6 mM) six times more potently than it inhibited 30 pM [3H]epibatidine binding to the native brain receptors (IC_{50} = 330 ± 60 mM). Thus, native α4-containing rat brain nicotinic receptors differ from the recombinant α4β2 receptors in regard to [^{3}H]epibatidine dissociation and trimethaphan inhibition.

The use of recombinant receptor subtypes has assumed an increasingly important role in developing subtype-specific pharmaceuticals. Our results show that one must be cautious about extrapolating the properties of recombinant α4β2 nicotinic receptors to those of the native brain nicotinic receptors. Further research will focus on the determining the molecular basis for the differences between the recombinant and native brain nicotinic receptors.

C08

Knock-in mice with hyperactive nicotinic acetylcholine receptors

Labarca, C.; Deshpande. P.; Kofuji, P.; Nowak, M. W.; Dong, H.; Boulter, J. and **Lester, H. A.**
California Institute of Technology

The α4 subunit of the acetylcholine (ACh) receptor, in association with the β2 subunit, constitutes the most abundant form of nicotinic receptor in the nervous system, and the most sensitive to nicotine. We are examining the physiological role of these receptors by generating knock-in mice with a mutation in the α4 subunit that renders the ACh receptors much more sensitive to the effects of ACh and nicotine. The Leu9'Ser mutation in the M2 transmembrane region of the α4 subunit, increases the sensitivity to ACh about 10-fold for every subunit mutated. Neuronal α4β2 receptors with two mutated subunits have ACh sensitivity about 100-fold higher than wild type receptors when examined in the *Xenopus* oocyte system. Naturally occurring mutations of this type in the ACh receptor of human muscle produce myasthenic syndromes caused by excessive activation and characterized by a prolonged synaptic potential.

The M2 transmembrane region is in exon 5 of mouse α4 genomic DNA. A genomic fragment of 9.5 kb containing exon 5 was used to construct a targeting vector for homologous recombination. The Leu9'Ser mutation was introduced into M2 by site directed mutagenesis, and a neomycin cassette flanked by loxP sites was introduced immediately downstream from exon 5. Following homologous recombination, ES cell clones positive for the mutation were transfected with a plasmid carrying Cre recombinase to delete the neomycin cassette which might interfere with RNA splicing. ES cell clones with the neomycin cassette deleted (neo-deleted) and undeleted (neo-containing) were injected into blastocysts to generate chimeric mice carrying the mutation. Neo-containing heterozygous animals generated from mating chimeric males with C57BL/6 females showed no obvious phenotypic differences with wild type animals. These neo-containing heterozygous mice mated with each other produced litters with no homozygous mutant animals, and the heterozygous and wild type were present in proportion of 1.15 to 1 instead of 2 to 1. Neo-containing embryos however were present in the expected ratios of 1:2:1 for homozygous mutant, heterozygous and wild type. All the homozygous mutant and some of the heterozygous mice apparently die very soon after birth. Neo-deleted chimeric males mated to C57BL/6 females produced litters with only wild type animals. When the neo-deleted embryos were examined (n=61) wild type and heterozygous were found in a proportion of 2.8 to 1 instead of 1 to 1. All the neo-deleted heterozygous mice apparently die very soon after birth. Removal of the neomycin cassette aggravates the effect of the mutation.

We are investigating the cause of death of the mutated mice by examining the brains of neo-deleted and neo-containing embryos and neo-deleted heterozygous mice, and we analyzing the neo-deleted heterozygous mice for changes in physiology, nicotine responses, and behavior.

C09

Low concentrations of nicotine persistently activate interneurons in the rat hippocampus

Jia, Yousheng and **Sumikawa, Katumi**
University of California, Irvine

The long-term objective of this research is to understand how acute and long-term nicotine exposures alter the normal functioning of synapses in the hippocampus (through interactions with its receptors) and enhances cognitive function, a property that has been linked with the continued use of tobacco.

It has been demonstrated that most, if not all, nicotinic acetylcholine receptors (nAChRs) desensitize rapidly in the continued presence of nicotine. Because the smoker maintains a low level of nicotine, it is not clear whether most nAChRs in the smoker's brain are in their desensitized state, or in their resting state in which they can be activated by the next dose of nicotine by cigarette consumption. Using whole-cell recording techniques in visually identified CA1 interneurons in rat hippocampal slices, we observed that application of a brief puff of acetylcholine (ACh) produced rapidly activating and desensitizing inward currents in interneurons that were blocked by the α7-containing nAChR-selective antagonist methyllycaconitine (MLA). The currents could be desensitized by bath superfusion of nicotine at a concentration found in the serum of smokers. Thus, α7-containing nAChRs are likely in their desensitized state in the smoker's brain. We also observed that rapid bath application of nicotine (0.5 μM) or A85380 (1 μM) depolarized the cells and caused spontaneous firing of action potentials. The effects of nicotine were maintained during the application of nicotine (at least 20 min) and blocked by DHβE (1 μM), but not by MLA. The DHβE blockage of persistent nicotine action suggests that the nicotine-induced effect is unlikely mediated by activation of second messenger pathways. Furthermore, the effects of nicotine were still observed in chronic-nicotine treated rat hippocampal slices. These results suggest that nAChRs responsible for the effects are non α7-nAChRs and do not desensitize. Because smoking a single cigarette delivers an acute dose of approximately 0.5 μM nicotine that is superimposed on a chronic nicotine level of about 0.1 μM, it appears that the concentration of nicotine rises to effective levels to activate the nAChRs with each cigarette smoked. Thus, our results suggest that at least one subtype of nAChR could be persistently activated in the smoker's brain.

We will further examine the functioning of the two types of nAChR in the chronic nicotine-exposed and in the nicotine-withdrawn hippocampi, because the long-lasting functioning of one type of nAChR in the chronic nicotine-treated hippocampus and the functional recovery of desensitized nAChRs following withdrawal may contribute to the tobacco-seeking behavior.

C10

Age-dependent behavioral effects of nicotine

Leslie FM, Oliff HS, Khaja R, Qadeef K, Belluzzi J.
University of California, Irvine

Ninety percent of adult smokers begin using tobacco before their eighteenth birthday. Whereas social and cultural factors have been shown to be important in the early initiation of tobacco use, it is not yet clear whether an increased biological susceptibility to the behavioral effects of nicotine is also involved. In order to address this issue, we have compared the behavioral effects of nicotine during development with that in adult. Two separate experiments were conducted to evaluate possible age differences in sensitivity to the locomotor and reinforcing effects of nicotine. In the first experiment, 3 groups of male rats, aged postnatal day (P) 21, 30 or 60 (adult; n = 15 per group), were injected subcutaneously (s.c.) with either saline or nicotine (0.175 or 0.5 mg/kg) and monitored for locomotor activity over a 30 min period. Whereas both nicotine doses significantly reduced rearing in P21 animals, there was no significant drug effect on horizontal movement. In contrast, in the other two age groups there were significant drug-induced decreases in both vertical and horizontal locomotion. In a second experiment, animals aged P 36 - 40 and 70 - 75 (n = 12-13 per group) were tested for the reinforcing effects of nicotine (0.5 mg/kg s.c.) in a conditioned place preference apparatus. During a conditioning period, nicotine and saline injections were given in compartments that were paired with unique sensory cues. In a subsequent test, animals were allowed open access to both compartments and monitored for time spent in each. Consistent with previous studies using this experimental design, we found that adult rats exhibited no preference for the nicotine-paired compartment. In contrast, juvenile rats spent significantly more time in the nicotine-conditioned environment ($p<0.005$). Taken together, our findings suggest that nicotine has different behavioral effects prior to the onset of puberty than in adults. In particular, the balance of rewarding to aversive effects may be greater in juveniles than in adults. These data support the view that biological factors may predispose adolescents to the use of tobacco.

C11

Effects of clozapine on brain reward thresholds and somatic signs during nicotine withdrawal: relevance to schizophrenia

Semenova, Svetlana and **Markou, Athina**
The Scripps Research Institute

Cessation of tobacco smoking in individuals dependent on nicotine leads to a withdrawal syndrome characterized by both affective/emotional and somatic signs of withdrawal. The affective aspects of withdrawal include an inability to experience pleasure (i.e., anhedonia), and contribute to craving and relapse. Interestingly, the above mentioned symptom is common to nicotine withdrawal and schizophrenia. A high proportion of schizophrenia patients (88%) are heavy smokers compared to the general population (25-30%). It has been hypothesized that schizophrenia patients smoke in order to alleviate a cluster of symptoms, called negative symptoms of schizophrenia, that are not readily treated by the majority of antipsychotic drugs. Clozapine is an atypical antipsychotic that is more effective against the negative symptoms of schizophrenia than the typical antipsychotic drugs. Considering that nicotine withdrawal is characterized by a symptom (i.e., anhedonia) that is also one of the negative symptoms of schizophrenia, we hypothesized that clozapine or clozapine-related compounds may be useful in the treatment of nicotine withdrawal. The aim of the present study was to test the above hypothesis by investigating whether clozapine would reverse the affective and/or somatic aspects of nicotine withdrawal in rats. We used the intracranial self-stimulation paradigm to assess affective changes, and observational methods to assess somatic signs associated with nicotine withdrawal.

We have previously shown that nicotine withdrawal results in elevated brain reward thresholds reflecting diminished interest in rewarding electrical stimuli, and in somatic signs that include gasps, writhes and eye-blinks. In the present studies, we first investigated the effects of a wide range of clozapine doses (1.5-12 mg/kg) on brain reward thresholds in naïve non-withdrawing subjects. Administration of low doses of clozapine had no significant effect on reward thresholds, while the highest doses used (9 and 12 mg/kg) induced significant elevations of brain reward thresholds, indicating that at high doses clozapine diminishes reward. In the next study, we investigated the effects of clozapine on nicotine withdrawal. Rats were prepared with osmotic minipumps delivering 9 mg/kg/day nicotine hydrogen tartrate salt or saline. On the seventh day the minipumps were removed and the rats' brain reward thresholds and somatic signs were assessed daily after either clozapine (3 mg/kg) or vehicle treatment for six days. Then subjects continued to be tested daily for five days without clozapine or vehicle treatment. In replication of our previous findings, nicotine withdrawal resulted in elevated brain reward thresholds and increased number of somatic signs compared to saline-exposed rats. Clozapine appeared to potentiate the effects of nicotine withdrawal as reflected in threshold elevations and increased number of somatic signs. Nevertheless, clozapine administration induced threshold elevations and somatic signs even in non-nicotine withdrawing subjects. Ongoing investigations are exploring whether indeed clozapine potentiates the effects of nicotine withdrawal, or whether clozapine and nicotine withdrawal have an additive effect on thresholds and somatic signs. Finally, there was no withdrawal from chronic clozapine administration.

The results of these experiments will have important implications about the links of cigarette smoking to schizophrenia, and the treatment of nicotine withdrawal in psychiatric and non-psychiatric populations.

C12

Nitric oxide synthase inhibition attenuates acetylcholinesterase inhibitor induced myopathy *in vivo*

Jeyarasasingam, Gayathri
The Parkinson's Institute

Nicotine, a predominant component of tobacco, is known to activate nicotinic receptors on muscle cells. Excessive stimulation of these receptors, however, results in muscle deterioration as seen in slow channel congenital myasthenia. This disorder is characterized by a wasting and weakness of neck and shoulder muscles, due to mutations in nicotinic receptor genes which result in the over-activation of muscle cells. Similarly, injection of acetylcholinesterase inhibitors, increases available acetylcholine at nicotinic receptors thereby resulting in receptor over-stimulation and muscle degeneration.

Our long term goal is to identify the pathway through which excessive stimulation of nicotinic receptors leads to muscle degeneration. The gaseous molecule nitric oxide, which is responsible for many biological functions including vasodilation, platelet aggregation, as well as cellular toxicity, may be involved in this pathway. Both endothelial and neuronal nitric oxide synthase (NOS), enzymes which produce nitric oxide, have been localized to skeletal muscle cells. Our recent work showed that NOS inhibitors prevent cultured muscle cells from dying in response to nicotine exposure. In the present study, we examined the potential protective effects of NOS inhibition on nicotinic receptor mediated myopathy *in vivo.*

Rats, injected with the acetylcholinesterase inhibitor paraoxon (4 mg/kg daily for four days), demonstrate a 90-fold increase in the number of degenerating muscle cells as compared to control. Co-administration of the non-specific NOS inhibitor L-NAME (10 mg/kg b.i.d.) dramatically reduced the presence of degenerating profiles to less than 25% of that seen with paraoxon alone. Furthermore, co-administration of 7-nitroindazole (10 mg/kg b.i.d.), a NOS inhibitor specific for the neuronal isoform, also prevents paraoxon induced muscle cell degeneration.

Further studies were done involving treatment with tacrine, an acetylcholinesterase inhibitor used in the treatment of Alzheimer's disease. Tacrine administration to rats produced a 30-fold increase in the number of degenerating muscle cells as compared to control. Such degeneration was also prevented by co-administration with the NOS inhibitor L-NAME (20 mg/kg b.i.d.).

These results show that NOS inhibition significantly prevents muscle cell degeneration resulting from the over-activation of nicotinic receptors following paraoxon administration *in vivo* suggesting that increased nitric oxide production mediates such myopathy. Furthermore, we have shown that tacrine, an acetylcholinesterase inhibitor that is used therapeutically to counteract the cognitive decline observed in Alzheimer's disease, also produces muscle degeneration which can be attenuated by NOS inhibition. Continued work may help to identify potential therapeutic agents to prevent nicotinic receptor mediated muscle degeneration from acetylcholinesterase inhibitor exposure *in vivo* and slow channel congenital myasthenic syndrome.

C13

Alterations in nicotinic receptor mRNA in an animal model of Parkinson's diseas e

Quik, Maryka
The Parkinson's Institute

Tobacco use is usually associated with detrimental effects on health, such as an increased susceptibility to cardiovascular disease and cancer. In contrast to these negative findings, however, it appears that tobacco use may have beneficial effects in Parkinson's disease. Over 35 epidemiological studies, done over a 40 year period, report a lower incidence of Parkinson's disease in tobacco users. Furthermore, accumulating evidence indicates that nicotine may have a neuroprotective role against nigrostriatal degeneration in rodents. The component in tobacco responsible for this beneficial effect in Parkinson's disease has not been identified. However, the animal studies described above, together with work which shows that nicotine results in an enhanced dopamine release, suggests that it may be the nicotine in tobacco products that mediates the neuroprotective effects observed in Parkinson's disease.

There are multiple nicotinic receptors in the brain, all of which are activated by nicotine with consequent beneficial actions, as well as negative side effects. A knowledge of the alterations in nicotinic receptor subtypes after nigrostriatal degeneration is therefore essential for developing a rational drug therapy with nicotinic receptor ligands. Since this is a largely unexplored area, our objective is to identify changes in nicotinic receptor subunit mRNAs in the MPTP treated monkey model of nigrostriatal degeneration. Because the available nicotinic receptor ligands lack specificity, we used *in situ* hybridization to study the distribution of nicotinic receptor subunit transcripts after nigrostriatal degeneration in the monkey. This species was selected because monkeys exhibit behavioral, neurochemical and neuroanatomical deficits after MPTP treatment which are very similar to those observed in Parkinson's disease. Furthermore, our previous work indicates that there are significant differences in nicotinic receptor transcript expression in monkey as compared to rodent brain, with the distribution in the monkey CNS more closely resembling that in man.

Previous studies from our laboratory have shown that $\alpha 4$, $\alpha 6$, $\alpha 7$, $\beta 2$, $\beta 3$ and $\beta 4$ nicotinic receptor mRNAs are present in the monkey substantia nigra with a particularly intense and localized labelling of the $\alpha 6$ and $\beta 3$ subunit mRNAs to this brain region in the primate. The present experiments show that MPTP treatment resulted in a decline in baseline motor activity to $36 \pm 11\%$ of controls. Caudate and putamen dopamine levels were reduced to $51 \pm 15\%$ and $43 \pm 6\%$ of control values, respectively. The number of tyrosine hydroxylase positive neurons in the substantia nigra was $75 \pm 6\%$ of control. Despite the reduction in nigral cell number, there were no changes in $\alpha 4$, $\alpha 7$, $\beta 2$ and $\beta 4$ mRNA levels in the substantia nigra. Interestingly, $\alpha 6$ mRNA levels were increased ($136 \pm 7\%$) and $\beta 3$ mRNA levels decreased ($62 \pm 6\%$) in the substantia nigra after MPTP. These results show that there are selective changes in nigral nicotinic receptor subunit mRNA levels in response to nigrostriatal degeneration.

These data may suggest that nicotinic receptor ligands directed to these receptor subunits are of therapeutic relevance in Parkinson's disease.

C14

Neuroendocrine responses to nicotine

Karan, Lori D.
University of California, San Francisco

The objective of this research is to develop a quantitative method for studying the hormonal response to nicotine in humans. Hormones play a key role in the body's state of arousal, alertness, attention, anxiety and response to stress. When released, hormones not only have end-organ effects, but they also have a role in the feedback and regulation of neuronal circuits. By developing a methodology to depict individuals' different responses to nicotine, we hope to find a probe for central nervous system responsiveness to nicotine, and contribute to our knowledge about the central mechanisms by which nicotine produces chemical dependency.

Four smokers have undergone separate sessions in which they received 5, 10, and 15 µg/kg/min intravenous nicotine infusions between 9:30 and 9:45 am each over one minute. ACTH, cortisol, human growth hormone, prolactin, arginine vasopressin and venous nicotine levels were each measured at baseline and at 2, 5, 10, 20, 40, 60, and 90 minutes after the infusion. Blood pressure, heart rate and subjective effects were measured as well. The hormone responses varied amongst individuals and were the most significant for the 15 µg/kg/min nicotine dose. The different subjects responded to the nicotine infusions with different quantities of ACTH, cortisol, growth hormone, and vasopressin. A clear prolactin response was not ascertained. The timing of peak nicotine venous levels paralleled the timing for peak subjective effects, as well as increases in heart rate and blood pressure, when they occurred. Due to these results, the next set of four subjects received one minute intravenous nicotine infusions which were of increased dose: 8, 16, and 24 µg/kg/min. This hormonal data will be presented and compared with that of the first four research subjects.

Possible future directions in achieving a reproducible methodology of the hormonal responsiveness to nicotine include adding a training session to our protocol, and lengthening the nicotine infusion to five minutes. Oxytocin and other neurohormones will be added to our battery of assays when nonresponsive hormones can be removed from the current panel.

There is no report yet in the literature which specifies that individuals may have different hormonal response patterns to the same doses of nicotine, much less a study which quantifies these differences. Future studies comparing arterial and venous hormonal levels are planned to make sure that these differences are not due to differences in tissue uptake.

The methodology developed from this research can be used, in the future, to investigate individual differences in larger population samples across genetic and environmental factors. These differences in hormonal response may well influence the susceptibility to and/or the maintenance of nicotine addiction. Understanding individual differences in hormonal sensitivity and tolerance to nicotine may enable the subsequent development of more precise diagnostic, preventive, and therapeutic interventions.

C15

Approaches to measuring cigarette "tar" exposure: Analyses for potential biomarkers using liquid chromatography - mass spectrometry (LC-MS)

Jacob, Peyton
University of California, San Francisco

The goal of this project is to develop methodology for determining human exposure to toxic substances in tobacco smoke. Nicotine and its metabolites, particularly cotinine, are widely used as biomarkers for exposure to tobacco and tobacco smoke toxins. For example, cotinine levels in biolfluids are used to determine smoking status, or to estimate the extent of tobacco use. However, nicotine and its metabolites may not be suitable markers for certain biological questions. Carcinogens, such as polycyclic aromatic hydrocarbons and nitrosamines, differ from nicotine in their volatility and other chemical properties, and may differ in their extent of transfer from smoke to lung tissue and across cell membranes. Nicotine in freshly generated tobacco smoke is largely in the particulate ("tar") phase, but as the smoke ages, e.g., as in environmental tobacco smoke, nicotine volatilizes from the particulate to the vapor phase.

Ideally, one would like to measure exposure to all of the significant toxins found in tobacco smoke for assessment of risk. However, tar is a complex mixture, and the large number of different toxic substances present would make this a formidable task. For this reason, measuring levels of representative substances present in tar, whose concentrations can be related to concentrations of toxic substances of interest is a practical alternative. Progress in developing mass spectrometric methods for determination of potential tar biomarkers, including solanesol and metabolites of polycyclic aromatic hydrocarbons, will be presented. It is anticipated that this methodology will be used to monitor human exposure to tobacco tar from different types of cigarettes, as might be required by the FDA if it decides to implement a nicotine reduction strategy proposed to make cigarettes non-addictive; to evaluate the health risks of new, potentially safer tobacco-based products; to evaluate harm reduction in tobacco treatment programs that seek to reduce health risks by reducing cigarette consumption in smokers who are unable to quit, perhaps in conjunction with nicotine maintenance therapy; and to assess risks of developing tobacco-related diseases in different populations of smokers.

D01

Characterization of a-7 nicotinic receptors on cardiac fibroblasts and long term effects of nicotine on post-infarction cardiac remodeling

Villarreal, Francisco
University of California at San Diego

The goal of this research project is to examine for nicotine mediated adverse effects on cardiac remodeling (healing, scarring and growth) after myocardial infarction. Previous data from our laboratory has indicated that exposure of rats for 7 days to nicotine adversely affects cardiac remodeling following infarction. These alterations were evident 7 days after infarction and were characterized by heart cavity enlargement, thinning of the infarcted ventricular wall and altered scar material properties. To further investigate the effects of nicotine and its mechanisms of action on cardiac healing and remodeling we have pursued studies in cultured cardiac fibroblasts and in infarcted rats.

In order to explore for the mechanisms of action of nicotine we have begun to characterize the presence of nicotinic receptors on cultured rat cardiac fibroblasts. Our initial studies have focused on assessing for the presence of mRNA for α7 nicotinic receptors utilizing RT-PCR. Preliminary results indicate that cardiac fibroblasts express an α7 nicotinic receptor. These results have been verified by the use of restriction digest analysis of PCR products and by utilizing total RNA isolated from rat brains as control. To confirm this observation at the protein level we are performing studies utilizing techniques such as Western blot and binding assays

We have recently performed a new set of experiments to further assess for long term changes in cardiac remodeling in infarcted rats exposed to nicotine. For these experiments we have examined for nicotine induced changes in passive and structural properties of the left ventricular and scar tissue analyzed from rats 1 month following infarction. Left ventricular pressure volume curves, left ventricular pressure deformation data and the recording of morphometric parameters were used for this purpose. Nicotine treatment resulted in a significantly thinner infarcted wall. Furthermore, nicotine treatment also yielded a smaller heart by weight. This observation is surprising since the compensatory growth of the heart is thought to be a physiologycally necessary response to infarction. These results led us to examine for the capacity of nicotine to impair cardiac growth in normal, young developing rats. Results in indicate that in normal growing rats exposed for 1 month to nicotine heart growth (i.e. weight) can be impaired. These results are intriguing and further experiments will be designed to examine these issues. Future directions to be pursued include the characterization of the α7 nicotinic receptor on cardiac fibroblasts, the effects of nicotine on fibroblast function and assessment of long term effects of nicotine on cardiac remodeling in rats. We also anticipate to begin to analyze echocardiographic data derived from assessment of infarcted patients who are smokers.

The findings derived from these studies are important in that they begin to establish nicotine as a risk factor for adverse cardiac remodeling after infarction. To the extent that these findings are corroborated and extended in humans, important benefits will be derived from the awareness of these issues and their appropriate treatment.

D02

Mechanism of nicotine-induced increase of spontaneous breakup of activation wave fronts during ventricular fibrillation in hearts with chronic myocardial infarction

Ohara, Toshihiko; Ohara, Keiko; Kim, Youn-Hoon ; Naik, Ajai; Mandel, William J; Chen, Peng-Sheng;
Karagueuzian, Hrayr S - *Cedars-Sinai Research Institute*

Smoking increases the incidence of sudden cardiac death by promoting ventricular fibrillation (VF) in hearts with chronic myocardial infarction (MI). VF is a rapid and irregular rhythm that develops suddenly when the single large propagating wave front present during the normal heart rhythm, breakups spontaneously to multiple independent and smaller-sized vortex-like rotating wave fronts. These fragmented wave fronts cause an irregular and rapid rhythm in the ventricles (i.e., VF) with loss of synchronous contraction of both ventricles that immediately lowers the blood pressure to near zero. With such dramatic blood pressure reduction the patient faints requiring emergency electrical shock to convert the rhythm otherwise sudden cardiac death ensues.

Our hypothesis is that nicotine promotes spontaneous breakup of activation wave fronts in diseased hearts by a mechanism compatible with the action potential duration restitution (APD-R) hypothesis that measures the rate of recovery of cardiac repolarization (relaxation). The faster the rate of recovery the higher the likelihood of wavebreak. Recovery rate is conveniently determined by the slope of the APD-R curve, that relates APD to recovery interval.

We induced experimental myocardial infarction in anesthetized dogs by occluding the left anterior descending coronary artery and restudied them 6-7 weeks later. Using high-resolution computerized electrode mapping system to analyze the dynamics of fibrillation wave fronts subsequently recorded monophasic action potentials (MAP) to construct APD-R curves. All recordings were made in the diseased and the non-diseased sites of the left and right ventricle (LV and RV respectively) before and after nicotine infusion (5mg/ml/kg). Nicotine increased the incidence of spontaneous wavebreaks and significantly increased the number of wavelets from 4.3±0.2 to 5.1±0.2 in the diseased LV. Increased wavelet number was associated with an increase in the maximum slope of the APD-R and the interval during which the slope remained greater than one, a sign of wave front instability. Similar but less pronounced effects of nicotine were also seen in the non-diseased LV and RV sites. Vortex-like activation (reentry) could be seen in the diseased LV and the normal zones before and after nicotine. Nicotine arterial blood levels ranged between 60-200 ng/ml.

As nicotine mimics the major cardiovascular effects of smoking, our work provides an electrophysiological mechanism for the epidemiological findings that showed increased incidence of VF in smokers with chronic MI. The mechanism of nicotine-induced increase in spontaneous wave break in hearts with chronic MI is compatible with the APD restitution hypothesis. Since a large number of antiarrhythmic drugs are available that can modify the APD restitution curve, future research should be directed to reverse nicotine's profibrillatory effect on the APD restitution curve. Such drugs might be extremely useful in reversing nicotine's detrimental effect of increasing the incidence of sudden death in smokers with chronic MI that still remains a major unresolved public health issue.

D03

Noninvasive imaging of early atherosclerosis

Tsimikas, Sotirios
University of California, San Diego

Atherosclerosis is a chronic and progressive disease of the artery wall that is caused by accumulation of cholesterol and lipid deposits. It accounts for approximately 50% of deaths in the United States and other developed countries. Entry of cholesterol and other lipids into arterial walls, enhanced by high blood pressure, diabetes, smoking and elevated cholesterol levels, and other factors is the initiating factor of atherosclerosis. Noninvasive diagnosis of atherosclerotic lesions, especially at early stages, is not feasible. Our research focuses on developing a non-invasive method to detect atherosclerosis which may provide information that cannot be obtained with current techniques. Our approach is to use radiolabeled monoclonal antibodies that recognize and bind strongly to abnormal cholesterol deposits within atherosclerotic plaques for diagnostic imaging of atherosclerotic plaques prior to clinical symptoms. Once cholesterol enters the vessel wall it becomes modified (i.e., oxidatively modified low density lipoprotein, OxLDL) by processes such as oxidation, which significantly accelerates the formation of atherosclerotic plaques. OxLDL is present in atherosclerotic but not normal arteries and plays a crucial role in the pathogenesis and adverse consequences of atherosclerosis. MDA2 is a well characterized murine monoclonal antibody that recognizes malondialdehyde (MDA)-lysine epitopes (e.g. in MDA-modified OxLDL) within atherosclerotic plaques. These epitopes are recognition sites on atherosclerotic plaques to which MDA2 binds in vivo after it circulates in the blood and enters the vessel wall.

We have shown that when injected intravenously in animal models, MDA2 accumulates specifically within atherosclerotic lesions but not in normal tissue. We have also shown that this method of atherosclerotic plaque detection is quantitative in that the signal generated by the antibody within the atherosclerotic plaque is proportional to the degree of atherosclerosis present. We have also shown that this technique detects the depletion of oxidized LDL as a result of hypocholesterolemic diets and/or antioxidant intervention. We have performed a series of imaging experiments in animals and have shown that deposits of oxidized cholesterol can be imaged noninvasively in live rabbits with 99mTc-labeled MDA2.

Potential applications of these techniques may include noninvasive diagnosis of narrowed arteries (i.e. coronary, carotid, renal) due to atherosclerosis for early detection and intervention, selection of optimal candidates for drug/dietary intervention based on results of imaging to allow for more precise and cost effective treatments, and to follow the natural progression or regression of these lesions. Future studies will be assessing several other antibody candidates and various imaging protocols in anticipation of developing this technique to image atherosclerotic arteries of patients

D04

Regulation of the recruitment of white blood cells into atherosclerotic lesions in the mouse

Kim, Chee-Jeong ; Khoo, John C; Li, Andrew; Palinski, Wulf; Glass, Christopher C;and **Steinberg, Daniel**
University of California, San Diego

Atherosclerosis is the major cause of death in this country, particularly among cigarette smokers. They die of heart attacks (myocardial infarctions) that occur when a blood clot forms at the surface of a damaged artery feeding heart muscles. A key step in the development of atherosclerosis is the entrance of white blood cells (monocytes) into the site of the developing arterial lesion. Factors that increase the rate of monocyte entry would be expected to accelerate the disease process. Until recently there were no methods sensitive enough to allow measurement of the rate of monocyte entry into atherosclerotic lesions in the intact animal. Over the past two years, our laboratory has developed and validated a new method that makes such measurements possible. The method utilizes a “trick” commonly used in molecular biology that allows one to make literally millions of copies of any particular segment of DNA (polymerase chain eaction; PCR).

Despite the sensitivity of the method, we could detect no donor monocytes in the arteries of normal mice nor in the unaffected segments of the aortas of mice with hypercholesterolemia and atherosclerotic lesions. Because test tube studies suggested that tumor necrosis factor-a and interleukin 1-b might be involved, we tested the ability of these so-called cytokines, injected simultaneously, to increase the rate of monocyte entry into lesions. In fact they more than doubled the rate at which monocytes accumulated. Interestingly, the magnitude of the increase was inversely related to the severity of the lesions.

The availability of this new method should now make it possible to evaluate under “real-life conditions” the importance of various factors that may influence atherosclerosis by affecting monocyte recruitment. Once identified, these factors become “targets” for pharmacologic intervention to prevent arteriosclerosis and, hopefully, heart attacks.

D05

Identification and characterization of a 315-bp enhancer, located more than 55 kb 5´ of the apolipoprotein B gene, that confers expression in the intestine

Antes, Travis J; Goodart, Sheryl A; Huynh, Cathy; Sullivan, Meghan; Young, Stephen G; and **Levy-Wilson, Beatriz**
Palo Alto Medical Foundation; Stanford University; Gladstone Institutes for Cardiovascular Disease; University of California, San Francisco

We recently reported that an 8 kilobase (kb) region, spanning from –54 to –62 kb 5¢ of the human apolipoprotein B (apoB) gene, contains intestine-specific regulatory elements that control apoB expression in the intestines of transgenic mice. In this study, we further localized the apoB intestinal control region (ICR) to a 3-kb segment (–54 to –57 kb). DNaseI hypersensitivity (DH) studies uncovered a prominent DH site, located within a 315-bp fragment at the 5¢ end of the 3-kb segment, in transcriptionally active CaCo-2 cells but not in transcriptionally inactive HeLa cells. Transient transfection experiments with CaCo-2 and HepG2 cells indicated that the 315-bp fragment contained an intestine-specific enhancer, and analysis of the DNA sequence revealed putative binding sites for the tissue-specific transcription factors HNF-3b, HNF-4, and C/EBPb. Binding of these factors to the 315-bp enhancer was demonstrated in gel retardation experiments. Co-transfection experiments with expression plasmids for HNF-3b, HNF-4 and C/EBPb demonstrated that these transcription factors act synergistically and are responsible for the activity of the apoB intestinal enhancer. The corresponding segment of the mouse apoB gene (located –40 to –83 kb 5¢ of the structural gene) exhibited a high degree of sequence conservation in the binding sites for the key transcriptional activators and also exhibited enhancer activity in transient transfection assays with CaCo-2 cells. In transgenic mouse expression studies, the 315-bp enhancer conferred intestinal expression to the apoB transgenes.

D06

Role of PAI-1 in the vascular response to injury

DeYoung, Mary Beth and **Dichek, David A**
Gladstone Institutes for Cardiovascular Disease; University of California, San Francisco

Introduction: Plasminogen activator inhibitor-1 (PAI-1) is a protein that prevents blood clots from being dissolved and may also promote thickening of artery walls, resulting in decreased blood flow. PAI-1 is increased in the plasma of smokers, and may be involved in their poor response to surgical treatment of vascular disease. PAI-1 may increase accumulation of fibrin, a component of blood clots, in injured vessels, which in turn can cause thickening of vessel walls. The purpose of our study is to test the hypothesis that PAI-1 alters healing after vascular injury.

Methods: We used a gene transfer technique to increase production of PAI-1 in balloon-injured rat carotid arteries. The DNA coding for PAI-1 was inserted into a modified cold virus, and the virus was used to transfer the DNA to the artery wall. Increasing PAI-1 DNA should increase the amount of PAI-1 protein made. We measured the amount of PAI-1 mRNA and protein in these arteries and also measured the thickness of the vessel walls. Special staining techniques were used to detect cell multiplication in the arteries and the amount of fibrin associated with the vessel wall.

Results: The gene transfer technique increased both PAI-1 mRNA and protein in the arteries by 50 to 100%. Two weeks after surgery, the PAI-1 treated arteries were significantly thicker. The increase in wall thickness was due to an increase in the number of cells in the artery wall. Our staining technique demonstrated that artery wall cells were multiplying more rapidly in the PAI-1 treated arteries than in arteries that had the normal amount of PAI-1. The staining technique that detects fibrin also stained PAI-1 arteries more strongly than control arteries. Thus, increasing vessel PAI-1 increases fibrin or fibrin-related proteins, cell multiplication, and the overall thickness of artery walls.

Future directions: We hypothesize that PAI-1- induced increases in fibrin are responsible for the increased number of new cells which increases the thickness of the vessel wall. Correlation between areas of fibrin staining and cell multiplication will be investigated. Additionally, the experiment will be repeated using a virus made with DNA that expresses a defective PAI-1 which does not affect fibrin. If PAI-1 is acting mainly through its effects on fibrin, no changes in vessel thickness will be observed after treatment of blood vessels with this virus.

Significance: The results of our study suggest that smoking-induced increases in PAI-1 may contribute to poor outcome after vascular surgery. Definition of the mechanism by which this occurs may help in the design of new treatments for smoking-related vascular disease. The gene transfer techniques we are developing may also be useful for improving the outcomes of vascular surgery in smokers.

D07

Vitamin E kinetics in smokers and non-smokers

Traber, Maret G; Winklhofer-Roob, Brigitte M; Roob, Johannes M; Khoschsorur, Gholamali; Aigner, Reingard; Cross, Carroll; Lodge, John; Ramakrishnan, Rajasekhar; Brigelius-Flohe, Regina

University of California, San Diego; Oregon State University; Karl-Franzens University of Graz, Austria; Columbia University College of Physicians & Surgeons; German Institute for Human Nutrition

Smokers have an increased incidence of chronic diseases that are associated with oxidative stress. Smokers also have lower plasma concentrations of some antioxidant nutrients and these nutrients may confer protection against such diseases as atherosclerosis and cancer. Because smokers are exposed to oxidants and their secondary products in cigarette smoke, and to increased phagocytic oxidant production as a consequence of cigarette smoke exposure, we hypothesized that smokers' antioxidant status is compromised as a result of this oxidant exposure. Therefore, we asked the question:

♦ ***Does cigarette smoking increase utilization of vitamin E in vivo?***

A preliminary trial was carried out in 12 healthy subjects (6 smokers and 6 non-smokers). Non-smoking subjects were matched with smokers (35 ± 6 cigarettes per day) with respect to age (30 ± 6 vs 24 ± 4 y) and serum lipids (cholesterol (mg/dl) 182 ± 23 vs 195 ± 44 and triglycerides (mg/dl) 88 ± 38 vs 117 ± 52). None took vitamin or antioxidant supplements.

The subjects consumed stable isotope labeled-vitamin E-150 mg each d_3-*RRR* and d_6-*all rac*-α–α-tocopheryl acetates (natural and synthetic vitamin E, respectively) daily for 7 days with standardized breakfast. Fasting blood samples were obtained daily in the morning on days -7, -6, -5, -4, -3, -2, -1, 0, 1, 2, 3, 4, 5, 6, 7, 9, 14, 21 (negative values indicate days of vitamin E supplementation) Spot urine samples were obtained in the morning of days -7, 0, 7, 14 and 21; 24-hour urine collections were obtained prior to (completed on day -7), on the last day of vitamin E dosing (day 0) and at days 7, 14 and 21.

During the study, the dietary vitamin E intakes were assessed from daily dietary records. The two groups of subjects had similar vitamin E intakes (nonsmokers 9.7 ± 3.9 vs smokers 9.3 ± 3.3 alpha tocopherol equivalents/day). Urinary cotinine, expressed per mg creatinine, was 2427 ± 848 in smokers compared with 8 ± 7 ng/mg in non-smokers, and verified that the smokers and non-smokers were correctly identified.

Plasma deuterated and unlabeled alpha-tocopherols were measured by gas chromatography/mass spectrometry. Deuterated tocopherol disappearance rates were estimated by fitting the post-peak plasma %d_3-RRR/total alpha-tocopherols (%d_3) using computer-assisted kinetic modeling. Statistical comparisons were made between smokers and non-smokers using an unpaired t-test.

Plasma d_3-*RRR* concentrations were approximately double those of d_6-*all rac*-α-tocopherol in both smokers and non-smokers. The maximum concentrations were similar in both groups. Smokers were found to have a significantly ($p<0.002$) faster rate of %d_3 disappearance during the first week following isotope dosing. These data suggest that smoking causes an increase in vitamin E utilization. Further studies are needed to confirm this provocative finding.

D08

Nicotine is an agent of angiogenesis: Role of nitric oxide and prostacyclin

Heeschen, Christopher;. Jang, James J; Ho, Hoai-Ky V; Kaji, Shuichiro; Yang, Phillip; Hu, Bob S; **John P. Cooke**

Stanford University

Background: To investigate the effect of nicotine upon angiogenesis, we used a disk angiogenesis system (DAS) and a murine model of hindlimb ischemia.

Methods: The DAS was subcutaneously implanted in 12-week old C57BL/6J mice. Some discs were treated with nicotine solution (n=15; 20 µL; 0.1 mM) in the presence or absence of either indomethacin (n=5; 20 µL, 10-5 M) or L-nitroarginine (n=5; 20 µL; 10-5 M). Discs were harvested after 2 weeks and the area of fibrovascular growth was measured as an index of angiogenesis. In the hindlimb ischemia model, right femoral arteries were excised. Mice were treated with nicotine, epibatidine, or saline by daily intramuscular injections into the ischemic hindlimb (n=8 each group). Three weeks after surgery, angiogenesis was quantified by magnetic resonance (MR) perfusion imaging as flow index, and by determination of capillary density (capillary/myocyte ratio) in skeletal muscle.

Results: In the DAS, fibrovascular growth was doubled by nicotine (20.1±3.2 mm2 vs. 12.2±1.2 mm2; P<0.001). The effect of nicotine was blocked by indomethacin (3.7±0.4 mm2) or by L-nitroarginine (7.3±0.2 mm2). Capillary density in the hindlimb ischemia model was significantly increased by either nicotine (0.1 µg/kg; 0.48±0.126; P=0.005) or epibatidine (0.001 µg/kg; 0.56±0.065; P<0.001) as compared to saline treated mice (0.29±0.056). In contrast, at higher concentrations of nicotine (3.0 µg/kg), cytotoxicity was observed with reduced numbers of myocytes per microscopic field and no significant increase for capillary/myocyte ratio. Concordantly, limb blood flow as assessed by MR perfusion was also higher in nicotine (0.26±0.075; P=0.031) and epibatidine (0.25±0.031; P=0.004) group as compared to saline (0.12±0.091). This effect of nicotine on angiogenesis was abrogated by the nicotinic receptor antagonist mecamylamine (0.29±0.06 vs. 0.34±0.08; saline vs. nicotine + mecamylamine; P=0.82).

Conclusions: Nicotine stimulates angiogenesis in vivo. The response to nicotine is bimodal, with angiogenesis at lower doses and cytotoxicity at higher concentrations. The angiogenic response to nicotine appears to be mediated by the nicotinergic receptor and depends on products of the cyclooxygenase and NO synthase pathways. The observed angiogenic effect of nicotine is equivalent to that of other growth factors, and may have implications for plaque growth, as well as neoplastic or therapeutic angiogenesis.

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D09

Chronic exposure to environmental tobacco smoke modifies the artery wall and increases low-density lipoprotein accumulation

McDonald, James M.; **Rutledge, John C.**
University of California, Davis

Background: Environmental tobacco smoke (ETS) has been strongly linked with the development of atherosclerosis. Female sex hormones, and specifically estrogens, appear to be atheroprotective. The goal of this project was to determine the actions of environmental tobacco smoke on the artery wall and to study the regulation of this interaction by the female sex hormone 17 beta-estradiol. **Methods:** Intact female rats and ovarectomized rats treated with placebo or 17 beta-estradiol pellets placed subcutaneously were exposed to ETS (nicotine 5.3 mg/m^3, carbon monoxide 90 ppm, total suspended particulates 30.8 mg/m^3) or filtered air for 6 weeks (6 hours/day, 5 days/week) in the exposure chambers at UC Davis. The rate of fluorescently labeled LDL accumulation in the carotid artery wall was determined by quantitative fluorescence microscopy. Results: Exposure to ETS significantly increased LDL accumulation as compared to exposure to filtered air (4.01 +/- 0.99 vs 0.81 +/- 0.08 ng cholesterol/min/cm^2; p=0.001). Moreover, the presence of female sex hormones did not prevent increased LDL accumulation in animals exposed to ETS. In the same arteries compliance indices were determined by hydrostatic pressure/volume relationships. Although a trend was note, compliance indices of arteries from animals exposed to ETS or filtered air did not differ significantly. **Conclusions:** Exposure to ETS modifies arteries thereby increasing LDL accumulation. This effect is likely due to LDL binding modifications in the arterial wall and/or increases in vascular permeability to LDL. Furthermore, in respect to lipoprotein flux, estrogens do not appear to protect the artery wall from atherogenic effects related to ETS-induced increases in LDL accumulation. **Future Directions:** Although the mechanisms by which ETS initiates atherosclerosis are becoming more clearly understood, many questions remain unanswered. Our future endeavors will include determining the relative contributions of binding and alterations in vascular permeability in increasing LDL accumulation. In addition, we will continue to examine the potential of ETS to alter vascular compliance via alterations in the arterial wall. Finally, we will examine the potential for antioxidants to attenuate ETS induced changes of both lipoproteins and the arterial wall. **Significance:** Our work will contribute to the understanding of the mechanisms by which ETS induces atherosclerosis. By understanding the mechanisms of this disease process, it will be possible to develop therapies which will be used to reduce both the extent of ETS-induced vascular disease as well as overall mortality.

E01

Tobacco control in Latino communities

John P. Elder
San Diego State University

The Tobacco Control in Latino Communities (TCLC) Integrated Research Project (IRP) comprises three interwoven individual projects (IPs). Two of the IPs are field interventions that use a community health advisor (CHA) or *promotor* model to promote smoking cessation and environmental tobacco smoke reduction, respectively. The third IP studies tobacco taxation in Latino communities and its effect on tobacco consumption. Among its primary aims, the ***TCLC Core*** provides support to the two intervention IPs by assisting with program implementation for the *promotor*-delivered programs. The ***TCLC Core*** also works to conduct a process evaluation of *promotor* characteristics and how those characteristics may influence program outcomes.

The process evaluation involved a collaborative effort between the two IPs and the ***TCLC Core*** to develop a paper and pencil measurement tool. This instrument collected information about *promotor* characteristics to describe the type of people who volunteer in their community, and to identify factors associated with positive participant outcomes. The instrument measures eight intervention-oriented constructs (i.e., intentions, environmental constraints, anticipated outcomes, perceived normative pressure, self-standards, self-efficacy, emotional reaction, ability/skills) drawn from several major theories of behavior change that may predict successful *promotores*. The instrument also measures intervention content knowledge, general self-esteem and self-efficacy, motivation, effects of the program on *promotor* interactions within their social networks, and satisfaction with their function within the IP.

The TCLC core and the two *promotor*-delivered intervention IPs have trained and evaluated four groups of *promotores*. Positive pre-post training effects were seen in knowledge, several intervention-oriented constructs, and general self-esteem and self-efficacy. In addition, after the training, topics needing additional attention were identified and booster sessions were conducted to address them. Results of training and evaluation will be presented.

These studies address a broad spectrum of potentially effective direct and indirect methods for tobacco control in the Latino community primarily through the use of *promotores* to promote skill building and behavior modification procedures for cessation and ETS prevention. Cessation and other behavior change efforts, however, are strengthened when undertaken in the context of broader policy initiatives. If effective, these programs will have important implications for controlling the ill effects of exposure to tobacco smoke.

E02

Smoking cessation in Latinos using community health advisors

Talavera, Gregory A.
San Diego State University

Although studies have demonstrated the need to develop culturally appropriate cessation programs that take into account the specific cultural beliefs and experiences of the Latino smoker, few resources and strategies have been developed to help Latinos quit smoking. The present study recruits Spanish-speaking Latino smokers living in San Diego County. Smokers are randomized into either an intervention group or a comparison group. The first group receives a culturally sensitive intervention that promotes smoking cessation and maintenance. Those assigned to the intervention group receive a 4-month smoking cessation program (that includes 4 home visits and 3 phone calls) delivered in the home by community health advisors or *promotores*. Participants in the comparison group are referred to the California Smoker's Helpline via mailed postcards.

To date, the project has trained 17 *promotores* of which 14 are actively participating in the project. Active *promotores* successfully completed a 5-week training course in which they met twice a week and learned about basic smoking facts especially those pertaining to Latinos; smoking as an addiction; smoking cessation techniques used in the program (choosing a quit date, using positive self-talk, using a quit buddy, and using the quit kit); one-on-one communication skills; and role playing of each home visit and phone call. Currently, *promotores* meet monthly to brainstorm solutions to problems that arise in the field, support each other's successes, and receive feedback and intervention materials from project staff.

Project staff have instituted new participant recruitment methods, such as the use of print advertising in primarily Spanish-language publications and participation in local community events (e.g., swap meets, festivals, and health fairs) to enhance recruitment rates. A total of 104 study participants have been recruited as of this writing. Preliminary demographic data indicate that the majority were born in Mexico, more than half are women, average age of study participants is 43 years, and the average acculturation level is 2.2 on a 5 point scale (low to high). In terms of baseline smoking indices, the average age of smoking initiation is 17 years, 85% of participants consider themselves daily smokers with an average of 6-10 cigarettes per day, 75% have tried to quit in the past, and 82% intend to quit in the next 30 days.

Data on 28 study participants for whom pre- and 4-month post-test data have been collected, reveal that participants in the intervention group are much more likely to report abstinence from smoking in the past week than participants in the comparison group. At post-test, intervention group participants report slightly more quit attempts during the past 3 months, report greater reductions in amount smoked, and are more likely to answer correctly smoking/cessation related knowledge questions than comparison group participants. Admittedly, the longitudinal sample size is still small, but the data suggests change in the desired direction. Future efforts will focus on participant recruitment and retention for the 1-year follow-up.

E03

Reducing environmental tobacco smoke in Latino children

Conway, Terry L. - *San Diego State University*

Environmental Tobacco Smoke (ETS) has been associated with a variety of illnesses in children, including increased rates of respiratory illness, middle ear infections, decreased lung function, asthma, and Sudden Infant Death Syndrome. Many children, however, continue to be exposed to ETS in the home, often by parents or relatives who may not fully realize the possible harm to their children. A recent survey in San Diego County, for example, found that 43% of Latinos reported having either no restrictions or only a partial ban on smoking inside their homes.

The primary goal of this research is to evaluate a behavioral problem-solving approach, based on operant and social learning theory, to reduce ETS exposure among young Latino children identified through Head Start programs in San Diego County. Intervention efforts are being directed toward a key member (e.g., the mother) of the household in which the child lives. This person is guided by trained bicultural and bilingual Latina promotoras to do problem solving aimed at lowering the target child's exposure to ETS in the household.

Participants are randomly assigned either to the control group or the 6-session intervention group. Intervention sessions involve behavioral problem-solving techniques such as contracting, shaping, positive reinforcement, and stimulus cues to assist the household contact in achieving ETS reduction goals. Reports of the child's exposure to ETS in the home and hair cotinine levels are the primary dependent variables.

Eight promotoras, who were recruited from the community and successfully completed a 4-week, 20-hour training course, are currently recruiting Latino households and delivering the intervention to participants randomly assigned to the intervention condition. Because of difficulties identifying enough eligible participants through Head Start alone, screening efforts have been expanded. To date, we have worked with 18 Head Starts, made presentations and distributed flyers to local preschools, kindergartens, health fairs, and swap meets, and received referrals from other participants.

Promotoras have made over 690 calls to potential participants to assess their eligibility and willingness to participate. Of these calls, only 11% were eligible (i.e., had a 2-5 year old child living in household and exposed to at least 6 cigarettes per week in home). Of the 80 eligible households, 64 (80%) were willing to participate. Preliminary analyses on 43 households (23 intervention and 20 control) with baseline and post-intervention follow-up data have indicated a greater reduction in the past 30-day cigarette exposure of target children in the intervention group than in the control group. Positive trends in outcome expectancies and perceived environmental constraints related to ETS reduction for the target children have also been found.

Previous research has shown that lay community health advisors (e.g., promotoras) can be effective in creating awareness of health issues and providing support for behavior change within their community, often helping those who do not have ready access to health care. This study is the first to extend the community health advisor model into the area of ETS reduction and, if found effective, will have important implications for controlling the ill effects of exposure to tobacco smoke.

E04

Healthy generations: Initial results from a study on parental prompting to smoke among Latino youth

Raphael Laniado-Laborín
San Diego State University

In previous studies with Latino youth, we learned that some parents were prompting their children to engage in behaviors that put them in direct contact with cigarettes or, without meaning to, encouraged them to "practice" smoking-relating behaviors. The most direct of these parental prompts was to ask a child to light the cigarette in their mouth and then pass it to the parent. We are currently conducting a study to validate children's reports of parental prompting with parents' reports, to identify what parental characteristics are associated with prompting, and to see if children who report parental prompts early in the study are more likely to become smokers.

Surveys have been completed in schools by 3600 children to collect information on parental prompting and the child's smoking behavior. Through this process, over 600 parents have been identified for parent interviews (n=600) currently . Student surveys and parent interviews assess a) initial smoking behavior among children, b) children's reports of parental prompting, c) parent's reports of parental prompting, d) agreement between child and parent reports of parental prompts, and e) factors associated with parental prompting.

Initial analysis with 1125 students showed that requests for the child to empty ashtrays (11.2% for mothers and 10.7% for fathers) or bring cigarettes (10.1% for mother and 13.2% for fathers) were the two most common prompts. Latino students (n=755) reported similar prevalence of prompting as seen for all participants. Mothers and fathers were not different in their level of prompting with the exception of requests to bring cigarettes (fathers were higher). Boys and girls were equally likely to be prompted. The poster will include results of the remaining student surveys in addition to those presented here.

E05

Smoking predictors in Hispanic youth

Marín, Gerardo, Balls Organista, Pamela, and Gamba, Raymond J.
University of San Francisco

The purpose of the current investigation is to: (1) identify predictors of each of three stages of cigarette smoking behavior (Trying, Experimentation, and Regular Use) in a sample of Hispanic youths, and (2) evaluate the role of seven domains of variables in predicting each of the three stages of cigarette smoking. The predictor domains are social environment (e.g., smoking modeling by adults peers, normative beliefs about smoking prevalence, acculturation); (b) intrapersonal characteristics (e.g. depression, risk taking, religiosity, deviancy); (c) knowledge about the health effects of cigarette smoking; (d) attitudes (e.g., expectations about smoking, stereotypes about smokers, and optimistic bias); (e) normative beliefs about smoking (i.e., beliefs of important others regarding cigarette smoking); (f) intentions to try, experiment or smoke cigarettes; and (g) previous or current use of cigarettes, alcoholic beverages, marijuana, or other substances. These domains were chosen because they have shown promise in previous studies of cigarette smoking onset, and in general are susceptible to modification through short-term prevention intervention.

Our research group has recently completed the questionnaire development and pre-testing phase of the project. In order to include culturally relevant questions regarding cigarette smoking, open-ended interviews with 24 Hispanic adolescents and 20 non-Hispanic White adolescents were conducted in their homes. Questions pertained to the seven domains (e.g., "think of people your age who smoke cigarettes…why do you think they don't smoke cigarettes?"). If a response was frequent it was included in the closed-ended telephone questionnaire. This procedure helped to ensure the inclusion of questionnaire items that were important for Hispanic adolescents.

The second phase of the project will include a telephone survey of Hispanic boys and girls (10 to 17 years of age). Children will be selected from residents in the San Francisco Bay area. The parent(s) of the children will also be interviewed at the same time. The study will use culturally appropriate interview instruments available in English or Spanish.

This research directly addresses the prevention of tobacco related diseases among Hispanics (the soon to be largest ethnic group in the United States), most notably addiction to nicotine, by providing basic research on the predictors of smoking in young people. The research will address the complex and less than optimally understood processes that affect Latino youth and lead to tobacco use. The long-term aim of the study will be the development of multiple, culturally appropriate, intervention strategies designed to prevent tobacco use and dependence among Hispanic youth.

E06

Predictors of smoking in African American adolescents

Alexander, Mark; Balls, Organista, Metz, Pamela, Marilyn, Jones, Jennifer; Forte, Deirdra, Perez-Stable, Eliseo
University of California, San Francisco

According to recent data from the Centers for Disease Control, smoking among teenagers was on the decline until the early 1990s, when the rate of smoking abruptly increased. Although rates for African American female teenagers continue to decline and the *overall* smoking rate for African American youth is lower than those rates for White and Hispanic youths, cigarette smoking has nearly doubled among African American *male* teenagers since the early 1990s. The reasons for these striking changes are unexplained at this time. The purpose of the current study is to examine characteristics of African American youth and "African American youth culture" that positively and negatively predict cigarette smoking initiation and continued use. We propose two specific approaches: 1) *To identify predictors of each of the three stages of cigarette smoking behavior* (Trying, Experimentation, and Regular Use) in a cohort of African American youth over a four-year period and 2) *To evaluate the role of seven domains of variables in predicting* each of the three stages of cigarette smoking in the same cohort. These seven domains include: (a) social environment (e.g., modeling by adults, parents and peers); (b) intrapersonal characteristics (e.g., depression, risk taking); (c) knowledge about health effects of cigarette smoking; (d) attitudes (e.g., expectations, stereotypes); (e) common beliefs (e.g., of important others in the community) about cigarette smoking; (f) intentions to try or experiment with cigarettes; and (g) previous or current use of cigarettes or other drugs.

The study targets African American 10-12 year olds who live in the San Francisco Bay Area. We have begun recruitment of this cohort with the help of the San Francisco Unified School District and several community organizations that cater especially to African American youth (e.g., West Oakland Boys and Girls Club and Making Waves Education Program in Richmond). Each respondent will be administered a survey that contains questions related to the pertinent domains. Participants' parents or guardians will also be interviewed. Children will be asked to provide a saliva sample for laboratory analysis in order to verify their smoking status. Interviews will begin in the winter of 1999.

In order to develop an age-appropriate, culturally relevant questionnaire, we recently completed five focus groups with nearly 40 African American 10-12 year olds. These groups employed a standard set of questions related to smoking behavior. Most participants reported that a major reason children might smoke is "to look cool;" however, the majority of children did not personally believe that cigarette smoking was "cool." Many felt that there were too many negative health risks associated with smoking. Examples of reported risks included not only "lung disease" and "cancer," but also "heart disease," "birth defects," "bronchitis," and "bad breath." The survey will include questions based on frequently mentioned responses from these focus groups.

This research addresses the prevention of smoking and related illnesses among African Americans, and it will be useful in broadening our understanding of adolescent smoking. Its prospective design will allow us to assess processes that encourage or deter smoking over time. Eventually these findings can assist in the development of culturally sensitive preventive interventions.

E08

Does belief that smoking keeps bodyweight down impact cigarette smoking behavior among adolescents?

Zheng, Hong and Chen, Xinguang
University of Southern California

Cigarette use among adolescents has increased dramatically in recent years. Reports suggest that young people (especially females) tend to use cigarettes as a weight control measure. We hypothesize that the belief that smoking helps keep weight down is a risk factor for cigarette smoking. With data collected from 1997 California Youth Tobacco Survey (CYTS), this study examines the impact of beliefs that smoking keeps weight down, and peer's care about body weight on cigarette smoking behavior.

Methods: Data were analyzed from 2,692 adolescents (50.7% males), representing a survey response rate of 67%. The smoking variables used for this analysis included ever tried cigarette smoking, ever smoked a whole cigarette, and smoked in the last 30 days. Variables to be tested for their association with cigarette smoking included beliefs that "smoking can keep weight down", "if girls the same age care about keeping their weight down" and "boys the same age care about keeping their weight down". Self-reported body weight was used as a control variable. Relative risk was used to measure the association between the risk factors and smoking behaviors. In addition to simple risk ratio analyses using PROC FREQ with the options of CHISQ and MEASURES, logistic regression was used to test multiple variable models. Bivariate analysis was conducted by gender to see the gender differences.

Results: The mean age was 14.4±1.67 for males and 14.5±1.68 for females. The age range was from 12 through 17 for the male, and 12 through 18 for females. Among the 2,692 adolescents, 35.2% of males and 34.2% of females reported ever experimenting with cigarette smoking. 13.4% of males and 13.8% of females reported ever smoking one whole cigarette in their lifetime. 20.4% of males and 21.9% of females reported smoking cigarettes in the past 30 days. 18% of male and 24% of female reported that they believe cigarette smoking can keep weight down. More than 95% of both males and females considered that girls their age care about keeping weight down. 44% of the males and 33% of the females considered that boys their age care about keeping weight down.

Both simple bivariate and multiple logistic regression analyses indicated that belief in the effect of keeping weight down and belief in girls' caring about keeping weight down were risk factors for all three smoking behaviors examined. The corresponding risk ratios ranged from 1.5 to 2.2. Body weight showed no risk for cigarette smoking (risk ratio equals 1.0). Belief that boys care about keeping weight down was a protective factor for both boys and girls, and the risk ratio ranged from 0.79 to 0.88. Results from both bivariate and multiple logistic regression models were similar, indicating the additive feature of these risk factors on cigarette smoking behavior.

Conclusion: The more adolescents think that smoking helps keep their weight down, the more likely they have tried cigarette smoking, the more likely they have smoked one whole cigarette in their life, and the higher the probability that they smoked in the last 30 days. Tobacco control should take this into account. Perceptions that girls the same age care about body weight were found to be a risk factor for adolescent smoking while perceptions that boys the same age care about body weight appeared to be somewhat of a protective factor. Further studies should be conducted to examine this difference in perceived norms on cigarette smoking behavior among adolescents.

E09

Motivation-enhanced teenage tobacco use cessation

Sussman, Steven Y.
University of Southern California

High-risk youth typically have been left out of previous tobacco research efforts. The goal of the present study is to contribute to knowledge about the quitting process in high risk youth and develop a comprehensive tobacco cessation program designed specifically for high risk youth. A repertoire of motivation strategies to encourage adolescents to stop using tobacco now, rather than wait until the future, was developed, implemented, and evaluated at continuation high schools in southern California. The complete program includes an eight session tobacco cessation clinic and a tobacco cessation oriented "school as community" component. A three-condition field experimental design contrasted a clinic-only, clinic plus school-as-community and control condition in 18 continuation high schools. The clinic provides the intrapersonal motivational component, while the school-as-community component provides the interpersonal motivational component.

The first year of the project was devoted to assessing factors that may motivate high risk youth to quit tobacco use immediately. Focus group material gathered in a previous study formed the basis for developing a pool of written descriptions of motivation activities. These descriptions were presented to students for evaluations of like-ability and ability to motivate tobacco cessation (theme study). Based on the ratings of the self-reported smokers, some activities were eliminated from the pool. Next, a new pool of activities were actually implemented in classrooms for evaluation by students (component study). Based on self-reported smokers' ratings, eight motivation activities were chosen and incorporated into a previously developed five-session psychosocial and physical addiction oriented standard clinic, resulting in a revised eight session motivation-enhanced clinic. Two rounds of pilot studies were conducted, and the second pilot study included the school-as-community component.

The second year of the project was devoted to implementing the three-condition design field experiment. Six schools received the clinic only, 6 received the clinic plus school as community, and 6 served as a standard care control condition. Volunteer students were recruited to participate in the clinics and school as community groups. A total of 259 students enrolled in the cessation clinic across the two program conditions. At total of 78% were retained; 202 completed 4 or more of the 8 sessions. A total of 17% of enrollees quit at immediate posttest and, surprisingly, 21% of enrollees quit at an average 5-month follow-up. CO monitors were utilized to provide bio-validation. This is the first study to indicate that teen cessation clinic programming can approach the follow-up quit rates achieved by adults.

E10

Development of a web-based smoking cessation intervention

Stoddard JL, **Muñoz RF**, Lenert L, Perez-Stable E, Delucchi K, Collins N
University of California, San Francisco

The principal objectives of this project are 1) to evaluate the feasibility of conducting randomized controlled clinical trials evaluating methods for smoking cessation entirely via the World-Wide-Web (WWW), 2) to compare the individual and combined effectiveness of cognitive-behavioral and group therapy interventions delivered via the WWW.

Progress made toward these objectives include the development of: 1) cessation materials, including a Guide for quitting and Nicotine Replacement Therapy (NRT), 2) a structure for site navigation and questionnaires completion, and 3) a theme guided bulletin board that implements a virtual group therapy for subjects participating in the study. All materials are being tested on a designated server in order to resolve technical issues (e.g. operating system, server options, development software, security, and storage) related to implementation.

In additional to cessation materials, instructions for using the site were developed along with security features (password protection), and links to additional smoking cessation resources. The questionnaires developed for the study include: 1) Eligibility, 2) Informed Consent Form and Patient Bill of Rights, 3) Baseline Demographic and Tobacco Use, 6) Acculturation (Spanish), 7) Reasons for Quitting, 8) Confidence in Quitting, 9) Withdrawal Symptoms, 10) Major Depressive Episode (history and current), 11) Depression Symptoms, 12) Menstrual Cycle Symptoms, and 13) a Quit Calendar.

By automating these questionnaires, the data collected may be more accurate and complete compared with the more traditional paper-and-pencil methods. The lesser administrative burden associated with web based questionnaires (no materials, tools, handling) along with the added functionality of immediate to responses to questionnaires (e.g., comparisons to a group average) may increase subject's willingness to complete questionnaires. Moreover, feedback can also be programmed to cue participants when their responses appear to be inaccurate, illogical, or incomplete. Finally, the cumbersome and frequently error laden process of transcribing and entering data will be entirely eliminated. Together these features enhance the efficiency of smoking cessation research, particularly for populations who are hard-to-reach (e.g., rural, low income, immobile, or highly mobile) at a lower cost than mail or face to face interventions.

Despite these advantages, there are a number of challenges to collecting questionnaire data using the WWW including, 1) response validity, 2) presentation of information (attractiveness, size, space attention span), 3) subject response burden 4) differing access of certain populations to the Web (e.g. rural or low income), 5) security of submitted information, and 6) flexibility in development tools (e.g., web-authoring and database software). Efforts to address these challenges will be discussed at the poster session.

E11

A smoking cessation chat room for rural teen smokers

Woodruff, Susan I. - *San Diego State University*

Smoking among adolescents has tremendous public health importance. Onset and development of smoking occurs primarily in adolescence, and because tobacco is highly addictive, regular use in adolescence develops into nicotine dependency. Smoking among adolescents is likely to continue into the adult years, increasing the risk of numerous long-term negative health consequences. Yet, even after three decades of efforts to prevent smoking among children, large numbers of young people continue to smoke, particularly high-risk teens.

Unfortunately, adolescent smokers are difficult to recruit and retain in smoking cessation in interventions, and traditional classroom interventions may not reach those particularly in need of support Even more discouraging, cognitive-behaviorally oriented cessation interventions that have been effective with adults, when tried with adolescents in school clinics and classrooms, have not shown much promise. Many believe that advances in health promotion among young people, including motivating interest in smoking cessation, will focus on innovative and appropriate use of emerging technologies such as computerized communication.

This 13-month study pilot tested an innovative, relatively untested yet promising approach to adolescent smoking cessation using a one-group, repeated measures design. Young smokers interacted (i.e., chatted) in real-time in an Internet-based 3-dimensional "world" with a trained cessation facilitator and other teen smokers, capitalizing on the demonstrated benefits of facilitator-led structured motivational interviewing strategies, as well as peer-to-peer interaction. Using school computers, 25 participants interacted in six 1-hour intervention chat sessions addressing topics shown to be effective and unique to the cessation experience of adolescents. The chat room was evaluated using responses to on-line self-report surveys and content analyses of results from chat session monitoring. Feasibility (e.g., use and acceptability), as well as short-term effectiveness (e.g., changes in self-efficacy, intentions, and self-reported quit rates) was tested with high-risk teen smokers recruited from six alternative education high schools in nearby rural communities. Results showed positive pre-post changes in smoking status, amount smoked, number of recent quit attempts, intentions, and attitudes toward quitting. These changes were maintained or even enhanced at a l-month follow-up. At the post-test assessment, 22% of the teens reported abstaining from smoking during the past week compared to 11% reporting past-week abstinence at pre-test. Participants rated the chat room positively on a number of factors, such as ease of use, appeal, and relevance. The chat room approach will be further developed and a proposal will be written to test it in a larger sample with a comparison group.

The 3-D chat room approach for teen smoking cessation has both public health potential and practical appeal. Computer access for health promotion is a particular advantage for adolescents in more rural areas since there are fewer community-based resources to help them quit. One other advantage of the computer chat room approach is based on evidence that people who feel constrained by face-to-face interventions or programs that are implemented in group settings may be stimulated and less inhibited in a "virtual" environment. In addition, conducting the intervention research via a chat room environment holds promise for reasons related to research methodology and for reaching large numbers of smokers.

E12

Tomando control de su salud (Taking control of your health)

Lorig, Kate
Stanford University School of Medicine

There are three crucial components to the care of tobacco-related chronic diseases. These are traditional medical care, surgical care and patient self-management education. The latter is a potentially critical ingredient of appropriate health services, especially for the Latino population, who not only carries a heavy burden of chronic disease comorbidity, but also encounters limited access to health care and health education. A Spanish language chronic disease self-management program called Tomando Control de su Salud (Taking Control of Your Health) is a low cost and cost effective means of providing such services to Spanish-speaking people with chronic disease.

The major aim of the study is to: develop, implement and evaluate, in a 4-month randomized trial, a 6-week experimental, Spanish language self-management education program for people with coronary artery disease, chronic lung diseases, and diabetes.

Based on information obtained from seven focus groups of Spanish speakers with the target conditions, we developed a 6-week program and accompanying educational materials. The following is the program's progress to date.

A. Twenty-three Spanish-speaking leaders have been trained to teach the program. Training was approximately 24 hours over 4 days. Sixteen of these leaders have taught one or more programs.
B. One hundred ninety-seven persons have been recruited, enrolled into the study and randomized to either attend the program or wait 4 months before attending the program. All subjects have completed baseline questionnaires either through the mail or by phone.
C. Thirteen programs have been given in the San Francisco Bay Area. The mean attendance has been 4.2 of the total 6 sessions.
D. Seventy-four percent of the subjects have completed 4 months in the study and have completed 4-month data.

To date, 79.8% of the subjects are female. Their mean age is 57.8 with an average of 1.9 diseases (25.2 % heart disease, 48.5% hypertension, 48.5% diabetes, and 19.2% with chronic lung conditions). Their mean education level is 7.1 years, with an acculturation level of 1.4. Currently, 9.4% are smokers and 28.3 % were past smokers.

Conclusion: This is a study in progress. Outcome data analysis will not start for another year. To date, we have learned:

1. It is possible to recruit and train Spanish-speaking community people to deliver a community- based chronic disease program.
2. It is possible to recruit and collect data from Spanish-speaking subjects.
3. Spanish-speaking participants will attend a multi-week, community-based chronic disease self-management program.
4. It is possible to collect 4-month follow-up data.

E13

Anti-tobacco programming: reaching the deaf and hard-of hearing

Berman, Barbara A.; Bastani, Roshan; Kleiger, Heidi; Eckhardt, Elizabeth; Lipton, Douglas S.; Barkin Shari; Wong Glenn; Abbott, Laruen; Hoang, Tuyen
Division of Cancer Prevention and Control Research, Jonsson Comprehensive Cancer Center and School of Public Health, UCLA; Greater Los Angeles Council on Deafness, Inc. (GLAD); National Development and Research Institutes, Inc. (NDRI).

An understanding of the determinants and patterns of tobacco use among ethnically, culturally and economically diverse groups of children and adolescents has been critical to developing strategies for preventing and reducing tobacco use among young people in the United States. Unfortunately, communication, language and other barriers have hindered collection of tobacco-related information among Deaf youth. Thus, we know almost nothing about their knowledge, attitudes and practices, exposure to anti-tobacco programming, or, as a result, about the strategies for best delivering smoking prevention and cessation education in this population.

Our goal is to conduct a tobacco related survey among 500 Deaf children, adolescents and young adults in California to obtain this information. We are using a specially designed innovative interactive computerized video questionnaire technology, the Interactive Video Questionnaire (IVQ™), not previously used among youth populations. This technology allows us to communicate in all of the languages that Deaf people use: American Sign Language (ASL), Signed English (SE), Oralism/Speech Reading (SR). We describe here the challenges faced in developing the IVQ survey (i.e, drafting an appropriate English language questionnaire, videotaping the full translations of the questionnaire into each of these languages, transforming the resulting videotapes into interactive, multimedia formats for use with the IVQ, etc.) and in administering the survey through the thirteen sites that have agreed to take part in this study. Results from our first 150 completed surveys will be reported.

We also describe the script of the telephone interview that we are conducting with administrators of residential and day schools for the Deaf, and of mainstream schools and colleges in California which have Deaf students. These interviews will allow us to learn about anti-tobacco programs, if any, that are already available to the Deaf, and to identify programming needs of this population. Preliminary results from these interviews will be reported. Finally, we outline future steps which will be taken, based on the results from the youth and administrator surveys, to develop effective anti-tobacco interventions tailored to the unique culture and communication needs of Deaf children, adolescents and young adults.

E14

Interdependence of smoking and mood in natural settings

Olmstead, Richard E.
University of California, Los Angeles

It has been theorized that one of the reasons people chose to smoke cigarettes is as an attempt to regulate their mood states. Previous research has commonly supported the tenet that smoking, and nicotine specifically, can affect mood and that changes in mood state can lead to smoking behavior, at least in a laboratory setting. The present series of studies examines smoking's effects on mood but in a natural environment. Of interest is the potential of smoking to affect diurnal patterns in mood levels and mood variability and whether changes in mood are contingent on smoking and/or vice versa.

Subjects were observed during two sessions each lasting a full waking day. During this time, approximately once every 30 minutes, they will provide mood ratings, urge to smoke ratings and note any cigarette consumption via 2-way pagers. Diurnal variation in mood was examined by dividing the waking period into four time frames. Repeated measures analysis of variance were used to examine changes in mood level (mean) and variability and the impact of the exogenous factors (e.g., smokers vs nonsmokers, depressive symptomology etc.). State space modeling of smoking, urge to smoke and mood variables tested possible contingencies. Several studies are ongoing.

Results are presented that compares intra-day mood variation in high-dependence smokers, low-dependence smokers and never-smokers within a young adult population.

E15

Transdermal nicotine replacement for hospitalized smokers: A randomized trial

Simon JA, Carmody TP, Snyder E, Murray J., Solkowitz S.
University of California, San Francisco

BACKGROUND: There are at least 6.5 million adult smokers hospitalized yearly in the US, providing doctors and health educators a window of opportunity to reach smokers with smoking cessation interventions. Patients are often hospitalized for a smoking-related illness, may not be able to smoke while hospitalized because of restrictive smoking policies, and may, therefore, be more open to quitting smoking. To date, relatively few controlled clinical trials have examined whether hospital-based smoking cessation interventions are effective in helping smokers quit. Because the effectiveness of the nicotine patch for in-patient smoking cessation has not been studied previously, we are conducting a clinical trial in which study participants will begin use of the nicotine patch during their hospitalization.

METHODS: Patients who are current cigarette smokers and who are hospitalized for at least 48 hours are being randomized to two groups - an intervention group and a minimal-contact comparison group. Patients in both groups receive the nicotine patch for 2 months. The intervention group is given face-to-face advice on practical smoking cessation strategies by a counselor, self-help booklets and is shown a 10-minute stop-smoking videotape prior to hospital discharge. The intervention group is followed up by phone weekly for one month and then monthly for 3 months. The minimal-contact comparison group is given the self-help booklet only. All patients are being followed by
study personnel during the 12 months following hospital discharge to determine rates of tobacco abstinence at 6 and 12 months. To confirm tobacco cessation histories bio-chemically, cotinine levels are being obtained at one year in all those study subjects who report smoking abstinence.

RESULTS: This study will be recruiting participants through March 2000. A total of 179 current smokers admitted to the San Francisco VA Medical Center between October 1997 and September 1999 have been enrolled to date. Six-month follow-up data are available for 56 intervention participants and 57 comparison participants. A total of
40% of the intervention participants (n = 20) and 13% of the comparison participants (n = 7) have quit smoking by self-report ($p < 0.01$). One-year self-report follow-up data are available for 33 intervention participants and 31 comparison participants. A total of 30% of the intervention participants (n = 10) and 23% of the comparison participants (n = 7) have quit smoking by self-report ($p = 0.49$).

CONCLUSIONS: By self-report, smokers enrolled in an intensive hospital-based smoking cessation intervention that includes the nicotine patch are more likely to have quit smoking at 6 months. If the 6-month trends continue through 12 months of follow-up and are confirmed by biochemical validation, our findings would suggest that hospitalized smokers should be offered the opportunity to participate in intensive hospital-based programs to increase smoking cessation rates after hospital discharge.

E16

Bupropion for smoking cessation: A randomized trial

Simon JA, Carmody TP, Duncan C, Snyder E.
University of California, San Francisco

BACKGROUND: Standard smoking cessation interventions include counseling, nicotine replacement therapy, and self-help literature. Because smokers are more likely to have a past history of depression and smoking cessation may increase symptoms of depression, the use of antidepressant medication for smoking cessation has been proposed as a possible aid for smoking cessation. A few studies have reported that the antidepressant bupropion is an effective adjunct for smoking cessation. However, the effectiveness of bupropion has to date been studied in only a few populations.

METHODS: We are conducting a randomized blinded clinical trial in which approximately 360 veteran smokers will receive standard treatment that includes 2 months of the nicotine patch, counseling, and use of self-help literature. In addition, approximately 50% of participants will be randomly assigned to receive 7 weeks of
bupropion whereas the remaining 50% of participants will be randomly assigned to receive an identical placebo. Neither the participants nor the investigators will know who has been assigned to receive the active drug. If the addition of bupropion to standard therapy increases biochemically-confirmed smoking cessation rates at one year, it may become an important addition to smoking cessation programs targeted at diverse populations of heavily addicted smokers.

RESULTS: This study is currently recruiting participants. A total of 121 current out-patient smokers at the San Francisco VA Medical Center have been enrolled to date. Six-month follow-up data are available for 70 participants. By self-report, 54% of the participants (n = 60) have quit smoking at the end of treatment (8 weeks) and 39% of the participants (n = 27) have quit smoking at 6 months of follow-up. Participants will be followed for 12 months at which time their treatment status will be disclosed and self-reported smoking cessation confirmed biochemically.

CONCLUSIONS: If the addition of bupropion to nicotine replacement and counseling significantly increases quit rates among our population of heavily addicted veteran smokers, we would recommend that such therapy being offered routinely in VA-sponsored smoking cessation programs.

E17

Helping tobacco users to quit: Pharm-assists' counseling role

Hudmon, Karen S.
Center for Health Sciences, SRI International

Since nicotine patches and nicotine gum became available without prescription in 1996, their sales have increased substantially. Although the packaging of the products have been designed for use by the general public, patients now are able to self-prescribe and self-treat their addiction without contacting a health care professional. Thus, tobacco users are able to make quit attempts without the proven positive effects of professional intervention.

Because nicotine patches and gum are almost exclusively available in pharmacies, and because new prescription-only pharmacological agents recently have been introduced as aids for tobacco cessation, the pharmacist is a logical candidate for providing intervention to tobacco users. However, previous studies have shown that pharmacists typically do not take an active role in providing this service. The goal of this study was to gain a clear picture of pharmacists' ability to provide and attitudes toward providing tobacco cessation counseling, with an emphasis on counseling patients for use of nonprescription patches and gum. Using a cross-sectional, descriptive survey design, questionnaires were mailed to all licensed pharmacists practicing in 4 selected Northern California counties. The questionnaires assessed (1) the extent to which pharmacists provide smoking cessation counseling and the characteristics of typical counseling sessions, (2) confidence in tobacco cessation counseling skills and stage of change for counseling, (3) feasibility of providing cessation counseling, including time limitations and physical proximity of nicotine replacement therapy products to the pharmacy, (4) pharmacists' perceived benefits of and barriers to providing tobacco cessation counseling to nonprescription customers, and (5) pharmacists' perceptions of the role that the profession of pharmacy should take in tobacco control.

Data from this study are being combined with data from a concurrent study that characterizes nicotine patch and gum users and their perceptions of the pharmacist's role in providing tobacco cessation counseling. In a third study, we are measuring the extent to which providing pharmacists with specialized tobacco cessation training alters their practice behavior; specifically, we are measuring changes in the number of patients that they counsel for tobacco cessation and quality of the counseling that is provided. Results from these three studies will be integrated and applied in an effort to design and launch broad-scale programs that encourage and better equip pharmacists with the skills that they need to provide tailored, motivational interventions to their patients who wish to quit using tobacco.

These projects combine efforts of tobacco researchers, the University of California San Francisco School of Pharmacy, representatives from the California State Board of Pharmacy and the California Pharmacists Association, and practicing pharmacists across California. This collective effort lays the groundwork for our goal of enlisting the profession of pharmacy as an ally for California's anti-smoking efforts.

F01

Case studies of tobacco control regulations: The role of research in the development of Maryland and Washington indoor air regulations

Bryan-Jones, Katherine; Mangurian, Christina; **Bero, Lisa**
University of California, San Francisco

The purpose of this study is to provide information to policy makers and health advocates on effective ways to use empirical research in the formation of tobacco control policies. We are comparing the regulatory policy development in two states and the extent to which policy makers utilized research findings when developing indoor air regulations.

We analyzed all transcripts of public testimony and written commentary submitted for the Maryland Indoor Air regulation and the Washington Clean Indoor Air Act. We coded each submission for position towards the regulation, arguments used, and referenced cited to support the arguments.

Among the Maryland commentaries, 43% (108/252) were in opposition to and 52% (132/252) were in support of the regulation. In Washington, 22% (29/133) of commentaries were against the regulation, while 76% (101/133) were for the regulation. In both Maryland and Washington, the tobacco industry submitted nearly one quarter of the commentaries opposed to the regulations. Overall, 16% (38/240) of the Maryland commentary and 34% (44/130) of the Washington commentary referred to science evaluation. These arguments included the quantity, reliability, quality, and validity of the scientific evidence. Commentaries against the regulations were more likely to include scientific arguments than those in favor. Those opposed to the regulations used over 12 different arguments to suggest that research on passive smoking health effects is invalid; commentaries in favor of the regulation rarely mentioned or countered these scientific arguments. Extra-scientific arguments were mentioned more often in Washington (99% of commentary) than in Maryland (51%). However, in both states, extra-scientific arguments such as economic, building management, political and procedural arguments were more often used in opposing commentary than in supportive. Analysis of hearing transcripts produced similar results as the commentary.

We have assessed the type and quality of the references that were submitted with the Maryland commentary or hearings. While those opposing the regulation submitted more documents than those in favor (356 vs. 172), 31% (110/356) of the references in opposition were from non-peer reviewed publications such as conference presentations and symposia compared to 5% (9/172) of those in favor. Journal articles cited in support of and against the regulation had similar median years of publication (1991 vs. 1989) and percentages that were peer reviewed (95.7% vs. 97.3%). However, references cited by those against the regulation had a lower mean impact factor (1.6) than those cited in support of the regulation (3.5). Similar data for Washington will be analyzed.

Discussions of health effects and economic research play a substantive role in smoking restriction regulatory policy development. By inundating regulatory officials with research of questionable credibility and quality, the tobacco industry attempts to slow and obfuscate the regulatory process. Our findings suggest the need for tobacco control advocates to counter these tactics by keeping the debate focused on the adverse health effects of passive smoking by citing the strong body of literature in this area.

F02

Scientific research used in risk assessments: Case study of the California environmental tobacco smoke risk assessment

Barton-Elson, Marieka, and **Bero, Lisa**
University of California, San Francisco

We studied the extent to which empirical research findings were used to develop the Environmental Protection Agency of California risk assessment of environmental tobacco smoke. The risk assessment was a collaborative effort by the Office of Environmental Health Hazard Assessment (OEHHA) and the Air Resources Board (ARB) to do a comprehensive review of the scientific data with collected public input. The report was also independently reviewed by the Science Review Panel. Our data consisted of public testimony at the workshops sponsored by OEHHA and ARB, as well as public commentary and citations submitted during the five years (1992-1997) of the risk assessment process. We included in our analysis all workshops and commentaries that referred to the risk assessment chapters on respiratory health effects, carcinogenic effects and cardiovascular health effects.

For the 3 workshops that were analyzed, all 9 presenters were tobacco industry representatives who were critical of the risk assessment. Analysis of the workshops suggest a strong emphasis on scientific research: 8 of the 9 presenters referred to science evaluation criteria in their arguments, including the quantity (5/9), reliability (6/9), quality (7/9) and validity (4/9) of the evidence presented. All 9 presenters used study selection and misclassification arguments to criticize studies presented in the risk assessment. Other scientific criticisms included exposure (6/9), confounders (7/9), misrepresentation or misappropriation of data (6/9) and general bias (6/9). No extra-scientific arguments were presented.

Preliminary analysis of the commentary suggests similar patterns to the workshops. Of the 35 commentaries that were received, 25 were critical of the risk assessment, 8 were supportive and 2 were neutral or missing. Of the 25 criticisms, 68% (17/25) were from the tobacco industry. In comparison, the 8 supportive commentaries were sent by a variety of different organizations, including federal and state governments (3/8), health professionals' organizations (2/8), private citizens (2/8) and non-smokers' lay organizations (1/8). Of the citations coded to date, 90% (423/470) were included with or referenced in commentary submitted against the risk assessment. Journal articles constituted 70% (328/470) of the total citations. Our current analysis suggests that articles cited by those against the regulation tend to be older, have a similar impact factor, and are less likely to be peer-reviewed, thus presenting a lower quality of citation.

Initial results show that the tobacco industry presented a strong response, although supported by poor quality research. In contrast, the public health community had little or no counter for this inundation of research. Future recommendations for public health advocates would include a stronger response with high quality research to support their position.

Risk assessments are important in reducing both the human and economic cost of tobacco use. Our previous work has shown that government reports, such as the United States EPA risk assessment of passive smoking, are heavily relied upon to support smoking restrictions. However since this risk assessment was "vacated" by the North Carolina courts in July 1998 on procedural grounds, the California risk assessment may now play a more important role in future smoking restriction debates. Furthermore, the California risk assessment was the first to include a section on the cardiac effects of passive smoking, thus making this risk assessment an important case study to support current and future smoking policies.

F03

Vapor-phase organics in ETS: Dynamics and Exposure

Singer, Brett C.; Hodgson, Alfred T.; Nazaroff, William W. and **Daisey, Joan M.**
Lawrence Berkeley National Laboratory

Environmental tobacco smoke (ETS) is the complex mixture of chemical vapors and particles to which non-smokers are exposed when in a room or house with smokers. ETS exposure is associated with increased risk of lung cancer, heart disease, and childhood asthma, but great uncertainty remains about which ETS components are most responsible for adverse health effects. The relative and absolute room air concentrations of individual ETS components are influenced by smoking frequency, ventilation, and the tendency of some less volatile gases to stick (sorb) to indoor surfaces. Sorbing compounds are selectively removed from the air during smoking - thus reducing exposure - but can later desorb from surfaces, resulting in exposure long after active smoking has ceased. Significant ETS exposure can also occur if non-smokers enter a room shortly after smoking has occurred.

The overall objective of this project is to further characterize ETS exposure under realistic home conditions. We are pursuing this goal through a framework of experiments and model development. The model will track ETS emissions from smoking, removal by ventilation, and will include a semi-empirical characterization of sorption and desorption for ETS gases. Sorption rates will be determined experimentally for a suite of over 30 gases in a 15 m^3 chamber lined with typical indoor materials such as carpet and wallboard. The effect of variations in smoking and ventilation rate will also be determined experimentally using a 50 m^3 chamber constructed and furnished to simulate a typical home environment. Our work during the first year has focused on chamber preparation, analytical method development, model development, and the execution and analysis of data for 2 experiments in which a specified number of cigarettes were smoked each day for 2-3 weeks in a ventilated, ETS-conditioned chamber.

The results of this research should aid toxicologists focus on the most important disease-causing agents in ETS and provide guidance for epidemiologists to better assess ETS exposure based on household smoking and ventilation rates. We also hope to describe quantitatively how much nonsmokers may reduce their exposure to ETS through control of ventilation and temporary avoidance of the room or rooms where smoking occurs.

F04

Arterial stiffness is increased in nonsmoking females exposed to environmental tobacco smoke

Mack, Wendy J.
University of Southern California

Pulse pressure (the difference between systolic and diastolic pressures) as a measure of arterial stiffness is associated with an increased risk of all-cause and coronary mortality. Arterial stiffness is also higher in persons with coronary artery disease compared to persons without coronary artery disease. Although active smoking has been shown to acutely increase arterial stiffness, the association between arterial stiffness and exposure to environmental tobacco smoke (ETS) has not been well studied.

We used baseline data from subjects randomized to the Vitamin E Atherosclerosis Prevention Study (VEAPS) to evaluate the association between ETS exposure and arterial stiffness in a sample of 227 (102 males, 125 females) healthy adult subjects who had never smoked. We utilized B-mode ultrasonograms of the common carotid artery to measure arterial diameters over the cardiac cycle. We then computed Peterson's elastic modules (Ep) as the ratio of pulse pressure to percent diameter change over the cardiac cycle. Higher values of Ep indicate stiffer arteries. Ep increased with age ($r = 0.46$). Adjusting for age and gender, Ep was nonsignificantly higher in persons who lived with a smoker (mean [SEM] Ep = 189.6 [21.3] kPa in exposed vs. 153.8 [10.8] kPa in unexposed, $p = 0.15$). ETS exposure at work was not related to Ep. Ep was significantly higher among persons regularly exposed to ETS outside of home and work (mean Ep = 184.7 [14.5] kPa in exposed vs. 145.5 [12.2] kPa in unexposed, $p = 0.04$). When analyzed by gender, the relationship with Ep outside of the home was found in females (mean Ep = 222.6 [25.3] kPa in exposed vs. 149.1 [19.7] kPa in unexposed, $p = 0.02$) but not males (Ep = 141.9 [11.7] kPa in exposed vs. 144.5 [10.8] kPa in unexposed, $p = 0.87$). In females only, Ep increased with number of smokers living in the home (age-adjusted $r = 0.16$, $p = 0.07$) and with greater average daily hours of exposure to ETS in the home (age-adjusted $r = 0.21$, $p = 0.02$). Because only 6% of the males were exposed to ETS in the home, the relationship with arterial stiffness could not be evaluated.

Using data from the larger sample of 615 nonsmoking subjects screened for the VEAPS trial, we have previously reported that exposure to ETS is also associated with greater intima-media thickness (IMT) of the carotid artery, a measure of subclinical atherosclerosis. These current data on arterial stiffness in women, a physiological measure related to cardiovascular morbidity and mortality, are consistent with our previous report. Future work will attempt to expand to larger samples to be able to more adequately address ETS exposures among males as well as females.

F05

Alternative sources of ETS exposure in infants

Matt, Georg E. and Quintana, P.J.E.
San Diego State University

Exposing infants to environmental tobacco smoke (ETS) significantly increases their morbidity and mortality risks. In California, approximately 1 in 3 children under age 5 are exposed to secondhand smoke in the home. During their first year of life, infants are in close and frequent physical contact with their parents, spend significant amounts of time in close proximity to floors, carpets, or blankets, and frequently handle and insert various objects in their mouth (e.g., toys, pacifier). This places infants at risk of ETS exposure even if no smoking takes place in their presence and even if they do not receive a smoker's breast milk.

This study explores whether inhaling contaminated indoor dust and ingesting tobacco constituents from contaminated objects and surfaces at home present significant sources ETS exposure in infants. Our study has been designed to explore the amount of ETS exposure from these sources and the relative contribution of different sources to the overall absorption of ETS in infants. To investigate these sources of secondhand smoke exposure, three types of homes with infants under 1 year old are being investigated: (1) Homes of nonsmokers where the infant is not reportedly exposed to secondhand smoke (No Exposure Group; N=15). (2) Homes of smokers where no smoking reportedly takes place in the presence of the infant either at home or outside of the home (Indirect Exposure Group; N=15). (3) Homes of smokers where smoking reportedly takes place in the presence of the infant (Direct Exposure Group; N=15). Dust, air, and surfaces are being examined in these homes to assess nicotine and cotinine contamination. Urine and hair samples of infants are being collected to determine secondhand smoke exposure. Behavioral interviews are being conducted with the infants' mothers to collect data on smoking histories, smoking and exposure patterns, and cleaning habits. Major physical characteristics of the infants' homes are also being recorded. We are currently in the process of completing data collection. We will present preliminary data, comparing the contamination of indoor environments and secondhand smoke exposure of infants in home with smoking and nonsmoking parents.

This study will provide much-needed data on potentially important alternative sources of ETS exposure for infants. It is important to better understand these sources of exposure because they may pose serious health threats even if no smoking takes place in the presence of an infant and long after smoking has ended. Findings could have significant implications on measuring, reducing, and preventing ETS exposure in infants.

F06

ETS exposures in the California Teachers Study Cohort

Reynolds, Peggy
California Department of Health Services

The primary objective of this project is to evaluate the baseline characteristics associated with reported historical environmental tobacco smoke (ETS) exposure, and to conduct a preliminary assessment of their relationship to selected short-term chronic disease outcomes in a large well-defined cohort of California professional school employees. Specific aims are:

1. To characterize both temporal and cohort-specific pat terns of historical ETS exposures in the home, in the workplace and in other settings.
2. To characterize the correlates of reported ETS exposure.
3. To conduct a preliminary short-term assessment of the association between reported historical ETS exposures and targeted chronic disease outcomes, particularly for common cancers and acute asthma events.

Extensive data on active and passive smoking, as well as information on potential correlates of exposure, will be derived from a series of surveys completed by the California Teachers Study cohort. The first questionnaire (WaveI), which contained basic indicator questions for active and passive smoking, was mailed in 1995 to all active and retired female school employees in California. 133,400 women responded. In 1997, the second questionnaire(Wave II) which contains the more detailed ETS exposure questions, was sent to all Wave I respondents. We have received approximately 100,000 Wave II questionnaires. A comparison of the baseline characteristics of Wave II responders to non-responders revealed no substantial differences. While data cleaning is not yet complete, we have run some initial frequencies on the data. Frequency distributions on the sociodemographic characteristics and tobacco exposures have been generated. While the cohort is primarily non-Hispanic white(86%), there are sufficient numbers of Native Americans(n=1,284), Asian/Pacific Islanders(n=4,817), blacks (n=3,513) and Hispanics (n=5,292) to allow for the examination of ETS exposures in these ethnic/racial groups. Only 5% of the cohort are current smokers and nearly two-thirds are lifetime non-smokers. Among lifetime non-smokers, approximately two-thirds have lived with a smoker at some point in their lives, nearly a half have been exposed in the workplace, and about a third reported exposures in social/other settings. A preliminary assessment of the degree of consistency in reported ETS exposures on the Wave I versus Wave II surveys indicated good agreement between the two surveys. For example, over 90% of those who reported having ever lived with a parent who smoked on the Wave I survey also reported it on the Wave II survey. In general, agreement in responses was better for reported parental ETS exposure than adult sources of ETS. Analyses to date primarily have focused on characterizing the correlates of ETS exposures.

Efforts are currently underway to complete the data editing and reduction. Once this is complete, the temporal and cohort-specific patterns of ETS exposures will be examined and the correlates of ETS exposures further explored. To allow for sufficient follow-up time, the outcome analysis as part of specific aim#3 will not begin until the final year of the project.

Understanding the patterns and correlates of active and passive smoking is critical in planning targeted public health interventions. Establishing baseline exposure profiles is necessary to evaluate future initiatives to reduce ETS exposures.

F07

Indoor measurements of environmental tobacco smoke

Apte, Michael G.; Gundel, Lara A.; Chang, Gee Minn and **Sextro, Richard G.** - *Lawrence Berkeley National Laboratory*

Environmental tobacco smoke (ETS) - the smoke released from the burning end of the cigarette - is one of the most common sources of carcinogens to which the general public is exposed. However, little is known about the size and extent of potential exposures to ETS. The objective of this research project is to improve the basis for estimating ETS exposures in a variety of indoor environments. The research utilizes experiments conducted in both laboratory and 'real-world' buildings to: 1) study the transport of ETS species from room to room; 2) examine the viability of using various chemical tracers as tracers for ETS; and 3) evaluate to what extent re-emission of ETS components from indoor surfaces might add to the ETS exposure estimates.

We constructed a three-room environmental chamber using common building materials for examining multi-zone transport of ETS and its tracers in a house. One room (simulating a smoker's living room) has been conditioned extensively with ETS, while a corridor and a second room (simulating a child's bedroom) remained smoke-free. A series of 5 replicated experiments was conducted to simulate the movement of ETS between rooms. Four pairs of experiments were conducted with different door opening configurations (sealed, leaky, slightly ajar, and wide open) under natural flow conditions. The fifth pair of experiments was conducted under forced-airflow conditions with a leaky door setting between the living room and corridor.

When the door between the rooms were slightly ajar, the particles dispersed from the smoking room into the other rooms, eventually reaching the same concentration throughout. On the other hand, nicotine quickly adsorbed on unconditioned chamber surfaces of the corridor and bedroom, so that nicotine concentrations in these rooms remained very low, even during smoking episodes. All of the ETS particle tracers: ultraviolet-absorbing particulate matter (UVPM), fluorescent particulate matter (FPM), and solanesol, characterized the transport of ETS particles into the non-smoking areas much better than did nicotine. Of the particle tracers, UVPM was found to be the most sensitive and chemically stable, while solanesol proved to be the least stable but most ETS-specific tracer.

In practical terms, these results suggest that, because of nicotine interactions with the building materials, ETS exposures inferred from nicotine measurements may often be either underestimated or overestimated. For example, using nicotine to estimate a child's bedroom ETS exposure from smoking occurring in the living room may underestimate the child's particle-component of exposure. Furthermore, nicotine measurements made in the smoker's living room during non-smoking periods may be elevated due to re-emission of nicotine from surfaces, leading to a possible overestimate of ETS exposures during time spent in the living room.

In the third year of this TRDRP project we plan to investigate these issues in real buildings. The research will focus on: 1) monitoring the inter-room ETS transport phenomena in typical residences; 2) investigating the time-dependence of re-emission of nicotine from indoor surfaces after smoking is no longer allowed in the house; and 3) identify which tracers are the most appropriate to use in real buildings.

F08

Quantification of population exposure to secondhand smoke

Switzer, Paul, Klepeis, Neil and Ott, Wayne
Stanford University and University of California Lawrence Berkeley Laboratory

The goal of our research is to develop new methodologies to measure, understand, and predict the exposure of Californians to secondhand smoke, with emphasis on exposure at home and in motor vehicles. To determine the health risks from secondhand smoke, it is necessary to know the pollutant concentration levels to which people are exposed and the durations of their exposures. Despite public health concerns about secondhand smoke, surprisingly few studies exist that measure the pollutant levels to which people are exposed in their daily lives from smoking activity.

Secondhand smoke consists of 3,000 toxic pollutants, both gases and particles. Adults and children traveling in motor vehicles can be exposed to extremely high pollutant concentrations from smoking due to the small volume of the vehicle passenger compartment. Similarly, smoking at home can expose children and other residents to high pollutant concentrations over relatively long time periods. We have measured pollutant concentrations in motor vehicles, homes, stores, bars, restaurants, and other locations where Californians are exposed to secondhand smoke.

To measure secondhand smoke exposure in the home, we are developing a new instrument package that can operate in the home unattended, the Continuous Air Monitoring Package (CAMP). The CAMP is designed to measure automatically the temperature and humidity and four important indicators of secondhand smoke — carbon monoxide, particle-bound polycyclic aromatic hydrocarbons, particle mass concentrations, and particle count density in six size categories. Our studies show that the CAMP can detect and measure accurately the pollutants generated by a single cigarette in a home. We also are developing mathematical indoor air quality models that can predict the concentrations in different compartments of the home. Our indoor models show that closing doors between the smoker and other occupants substantially reduces the exposures of others in the home.

To predict levels of secondhand smoke exposure among the California population, we are examining activity patterns from the diaries of 11,800 people, which include their reported secondhand smoke exposure. We are combining the activity pattern data with pollutant concentration data that we have collected in the home and locations outside the home to produce mathematical models of population exposure. These models allow us to anticipate changes in population exposure resulting from different intervention strategies, such as reducing smoking in motor vehicles, homes, or bars and restaurants. Furthermore, using demographic information included with the activity pattern diaries, our models can assess the differential effects of alternative mitigation strategies for different demographic groups based on sex, age, or race, for example.

G01

Chronic exposure to sidestream smoke augments bronchopulmonary C-fiber inputs to nucleus tractus solitarius and reflex output

Mutoh T, Bonham AB, **Joad JP**
University of California, Davis

Children chronically exposed to environmental tobacco smoke (ETS) have more wheeze, cough and airway narrowing which may result in part from stimulation of C-fibers. C-fibers are nerve fibers thought to protect the lung from injury. We have previously shown that exposing young guinea pigs to sidestream smoke enhances the sensitivity of bronchopulmonary C-fiber endings. To determine whether this increased sensitivity at the level of the primary afferent endings has any physiological relevance to changes in breathing pattern in response to C-fiber stimulation, we determined whether the enhanced sensitivity was manifest in the brain and ultimately in breathing pattern.

Guinea pigs were exposed to sidestream smoke (the surrogate for ETS) or to filtered air for 5 days/week for age 1-6 weeks, the equivalent of human childhood. The guinea pigs were then anesthetized and a stimulator of C-fibers, capsaicin, was injected into the left heart to activate bronchopulmonary C-fibers. We simultaneously recorded the impulse activity of neurons at the first central synapse in the C-fiber pathway in the nucleus tractus solitarius (NTS), phrenic nerve activity (as an index of breathing output), tracheal pressure (as a global index of airway tone) and arterial blood pressure and heart rate (as cardiovascular indices).

Chronic sidestream smoke exposure markedly increased the responses of NTS neurons to C-fiber activation with capsaicin ($P = 0.03$). This exaggerated sensitivity at the first central synapse in the brain was also accompanied by an exaggerated reflexly-evoked cessation of breathing ($P = 0.03$). By contrast, sidestream smoke exposure had not effect on the capsaicin-stimulated increase in tracheal pressure, or decrease in heart rate and blood pressure.

These findings show for the first time that sensitization of lung sensory afferent fibers by chronic exposure to sidestream smoke is sustained in the brain and is associated with an augmented reflex output to change breathing pattern. The findings suggest that chronic ETS exposure changes respiratory function via a reflex involving the brain and provides a mechanism to explain some of the respiratory symptoms in children who live in the homes of smokers.

G02

Exposure of neonatal mice to environmental tobacco smoke enhances the allergic response to ovalbumin at adulthood

Seymour, Brian W .P. Friebertshauser, Kathy E. ,Coffman, Robert L. and **Gershwin, Laurel J**.
University of California , Davis and DNAX Research Institute

Epidemiological studies have shown that "second-hand smoke" is a risk factor for allergic diseases in childhood health. To understand how second-hand smoke may increase these risk factors, we have used an experimental animal model to study the immune response after exposure of neonatal mice to environmental tobacco smoke (ETS). Newborn BALB/c ('atopic') mice were housed in environmental chambers containing a specially generated and quantified concentration of ETS. At 6 weeks of age (adulthood) all mice were given immunogenic doses of ovalbumin (OVA) to induced an allergic response. This was done by the administration of 10 mg OVA in 2 mg of aluminum hydroxide (OVA/AL) intraperitoneally followed, 2 weeks later, by a 20 minute exposure to a 1% aerosolized OVA. We assessed the allergic response and found that mice housed in ETS had enhanced allergic (IgE, IgG1) antibodies when compared to those housed in ambient air. These antibodies were specific for OVA and not cigarette smoke components conforming epidemiological studies which state that atopic children from households exposed to ETS are at a higher risk for allergic sensitization compared to those from non smoking environment.

The early childhood years have been acknowledged as a period when exposure to allergens can result in sensitization with consequences of an allergic response. We and others have shown that primary aerosol exposures of adult mice to OVA over 10 days results in a TH2-response (chemical mediators that induce allergy) and IgE-specific unresponsiveness to subsequent immunogenic challenges with OVA. The same aerosol OVA treatment was not able to induce tolerance when begun in mice 3 days after birth. The ability of mice to be tolerized is acquired only gradually during the first 4 weeks after birth. Aerosol OVA exposure beginning 3 days after birth prevented mice from being tolerized by a second series of aerosol OVA exposures which began at 6 weeks of age. Instead, it primed them for an allergic response. This data implies that neonatal mice up to 3 days of age are at high risk for T cell sensitization which, can result in allergic responses at adulthood. It also implies that the risk of atopy is associated with a delay in the development of the protective mechanism for inhalation tolerance. Presently, we are investigating the ability of ETS to further delay the establishment of the tolerance mechanism thereby increasing the time in which neonatal mice greater than 3 days old may become primed for allergic responses when exposed to aerosolized OVA.

This work is particularly relevant to increasing our understanding of the increase in allergy and asthma that has been observed in recent years in children from urban areas. Preliminary data from the animal studies suggest that human neonates in homes of smokers have an enhanced risk of allergic sensitization to allergens present in their environment.

G03

Identification of a novel *Cis*-acting regulatory element critical for the constitutive expression of the LTC_4 synthase gene in the monocyte-like cell line, THP-1

Bigby, Timothy D; Serio, Kenneth J. and Hodulik, Craig R.
Department of Veterans Affairs Medical Center and University of California, San Diego

This study was undertaken to examine the molecular mechanisms of regulation of constitutive expression of the (leukotriene C_4) LTC_4 synthase gene in the mononuclear phagocytes. This gene is expressed in cells of inflammatory origin, such as mononuclear phagocytes, but is not expressed in cells of epithelial origin. Previous work has demonstrated that an Sp1 site (-42 to –37 bp) was necessary but not sufficient for gene expression. THP-1 cells were transiently transfected with plasmid constructs (pGL3) containing elements of the 5'-untranslated region (5'-UTR) and ligated to a luciferase reporter gene. Analysis of a deletion series revealed that the first 92 bp of the 5'-UTR contained the majority of the promoter activity. Fine mapping of the first 92 bp of the 5'-UTR demonstrated that a region between –77 to –62 bp was critical for expression in THP-1 cells, as demonstrated by deletion analysis. Further analysis with this 16 bp region using EMSA revealed a single gel shifted band that was specific for cells expressing the gene (THP-1) and was not found in cells not expressing the gene (HeLa). Additional EMSA studies with this region revealed that 2-3 bp substitutions resulted in a loss in the THP-1-specific gel shift. Re-analysis of these 16 bp did not demonstrate a known consensus site for a *cis*-acting transcriptional element via any available database. Site directed mutagenesis in this region revealed a dramatic loss of cell-specific activity in promoter-reporter constructs. UV cross-linking studies revealed a single band suggesting a 30-35 kDa protein binding to this region. These studies identify a novel *cis*-acting transcriptional element responsible for cell specific constitutive expression of the LTC_4 synthase gene in the monocyte-like cell line, THP-1.

G04

Monocyte chemokine expression by airway epithelium is enhanced following exposure to environmental tobacco smoke

Miller, L.A., Li, C., Pinkerton, K.E. and Hyde, D.M.
University of California, Davis

The role of cigarette smoke as a primary risk factor for chronic inflammatory lung disease is well recognized. Although the precise molecular mechanisms by which chemical constituents of tobacco contribute to lung disease are unclear, inflammation is an early prognosticator of airway dysfunction. One consistent observation of the direct effects of cigarette smoking is a marked increase in number of alveolar macrophages within the lung. Resident alveolar macrophages likely play a supportive role in maintenance of immunological homeostasis under normal conditions. However, a persistent influx of new monocyte populations in response to cigarette smoking may promote chronic inflammatory lung disease by exaggerated production of oxidants, proteases, and leukocyte chemoattractants. The overall goal of this project is to characterize the cellular and molecular biology of monocyte recruitment to the lung in response to tobacco smoke. Mainstream tobacco smoke presents a significant health hazard to smokers, yet environmental tobacco smoke (ETS), or "secondhand smoke" can also have an impact on the health of nonsmokers. To determine if ETS may induce a similar inflammatory response, we investigated the effect of ETS on expression of monocyte chemokines by human airway epithelium. ETS was simulated by extracts collected from aged and diluted sidestream cigarette smoke and immediately added to human airway epithelial cell cultures. Following a 16 hour exposure to ETS extract, conditioned medium (CM) was collected and RNA was extracted from cell cultures for analysis of chemokine expression. mRNA for both RANTES and MIP-1β increased in response to ETS extract, whereas MCP-1 mRNA levels did not appear to change significantly. To address chemokine protein levels in CM, monocyte chemotaxis was utilized as a functional assay. Migration of monocytes to CM from ETS extract-exposed cultures was enhanced in a dose-dependent manner, as compared with CM from unexposed cultures. Treatment of monocytes with pertussis toxin significantly inhibited migration to CM from ETS extract-exposed cultures, suggesting that chemokines play a role in this process. Blocking antibodies against RANTES, MIP-1β, and MCP-1 had a moderate inhibitory effect on monocyte migration. Blocking antibodies against MCP-1 appeared to have the most potent effect on monocyte migration, suggesting that MCP-1 protein levels were enhanced following ETS exposure. Finally, exposure of airway epithelial cells to acrolein and acetaldehyde (volatile components of ETS) resulted in a dose-dependent production of factors that promote monocyte migration. Cumulatively, these data indicate that exposure of airway epithelium to ETS results in upregulation of monocyte chemokine mRNA expression, but MCP-1 appears to have the most significant role in monocyte chemotaxis. These preliminary findings suggest a putative mechanism by which persistent exposure to ETS can result in inflammation within airways. A clear understanding of the basic mechanisms by which monocytes traffic within the lung is imperative for the development of therapeutic modalities which specifically target the immunologic response to tobacco smoke.

G05

Modulation of neutrophil activation by cigarette smoke

Nguyen, Hung; Reznick, Abraham; Cross, Carroll and **van der Vliet, Albert** - *University of California, Davis*

Cigarette smoking is a risk factor for respiratory and cardiovascular diseases, but its contributing actions are incompletely understood. Cigarette smokers commonly have higher levels of inflammatory cells and the multiple oxidants and nitrogen oxides present within cigarette smoke (CS) are expected to interact with inflammatory components to generate novel oxidizing intermediates that induce and/or aggravate lung injury. Conversely, modulation of inflammatory processes by CS might underlie enhanced susceptibility to certain respiratory tract infections.

In past research, we have discovered that the neutrophil enzyme myeloperoxidase (MPO) is capable of converting the nitric oxide-metabolite nitrite (NO_2^-) to reactive nitrogen intermediates that can induce characteristic biomolecular modifications such as nitrotyrosine. As CS is abundant in nitric oxide and aqueous CS extracts contain high levels of NO_2^-, we anticipated that exposure to CS, in combination with the presence of activated inflammatory/immune cells that contain MPO or related peroxidases, could result in the formation of such nitrating intermediates, thereby resulting in unique modifications of critical lung constituents. To address this issue, we exposed isolated human neutrophils to gas-phase CS and analyzed the production of nitrating and chlorinating oxidants following neutrophil stimulation, by trapping with the aromatic substrate hydroxyphenylacetate (HPA). We observed that CS is able to induce oxidation and nitration of HPA, but not chlorination. In contrast, stimulation of neutrophils with the phorbol ester PMA caused oxidation and chlorination of this substrate, but no nitration. Stimulation of neutrophils in the presence of CS caused dramatically less oxidation and chlorination of HPA, indicating that one or more components within CS prevented activation of the NADPH oxidase or inhibited the activity of myeloperoxidase (MPO). The latter possibility was excluded based on unchanged peroxidase activity or unaltered MPO-dependent oxidation and chlorination of HPA upon addition of H_2O_2. The apparent inactivation of NADPH oxidase was found to coincide with depletion of cellular GSH, suggesting modification of critical cysteine residues in one or more of the NADPH oxidase components. In support of this notion, GSH depletion and inhibition of NADPH oxidase activation was also observed after neutrophil exposure to the a,b-unsaturated aldehyde acrolein, at levels comparable to those present in CS. Additionally, protein adducts of acrolein or similar aldehydes could be detected after neutrophil exposure to either CS or acrolein. One of the cytosolic NADPH oxidase components, p47phox, contains cysteine residues that appear to be critical for assembly and activation of NADPH oxidase. Immunoprecipitation experiments demonstrated the presence of carbonyl adducts within p47phox, suggesting addition of acrolein or related aldehydes to this protein, upon exposure of neutrophils to CS or to acrolein. We propose that such covalent modification of p47phox may be (in part) responsible for the observed inhibition of respiratory burst activation.

In summary, our results demonstrate that CS components are capable of negatively affecting the respiratory burst in neutrophils, which is critical in host defense against many lung pathogens. Despite increased local numbers of neutrophils, diminished neutrophil activity in smokers or in subjects exposed to environmental tobacco smoke may affect inflammatory/infectious conditions, and thereby contribute to CS-related disease.

G06

Synergistic effect of environmental tobacco smoke on ozone-induced lung injury

Yu, Mang; Witschi, Hanspeter and **Pinkerton. Kent E.** *University of California, Davis*

To determine the effects of environmental tobacco smoke (ETS) on the sensitivity of the lung to ozone, strain A/J and B6C3F1 mice were exposed to (1) filtered air, (2) ETS, (3) ozone, or (4) ETS followed by ozone (n = 6 / group). Exposure to ETS was at a total particulate concentration of 30 mg/m^3, 6 hours/day for 3 days; exposure to ozone was at 0.5ppm for 24 hours. Bromodeoxyuridine (BrdU) immunolabeling was used to identify proliferating cells. The percentage of BrdU labeled cells within the centriacinar regions of the lungs was found to be significantly elevated in both strains of mice following exposure to ozone (2 to 2.5-fold above control) and further augmented in mice exposed to ETS followed by ozone (3 to 3.5-fold above control). These differences in cell labeling between ozone alone and ETS plus ozone clearly demonstrated that prior exposure to ETS significantly increased cell turnover rates following exposure to ozone. In contrast, exposure only to ETS did not change BrdU labeling compared with filtered air control mice. We conclude that ETS exposure enhances the sensitivity of animals to ozone-induced injury in a synergistic manner.

G07

Effects of environmental tobacco smoke (ETS) on perinatal lung development in non-human primates

Pinkerton, Kent E.; Joad, Jesse P.; Yu, Mang; Chang, Aimin; Zhou, Yamei; Tarantal, Alice F.; Buckpitt, Alan R.
University of California, Davis

Exposure to ETS during early childhood development may have important consequences on the respiratory system. These effects include enhanced respiratory infection, decrements in lung function, and increased incidence and severity of asthma. The current work examined the effects of exposure to ETS during perinatal development in non-human primates. An exposure system was designed and constructed for the exposure of timed pregnant monkeys to aged and diluted sidestream smoke as a surrogate to ETS. Monkeys were exposed to ETS with the following characteristics: total suspended particulate (TSP); 1.02 ± 0.11 mg/m^3, nicotine; 190 ± 75µg/m^3 and carbon monoxide; 5.3 ± 0.7 ppm for six hours a day, five days a week, from 100 days gestational age (DGA) to 2.5 months postnatal age (PNA). Sonographic measurement of the fetuses (growth, hemodynamics) prior to exposure and at 120 and 150 DGA revealed normal growth and development of all animals assessed (n = 4 controls and 4 ETS). The time of birth was in the expected range of 165 ± 10 DGA with the exception of one dam exposed to ETS, which prematurely delivered at 139 DGA. At 2.5 months PNA, infant monkeys were deeply anesthetized and the lungs were prepared for examination. Exposure to ETS did not alter the number of cells recovered from lavage of a single lung lobe, but was associated with a significant shift to increase the proportion of monocytes and pre-macrophages (16.4 ± 3.2% in ETS vs. 7.0 ± 3.1% in controls). The volume of each lobe of the left lung was measured and demonstrated no significant differences between the control and ETS groups. Striking increases in micrsosomal cytochrome P4501A1 isozyme activity, measured with ethoxyresorufin as the substrate, were observed in all airway subcompartments of animals exposed to ETS compared to filtered air controls. In the parenchyma, a 175-fold increase in activity was measured in microsomes prepared from ETS (1618 pmol/min/mg) compared to filtered air controls (9 pmol/min/mg). The proximal, mid-level and respiratory airways were also induced, but to a lesser degree than observed in the parenchyma; the increases varied from 17- to 90-fold over control. In comparison, our previous experiments with rats exposed to an identical concentration of ETS demonstrated an increase in P4501A1 activity of only 2-fold above control value. We conclude that exposure to ETS during perinatal lung development under conditions relevant to actual human exposures (1) does not affect fetal growth, (2) is associated with a significant shift in cells of the immune system to a more immature form and (3) significantly elevates pulmonary metabolic function for cytochrome P4501A1 in a site-specific manner in the neonate. These findings suggest that ETS exposure during the perinatal period of lung development significantly affects the lungs of non-human primate infants.

G08

Aged and diluted sidestream cigarette smoke (ADSS) inhibits bronchiolar epithelial repair in the adult mouse

Van Winkle, Laura S; Evans, Michael J; Brown, Collete D; Ocampo, Eugenio T; Gunderson, Andrew D; Shimizu, Judy A; Pinkerton Kent E and Plopper, Charles G
University of California, Davis

Environmental tobacco smoke is a substantial public health hazard because of the large number of people who are involuntarily exposed. Aged and diluted sidestream smoke (ADSS) is a chemically characterized smoke that is used in experiments as a surrogate for environmental tobacco smoke. Our hypothesis is that exposure of nonsmokers to environmental tobacco smoke compromises the ability of the lung to repair when challenged with a second injurious agent. We are using an injury and repair model that we have defined (naphthalene in the mouse) to determine the effect of both prior and continuous ADSS exposure on injury and repair responses in the lung. The bronchiolar injury/repair response to naphthalene (NA) in mice includes a well-defined epithelial injury that repairs within 14-21 days. In our current study, we tested whether prior exposure and/or co-exposure to ADSS would alter the repair response of the airway epithelium. Adult mice were exposed to either filtered air (FA) or ADSS (TS) before and/or after injection with either vehicle (corn oil) or NA and the lungs were examined 1 and 14 days after injury:

FA+CO+FA: filtered air 5 days, corn oil, continue in filtered air
TS+CO+TS: smoke 5 days, corn oil, continue in smoke
FA+NA+FA: filtered air 5 days, 200 mg/kg NA, continue in filtered air
TS+NA+TS: smoke 5 days, 200 mg/kg NA, continue in smoke
TS+NA+FA: smoke 5 days, 200 mg/kg NA, continue in filtered air

Sections of lung, trachea and lobar bronchus were examined using high resolution histopathology to establish the extent of injury. The extent of Clara cell injury did not vary between groups. The major changes were confined to terminal bronchioles. Injured terminal bronchioles were examined using high resolution histopathology/morphometry, immunohistochemistry for Clara cell differentiation markers and scanning electron microscopy. FA+CO+FA and TS+CO+TS airway epithelium was similar to untreated controls at all timepoints. As expected, the epithelium in the NA treated groups contained many injured Clara cells and squamated ciliated cells within terminal bronchioles during the acute injury phase. Repair in the FA+NA+FA group (including redifferentiation of epithelial cells and restoration of epithelial thickness) was nearly complete 14 days after injury. In contrast, the TS+NA+TS and TS+NA+FA groups contained only minimal epithelial repair in terminal bronchioles; at 14 days many terminal bronchioles contained abundant squamated undifferentiated epithelium. We conclude that even ADSS exposure that occurs only prior to injury delays repair. Future directions of this work include evaluating ADSS exposure during lung development on the injury and repair responses. The potential impact of our current findings are that exposure to environmental tobacco smoke that precedes exposure to a second toxic agent (many of which are present in our daily environment as other forms of air pollution) results in inhibition of bronchiolar epithelial repair. This has implications for human exposures to environmental tobacco smoke that occur along with exposures to other pollutants.

G09

How does smoke activate mucin transcription in lung cells?

Basbaum, Carol B.
University of California, San Francisco

This project is aimed at understanding how tobacco smoke stimulates mucin production in lung epithelial cells. We observed that by incubating cell culture fluid with cigarette smoke, some of the smoke particles become dissolved in the fluid. When we apply the smoke-containing fluid to lung cells, the cells respond by producing mucin, the major component of mucus. This is an experimental model of the phenomenon in which human lung cells make mucus in response to smoke leading to sputum production and lung congestion.

We found that the stimulation of mucin by smoke is controlled at the level of mucin DNA transcription to RNA. We cloned the regulatory region of the mucin gene to permit identification of the precise fragments needed for the smoke induction of mucin. We have identified the fragment to within 200 nucleotides and are working to further specify the control site within these 200 nucleotides. Simultaneously we are trying to determine how the nucleotides control transcription. Inevitably the mechanism will require specific DNA-binding proteins. We have tentatively identified these proteins as jun-fos and NFkB.

At the same time we are analyzing the smoke-mucin response at the gene (DNA) level, we are trying to determine the series of biochemical events triggered by smoke at the lung cell surface. These events, largely mediated by enzymes, carry the information to the DNA and proteins in the lung cell nucleus to stimulate mucin production. We know that one of the earliest molecular events following smoke exposure is activation of the cell surface receptor called EGFR. This receptor has been studied previously for its role in causing cell growth in response to a hormone called EGF. We and others find that this receptor can play roles in the cell other than mediating the hormone response. One of our most pressing questions is how does activation of the receptor differ when stimulated by hormone vs by smoke. Because of the role of the receptor in cell growth, the discovery that it is activated by smoke may also lead to better understanding of how smoke causes lung cancer. In related experiments, we are fractionating smoke to identify the molecular component responsible for stimulating mucin production in lung cells.

G10

Increased gene expression in tobacco smoke exposed airway epithelial cell cultures

Yoneda, Ken Y.; Chmiel, Kenneth; Wu, Reen
University of California, Davis

The objective of this project is to identify and characterize genes in respiratory epithelial cells of the trachea and conducting airways of the lung that are affected by tobacco smoke. The initial phase of this study involves the identification of such genes in cell cultures utilizing the technology known as a cDNA micro-array system. The second phase will involve the characterization of these genes and the final phase will involve the study of these genes in the airways of tobacco smoke exposed humans. The identification and characterization of genes that are responsive to tobacco smoke in conducting airway epithelium will increase our understanding of the early effects of tobacco smoke. It is hoped that this information will lead to a better understanding and treatment of tobacco related lung diseases.

We utilized a calorimetric cDNA micro-array system to screen for changes in tobacco smoke induced gene expression. 9,600 genes were obtained from partially sequenced EST clones and were spotted on a nylon membrane approximately two by three centimeters in size. Primary conducting airway epithelial cell cultures were exposed to whole tobacco smoke for 12 hours. Smoke and non smoke exposed control cells were harvested for RNA. We then purified mRNA from total RNA and used reverse transcriptase labeling to tag the mRNA samples with two different color reaction labels, biotin and digoxigenin. The mRNA levels of ten clones were increased above a predetermined threshold by the tobacco smoke exposure. One clone is a partially sequenced but uncharacterized gene, three were replicates of DNAJ protein (heat-shock protein 40) and six were known genes. None of the genes have been previously described to be induced by tobacco smoke in airway epithelium.

The response of a group of heat-shock protein (HSP) genes to tobacco smoke was then investigated. Northern blot of a whole tobacco smoke exposure time course confirmed that the HSP-40, HSP-70 and HSP-90 alpha gene message increased optimally at 10-15 hours. Confirmation of the remaining genes is pending.

Using a cDNA micro-array system we screened 9,600 gene clones. We believe we have identified a group of genes whose expression is increased by tobacco smoke exposure of conducting airway epithelium. In addition to the HSP genes, we plan to confirm the remaining genes, characterize the nature of their regulation and confirm an increase in the expression of their corresponding proteins. We hope to confirm the increase in gene expression in vivo using a rat model and on endobronchial biopsies obtained from human volunteers. Also of interest are the genes whose expression was decreased by tobacco smoke exposure. Completion of the above experiments should add greatly to our understanding of genes that are affected in tobacco related lung diseases.

G11

Induction of CYP1A1 by environmental tobacco smoke occurs in the airway epithelium in the postnatal period and increases with duration of exposure

Royce, Fred H., Daftari, Pratibha; Nguyen, Vinh; Peake, Janice L.; Pinkerton, Kent E. - *University of California at Davis*

Previously we reported that exposure of the developing rat lung to aged and diluted sidestream smoke (ADSS), a surrogate for environmental tobacco smoke, induced the cytochrome P450 monooxygenase CYP1A1 early in the postnatal period in rat lung. Substances contained in ADSS are known to induce CYP1A1 transcriptionally through binding of the aryl hydrolase receptor (AhR). We found that exposure of timed pregnant rats and their pups to ADSS (1 mg/m^3) induced CYP1A1 mRNA by over 100-fold at postnatal day (PND) 7 and with ongoing exposure CYP1A1 mRNA was similarly induced at PND 19, but exposure *in utero* did not induce CYP1A1 mRNA. Previously, it has been shown that CYP1A1 activity is not induced at PND 7 but is significantly induced (1.5-fold) at PND 14. To understand the difference between mRNA induction and CYP1A1 activity, immunostaining was performed. Only occasional cells were found to contain immunoreactive CYP1A1 at PND 7, while at PND 19 nearly one half of nonciliated respiratory epithelial cells were positive. To identify whether the low levels of CYP1A1 induction in the airway epithelium were related to an immature cellular response, we administered ß-naphthoflavone (ßNF), an AhR agonist known to induce CYP1A1. We injected timed pregnant rats with ßNF (80 mg/kg) 28 and 4 hours prior to examination for the presence of immunoreactive CYP1A1 at 15 and 21 days gestation and at PND 1. We also injected rats at days 1, 2 and 12 of life and sampled 24 hours later. In the prenatal period, we found that CYP1A1 was highly induced in the vascular endothelium and was also induced in the airway epithelium on gestational day 21. In the postnatal period, immunoreactive CYP1A1 was identified in airway epithelial cells and remained abundant in the vascular endothelium. Recognizing that CYP1A1 mRNA is inducible by ADSS at seven days and that immunostaining shows little protein, we concluded that induction of CYP1A1 in the airway epithelium by inhalation of ADSS may be related to the dose of AhR ligand delivered. The focus of the vascular endothelium before birth followed by increased expression in the airway epithelium around the time of birth may be important for predicting CYP1A1 mediated toxicity. It is known that over expression of CYP1A1 in the neonatal period is associated with pulmonary edema - a process mediated by the vascular endothelium. Over expression of CYP1A1 may also increase susceptibility of the lung to other oxidants. Preliminary evidence in our laboratory suggests that mice pre exposed to ADSS have increased sensitivity to ozone, as evidenced by increased bromodeoxyuridine uptake in the airway epithelium. We are currently testing whether sequential exposure of the rat lung to ADSS and ozone worsens injury and we will test the hypothesis that this injury is mediated through induction of cytochrome P450 monooxygenase. Understanding the effect of co exposure to ETS and other common air pollutants such as ozone is important for understanding the development of chronic pulmonary diseases such as asthma.

G12

The physical origins of low surface tensions

Zasadzinski, Joseph A.; Lee, Ka Yee; Lipp, Mike; von Nahmen, Anja; Ding, Junqi and Warriner, Heidi

University of California, Santa Barbara

The minimum surface tension and respreadability of a surfactant monolayer is limited by a two to three dimensional instability called collapse. Liquid-condensed or solid phase monolayers collapse via fracture followed by loss of material. Liquid-expanded monolayers collapse by solubilization into the subphase. Monolayers that retain a continuous LE phase network surrounding islands of LC or S phase collapse at lower surface tensions via a localized, large amplitude buckling. The buckled regions coexist with the flat monolayer, remain attached to the interface and are reversibly reincorporated into the monolayer upon expansion. Even minor smoking induced damage to components of surfactant can lead to significant changes in surfactant function.

The area available to the monolayer can be decreased by imposing an external surface pressure, π, which lowers the normal air-water surface tension. As the monolayer is compressed into the liquid-expanded (LE) phase, the hydrophobic parts of the molecules come into contact with each other and lift from the water surface, but remain largely disordered. Further compression leads to a first order transition to the "liquid-condensed" (LC) phase, marked by a plateau in the isotherm corresponding to LE and LC coexistence. In the LC phase, the molecules exhibit long range order, are less compressible and less fluid than in the LE phase. A kink in the isotherm at higher compressions marks the transformation to a better ordered "solid" (S) phase, in which the area per molecule corresponds to the packing of three dimensional crystals of the amphiphile. Above a critical temperature that depends on the subphase, many phospholipids exhibit only the LE phase; the better ordered LC and S phases are absent.

Eventually, the molecular area (or surface pressure) reaches a limiting value beyond which the monolayer cannot be compressed further. At these high surface pressures, a flat monolayer is similar mechanically to a plate under compression; as the pressure is increased past the limiting value, the plate can (1) fracture and break, (2) buckle, or (3) lose material (and hence interfacial area) depending on the elastic and solubility properties of the monolayer.. The surface pressure at collapse, π_C, determines the minimum surface tension for a given monolayer; the collapse mechanism determines the reversibility; i.e. what fraction of the monolayer remains at the interface and how well the monolayer respreads to cover the interface as π is decreased. In general, fluid LE monolayers, such as those formed by phospholipids above their critical temperatures, collapse at relatively low π_C via the ejection of material to the subphase. More ordered and rigid LC or S phase monolayers collapse at higher π_C, usually by fracturing, followed by loss of portions of the monolayer in the subphase or formation of multilayered crystalline aggregates at the air side of interface. Collapse via fracture or solubilization is irreversible; the collapsed phase material does not reincorporate into the monolayer as π is decreased.

A combination of isotherms, optical fluorescence microscopy, and atomic force microscopy (AFM) measurements show that monolayers with a continuous LE phase separating islands of LC or S phase at π_C can undergo a novel large amplitude buckling into the aqueous subphase. The folded regions coexist with the flat monolayer - further compression changes the fraction of material in the folds relative to the flat monolayer

at constant π_C, indicative of a first order phase transition. The morphology of a continuous network of fluid LE phase separating LC phase domains alters the elasticity of the monolayer, which allows the monolayer to bend rather than break. The folds remain attached to the monolayer at the interface and are reincorporated into the monolayer upon expansion with little loss of material. This buckling transition can be readily induced in a variety of single component and mixed phospholipid monolayers by the addition of the SP-B and SP-C proteins found in lung surfactant (LS). Each of the components of native lung surfactant act to produce the proper morphology and phase behavior necessary for reversible collapse and respreading. DPPC when mixed with PA, forms the solid phase islands, and the proteins and PG's form the fluid phase network. We have also found that Survanta, a clinically used extract of cow lung surfactant, does not have this phase behavior unless supplemented by additional proteins. This added protein makes the Survanta perform significantly better in laboratory animals.

G13

Deposition, binding, and intracellular processing of cationic lipoplex in the mouse lung

Uyechi, Lisa; Barron, Lee; **Szoka, Jr., Francis C.**
University of California, San Francisco

Intratracheal instillation (i.t.) and intravenous (i.v.) administration of cationic lipoplexes promote gene transfer in the lungs of mice. Our objectives are to characterize lipoplex delivery and to elucidate the gene transfer mechanisms of the cationic lipoplex.

1,2 Dioleoyl-3-trimethylammonium propane and cholesterol liposomes are formulated with DNA at a net positive charge. To visualize the distribution, intracellular processing and expression of lipoplex, we use fluorescent tags and reporter genes. Mice are dosed with 30 ug of DNA by either the i.t. or i.v. route, and at various time points the mice were sacrificed and the lungs fixed, in situ. The lungs were inflated with low melting point agarose, sectioned and examined by laser scanning confocal microscopy. Gene expression levels are also determined using a luciferase reporter protein, and distribution of lipoplex is determined with radioisotope labels on the lipoplex components.

I.T. administration results in regional deposition of fluorescently labeled lipoplex, primarily around the bronchioles but also in the alveolar sacs. The lipids have a prolonged persistence, up to 44 hours in the lung. Expression of a gene encoding a green fluorescent protein (GFP) arises within 24 hours, is sparsely located throughout the tissue, and suggests that both Type I and Type II pneumocytes are transfected. Simultaneous delivery of fluorescent oligonucleotide , within the same lipoplex as plasmid DNA, reveals that a much greater population of cells are accessible to fluorescent oligo than express the gene (green fluorescent protein).

In contrast, i.v. administration of lipoplex gives broader, although not completely homogenous, distribution, generally within the capillaries of the alveolus. Cells expressing GFP are sparsely distributed. Pretreatment with heparinase (i.v.) significantly reduces gene expression but has no effect on the distribution of radio-labeled lipoplex to the lung at early time points. These data confirm that heparin-like binding sites are involved, but suggest that intracellular processing is a critical step in cationic lipoplex gene transfer in the lung.

This work is supported by the California Statewide Tobacco Research and Development Program, UC Office of the President - Research and Development Program, the CF Foundation, and NIH DK46052.

G14

IGF-I administration prevents corticosteroid-induced diaphragm atrophy in emphysematous hamsters

Fournier, Mario and Lewis, Michael I.
Cedars-Sinai Medical Center, Los Angeles, CA.

Patients suffering from emphysema (EMP)/chronic obstructive pulmonary disease (COPD) have decreased respiratory muscle function due to an impaired mechanical efficiency of the diaphragm secondary to dynamic lung hyperinflation. Furthermore, COPD patients are often treated with corticosteroids (anti-inflammatory drug). A major side effect of corticosteroid treatment is skeletal muscle atrophy (i.e., muscle wasting) which results in further weakness of the respiratory muscles. The exact mechanisms by which muscle atrophy takes place are not completely known, but involve both a decrease in the rate of protein synthesis and an increase in the rate of protein degradation (proteolysis). The aim of this study was to evaluate whether insulin-like growth factor-I (IGF-I) could attenuate or prevent diaphragm fiber atrophy during corticosteroid administration to EMP hamsters.

Nine to 10 months after induction of EMP the animals were divided into 3 groups: 1) EMP only; 2) EMP + triamcinolone (dose: 0.4 mg/kg/day); and 3) EMP + triamcinolone + IGF-I (IGF-I dose: 600 µg/day by constant infusion). Drugs were provided over 4 weeks. Diaphragm fibers types were determined histochemically and fiber cross-sectional areas (i.e., fiber size) determined with a calibrated computer-based image analysis system. The intensity of IGF-I staining (immunoreactivity) within single fibers was measured by microdensitometry. Lung volumes of EMP were increased (180 to 200%) compared to controls. Despite similar initial body weights, those of EMP + triamcinolone progressively decreased (-15%) during the study, while those of EMP and EMP + triamcinolone + IGF-I remained stable. Food intake was reduced to the same extent in both triamcinolone groups compared to EMP. Hypoglycemia (i.e., low serum glucose) was not observed with IGF-I infusion. Diaphragm weight was reduced with triamcinolone, but preserved with IGF-I administration. Diaphragm fibers proportions were similar among the groups. The cross-sectional areas of types I, IIa and IIx fibers were reduced (20 to 31%) with triamcinolone administration. By contrast, the concomitant provision of IGF-I prevented atrophy of all fiber types. The levels of IGF-I immunoreactivity of single fibers were similar across the groups but tended to be lower in animals receiving IGF-I.

We conclude that: 1) IGF-I infusion prevented diaphragm fiber atrophy induced by triamcinolone; and 2) that this effect was mediated in large part by the hormonal (i.e., circulating) influences of the administered IGF-I and not by enhanced local intramuscular IGF-I expression, as the latter was found to be reduced. These results may have important clinical implications aimed at preserving respiratory muscle strength in patients with COPD receiving large doses of corticosteroids during acute exacerbations or those requiring maintenance steroid therapy.

G15

Therapies for muscle dysfunction in obstructive lung disease

Casaburi, Richard
Harbor-UCLA Research and Education Institute

This project aims to improve the effectiveness of medical treatment for people with lung disease produced by cigarette smoking. Chronic obstructive pulmonary disease (COPD) is a disorder affecting approximately 14 million people in the United States. It is a disabling disorder and inability to exercise is usually the foremost problem. It is becoming clear that these patients suffer not only from poorly functioning lungs but from poorly functioning muscles as well. We are focusing on strategies that will improve the muscle's ability to tolerate exercise. Two specific strategies are being explored. First, in men the naturally occurring hormone, testosterone, is important in maintaining muscle mass. We have shown that men with COPD have low levels of this hormone. We are determining whether administering testosterone to COPD patients will increase muscle mass (and exercise tolerance) as we have demonstrated in healthy men. Second, we have observed that COPD patients usually are very sedentary; we are determining whether a vigorous program of strength training will improve exercise tolerance and muscle function.

We are randomizing 48 men with COPD to one of four groups that are receiving: no exercise training and placebo, exercise training (for one hour a day, three times a week) and placebo, no exercise training and testosterone (100 mg per week), or both exercise training and testosterone. A number of outcome measures are being assessed before and after the 10 week study period: 1) regional lean and fat body mass by DEXA scan and also by deuterium water and sodium bromide dilution, 2) muscle strength by the 1-repetition maximum method, force-EMG analysis and functional performance of several tasks, 3) exercise endurance by cardiopulmonary exercise testing, 4) quality of life by general and disease-specific measures, 5) respiratory muscle strength, 6) pulmonary function testing, 7) cardiac mass and contractility at rest and during exercise by electron beam computed tomography, 8) sleep quality and, 9) a full battery of hormonal measurements. Further, a second TRDRP-funded project is allowing us to determine changes in muscle structure and biochemistry from muscle samples obtained by needle biopsy. To insure safety, blood counts, plasma lipids, PSA levels and periodic prostate examinations are being monitored by an unblinded investigator.

To date, 17 subjects have completed this protocol and 10 subjects are currently enrolled. The study interventions and the procedures necessary to evaluate the outcome measures have been well tolerated. As the study remains blinded, no results are available at this time.

Programs of pulmonary rehabilitation are already in place to help patients with COPD. This study should be directly applicable to these programs and help to decrease the suffering of patients with this smoking-related disease.

G16

Comparison of staple LVRS with a combined staple/laser LVRS technique in an animal emphysema model

Brenner, Matthew; Ha, HP; O'Connor, S; Serna, D; Powell, L; Burney, T; Frayne, J; Jalal, R; Chen, J; Jones, B; DiMartino, L; Kennedy, T
University of California, Irvine

Staple lung volume reduction surgical procedures (LVRS) have been shown to improve lung function in emphysema patients to a greater degree than laser reduction procedures. However, studies have not addressed whether adjunctive laser treatment in addition to staple LVRS improves response over staple procedures alone. The purpose of this study was to compare staple LVRS outcomes to combined staple/laser LVRS in an animal emphysema model.

For this study, emphysema was induced in seventy-seven New Zealand white rabbits by aerosolizing porcine elastase through an endotracheal tube. Elastase causes emphysema by destroying alveolar walls, which serves as gas exchange surfaces, provide lung elastic recoil necessary for exhalation, and add structural tractional support for the bronchioles. After sustaining emphysema, the animals were divided into two surgery groups: staple and combined staple with laser treatment. All animals underwent pulmonary function testing (lung volumes, static respiratory system compliance, lung diffusing capacity, and forced expiratory airway flow) before induction of emphysema, pre-operatively (after emphysema development), and post-operatively (following surgical treatment).

Increased lung compliance, is a primary negative physiological result of emphysema due to loss of alveolar walls. Results show that the combined staple/laser group exhibited 35% greater improvements in lung compliance following surgery compared with the staple only group ($p<0.05$). Furthermore, the combined staple/laser technique appeared to have a larger impact at lower lung resection volumes than at higher resection volumes. However, there was higher morbidity and mortality in the combined staple/laser treated animals. Future investigations will focus on determining the role of adjunctive laser treatment of specific subgroups of patients undergoing staple LVRS. The effects of LVRS in conjunction with laser treatment could possibly lead to improved symptomatic and objective outcomes in emphysema patients. Economically, improvement in LVRS techniques may decrease high costs of ongoing medical treatment and care of emphysema patients.

CTRDRP #6RT-0158

H01

Fertility, smoking and early mammalian development

Melkonian G.; Riveles K.; Martins-Green M.; and **Talbot, P.**
University of California, Riverside

We have previously reported that the structure of the oviduct and the abundance of the vasculature in corpora lutea of females are affected by inhalation of mainstream and sidestream cigarette smoke (Reprod Toxicol 9:513, 1995). Subsequent studies have shown that smoke solutions alter ciliary beat frequency and oocyte pickup rate of oviducts and also disrupt angiogenesis when assayed using chick chorioallantoic membranes (CAMs). In this study, we have isolated the components in smoke solutions that produce these effects. Initially 12 polar and nonpolar Bond Elut solid phase extraction cartridges were screened for their ability to retain components that produce toxic effects on the oviduct. Based on data from this initial screen, a subsequent screen was done using combinations of two cartridges. The first cartridge was expected to retain non-toxic components but allow toxicants to pass through while the second cartridge was expected to retain toxicants. The toxic components were then eluted off the second cartridges with methanol and fractionated using HPLC. Because effects on the CAM were especially pronounced when sidestream gas phase smoke was tested, our initial isolations have been done from solutions containing the water soluble components in sidestream gas phase smoke. The eluant from one pair of cartridges (SCX and PH) when subjected to HPLC was found to produce 23 peaks, most with substantial yield, and was selected for further study. Eight of the 23 peaks collected by HPLC were blown down with nitrogen, resuspended to 200 ul in culture medium, and tested in the CAM assay. Unfractionated sidestream gas smoke solution, isolated fractions of sidestream gas smoke solution, or control culture medium were placed on day 5 CAMs which were then sealed with tape and incubated overnight. On day 6, CAMs were fixed for 20 hours in 3% glutaraldehyde then evaluated intact using video microscopy and evaluated in stained thick sections using brightfield microscopy. Three of eight peaks (#8, 9, and 10) showed no effect on CAM development and were not studied further. Peaks 3, 6, 7, and 11 significantly retarded the overall growth of the CAM. Peaks 3, 6, and 11 inhibited the formation of the capillary plexus, and peaks 3, 4, 7, and 11 appeared to stimulate production of fibroblasts in the CAM mesoderm. The structural alterations produced by peak 11 were identical to the alterations observed with unfractionated sidestream gas phase smoke solutions. The other peaks either produced isolated effects, for example peak #4 increased the number of fibroblasts, or produced effects that were more subtle than peak 11. The components in the peaks showing a significant effect on CAM development are currently being determined using mass spectrometry. Similar studies are being done to determine which peaks affect ciliary beat frequency, oocyte pick-up rate, and muscle contraction in the oviduct. These data show that multiple components in sidestream gas phase smoke solutions adversely effect development of the capillary plexus in CAMs and that more than one process in CAM development is affected by sidestream gas phase smoke. These data help explain our earlier observations on the corpora lutea of smokers and show that blood vessel formation is adversely affected by specific components in cigarette smoke. Our results are important in the context of female reproduction, development, and wound healing which normally require extensive angiogenesis.

H02

Maternal DNA repair of tobacco-induced sperm lesions

Marchetti, Francesco; **Wyrobek, Andrew J.**
Lawrence Livermore National Laboratory

Evidence from human studies indicates that father's cigarette smoking is associated with reduced semen quality, as well as DNA damage in sperm, spontaneous abortion, malformation, neonatal death, and childhood cancer. Little is known of the mechanisms by which smoking may induce DNA damage in sperm and why only some pregnancies are affected. The purpose of this research is to expand our understanding of the molecular and genetic factors that can increase the risk for abnormal pregnancies in couples where the father smokes.

Two important biological aspects of sperm biology that are relevant to this research are: 1) the last two weeks of spermatogenesis have diminished ability to repair DNA damage; and 2) all the enzymes needed for repairing and duplicating sperm DNA after fertilization are provided by the egg. We hypothesized that: (a) paternal exposure to tobacco smoke induces DNA lesions in sperm that accumulate during the repair-deficient period of spermatogenesis and are converted into chromosomal aberrations after fertilization; and (b) deficiencies in DNA repair genes of the female partner can increase the frequency of paternally transmitted chromosomal defects. We have selected the mouse as the model species because the genetics of the mouse are well understood and there is a high degree of similarity between mouse and human DNA repair genes. Also, we have developed a new Fluorescence In Situ Hybridization (FISH) procedure in mouse zygotes that allows the identification of specific types of chromosomal aberrations that are associated with various abnormal reproductive outcomes (embryo death, heritable chromosomal defects, malformations) such as those observed in human epidemiological surveys.

Research is being conducted to determine whether DNA lesions accumulate in sperm after chronic exposure to diepoxibutane (DEB), a component of tobacco smoke. Male mice were treated with daily doses of DEB and than mated with untreated females. Chronic exposure to DEB for 3 weeks had a detrimental effect on semen quality as indicated by a significant reduction in the number of eggs that were fertilized. We also conducted experiments to examine specific maternal DNA repair functions in the processing of DNA sperm damage. p53 (a cell cycle control gene), XPA (a gene involved in the recognition of DNA adducts) and XRCC1 (a gene involved in the repair of damaged DNA bases) were investigated in unfertilized and fertilized eggs using PCR-based methods. Preliminary results suggest that not all DNA repair genes are active during the early phase of embryo development.

This project represents an important step for understanding the molecular mechanisms linking paternal exposure to tobacco smoke, induction of genetic lesions in sperm, DNA repair capacity of the fertilized egg, and the risk of paternally transmitted chromosomal abnormalities. Work performed under the auspices of the U.S. DOE by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48.

H03

Role of passive smoke on sperm, ICSI and IVF

Klonoff-Cohen, Hillary
University of California, San Diego

An estimated 2.5 million couples are infertile in the United States, and approximately 50% of these are attributed to infertility in the male. Most infertility specialists currently recommend intracytoplasmic sperm injection (ICSI) and/or in vitro fertilization (IVF) for male infertility problems. ICSI represents the most powerful procedure available for treating male infertility; this technique has revolutionized the field, albeit, anecdotal studies report an increased rate of birth defects.

Men who smoke have abnormally high concentrations of DNA adducts in semen, which could result in an increased risk of birth defects and cancer in their offspring. Furthermore, smoking is more likely to have a detrimental effect on men with already-impaired spermatogenesis rather than on healthy men; sperm nuclei of infertile men may be more susceptible to oxidative stress than the nuclei of fertile men. To date, the toxic role of passive tobacco smoke on sperm indices or assisted reproductive technologies has never been investigated. This prospective study will examine the role of passive and direct tobacco smoke on routine (e.g., count, motility, morphology) and advanced sperm indices (zona- free hamster penetration test, computer-imaging) and reproductive endpoints (e.g., implantation, fertilization, implantation, embryo transfer, and pregnancy related outcomes including birth defects, spontaneous abortion, stillbirths, low birth weight, and prematurity) of 500 Caucasian, African-American, Asian, and Hispanic infertile men undergoing ICSI at 6 sites in southern California. All men will complete questionnaires and provide 2 urine samples (first clinic visit and during the procedure) and a sperm sample for hormone and cotinine analyses.

Currently, progress towards achievement of the specific aim consists of the following: institutional review board approval has been completed at each of the 6 sites; the protocol for patient eligibility and recruitment has been finalized and implemented by the 6 reproductive endocrinologists; the physicians and nurses have been trained; the 5 questionnaires, medical records form, and laboratory document have been developed and pilot-tested; the data base has been created; the laboratory courier system has been implemented; and the seminal fluid cotinine assay has been tested. In May 1999, patient recruitment, data abstraction, sample collection and analysis, and data entry began, and it is expected that data collection will be completed by the middle of 2001.

The biological, biomedical and health impact of second hand smoke (and direct smoke) on the male reproductive system is of paramount importance, and has public health implications for all male children, adolescents, and couples of reproductive age who are planning families.

H04

Maternal cigarette smoking harms human placental development

Genbacev, Olga and **Fisher, Susan**
University of California, San Francisco

Smoking by pregnant women significantly increases the risk of serious pregnancy complications such as abortion and failed fetal growth. Little is known about how smoking produces these effects. The placenta plays a pivotal role in pregnancy by connecting the embryo/fetus to the uterus. Pregnancy complications are often caused by placental defects. Accordingly, we hypothesize that maternal smoking harms the placenta, and our goal is to understand how this happens.

Our experimental strategy is to use a laboratory (tissue culture) model to predict the effects of smoking on the placenta. Then we determine if the same effects are seen in placental tissues collected from mothers who smoke during pregnancy. Previous results obtained from our laboratory model suggest that smoking alters the ability of the placenta s specialized cells, termed cytotrophoblasts, to proliferate and differentiate into anchoring villi. These defects are important because anchoring villi attach the embryo/fetus to the uterus. If this connection is not formed properly, the embryo/fetus is deprived of maternal blood and nutrients. Possible consequences are abortion and abnormalities in fetal growth.

Here we report findings from the analysis of placental samples collected from 75 women who smoked during the first 12 weeks of pregnancy (experimental) and from 12 women who did not (control). The experimental samples (25 per group) were obtained from the following women: group A smoked 20 or more cigarettes per day for 2-5 years; group B smoked 10-20 cigarettes per day for 2-5 years and group C smoked 5-10 cigarettes per day for 2-5 years.

First, we compared the number of anchoring villi in experimental and control placentas. In group A , the number per tissue section was 3.2 ± 0.8 times lower than in the control samples. The numbers in groups B and C were not different from the controls. Second, we used specialized reagents (antibodies; Ki67 and PCNA) to assess the number of proliferating cells in tissue sections. We also used a special reaction (TUNEL) to detect cell death (apoptosis). The results showed that the percentage of proliferating cytotrophoblasts in samples from groups A and B was approximately 25-35% of that observed in control samples; group C was unaffected. The TUNEL reaction showed that the percentage of apoptotic cytotrophoblasts in group A was 2 times higher than in controls. No differences from controls were observed in groups B and C. Together, these results suggest that heavy smoking during early pregnancy impairs cytotrophoblast proliferation and differentiation, confirming the results from our laboratory model. In the future we will determine the exact points at which these critical processes fail. These findings also offer a possible explanation namely, impaired placental development for the high rate of pregnancy complications among women who smoke. We envision that the results can be used to explain to pregnant women, in simple terms, why smoking harms the embryo/fetus. If women understand the risks and stop cigarette use, our work could lead to a reduction in smoking-induced pregnancy complications and their associated costs.

H05

Transport and disposition of nicotine in the human placenta

Lacayo, Catherine; Pak, Winnie; and **Kroetz, Deanna L.**
University of California at San Francisco

Smoking in pregnant women is associated with numerous health risks to the fetus, including fetal respiratory problems, perinatal death, and low birth weight. The placenta provides nourishment to the fetus and rids the fetal circulation of metabolic end products. In addition, the placenta also serves as a barrier against exposure to high levels of xenobiotics. The goal of these studies is to characterize the role of specific proteins involved in the elimination and transport of drugs, the cytochrome P450 enzymes and the transporters P-glycoprotein (P-gp) and multidrug resistance protein (MRP), in controlling fetal exposure to nicotine.

The mechanism by which nicotine is broken down (metabolized) and eliminated from the body involves a class of proteins that can act upon many drugs and environmental toxins, the cytochrome P450 enzymes (CYPs). The liver is the major organ in the body where most drug and toxin metabolism occurs. The placenta has similar functions although this is less well characterized. We proposed that CYPs in the human placenta can metabolize nicotine into less toxic compounds which will be eliminated from the body. To test this hypothesis we have measured nicotine metabolism in human placenta samples. Nicotine is metabolized by human placental samples to a less toxic compound. Inhibition studies suggest that this metabolism is catalyzed almost exclusively by a single CYP enzyme, CYP2A6. Analysis of CYP2A6 protein levels indicate that it is expressed in all placental samples examined. We hypothesized that nicotine metabolism would be increased in placentas from smoking women compared to those from nonsmoking women. Such an increase would be a direct consequence of the increased nicotine levels in the mother and the protective response of the placenta to protect the fetus from this harmful chemical. However, our analysis to date suggests that nicotine metabolism and CYP2A6 protein levels are similar in placentas from smokers and nonsmokers. A second mechanism which would determine fetal exposure to nicotine is the transport of this compound across placental membranes. The multidrug resistance proteins P-gp and MRP play an important role in the transport of numerous drugs into and out of cells and we proposed that these transporters might also be important mediators of nicotine transport into and/or out of the placenta. Our preliminary studies have identified both of these proteins in human placental samples. Future studies will look directly at whether either of these transporters can move nicotine across a membrane and whether the levels of these proteins in the human placenta is influenced by the smoking status of the mother.

A detailed characterization of how the placenta determines exposure of the fetus to nicotine is essential for understanding the consequences of fetal exposure to cigarette smoke. Increased knowledge of the biochemical processes which determine fetal nicotine exposure might explain the widespread variation in the severity of nicotine-induced toxicity in the children of women who smoke during pregnancy.

H06

Carbon monoxide and the brain growth spurt

Edmond, J., Korsak. R., Ma, A., Bassilian, S., Stockard, J. and Lee, W-N.,P. - *University of California, Los Angeles*

Carbon monoxide (CO) is one of the two most common of the more than 3000 active compounds in tobacco smoke. It has been reported that one cigarette can yield from 0.003 to 0.05 grams CO and one cigar up to 0.8 grams. Both nonsmokers and active smokers can be exposed to significant amounts of CO in the air they breathe depending on their environment. Inhaled CO binds to hemoglobin in the red cell and decreases their oxygen carrying capacity. CO inhibits respiration within cells. Physiologically, mammalian cells have the capacity to produce CO naturally in small amounts, and this gas is now considered to be a distinctive neuroregulator.

Our studies are designed to examine the consequences of exposure to CO on a critical period of growth in the brain. In this period brain undergoes profound cell division and the development of discrete structures with their intricate multi-cellular organization, particularly that of the nerve insulation membrane known as myelin. We have shown that brain is required to produce, exclusively, all the major lipids it needs to meet this dynamic growth. Developing brain can be vulnerable if lipid synthesis is arrested because of energy deficits. To test our hypothesis that exposure to CO causes growth deficiencies in brain, and to be able to study the vulnerable developing brain, we are examining exposure to CO using our animal model, the gastrostomy-reared rat pup. The important age for the brain growth spurt and onset of myelin formation takes place after birth when the pups are fed milk. The gastrostomy-reared pups are fed rat milk substitutes under carefully controlled conditions of diet and exposure to CO. In our initial experiments the rat pups received chronic exposure to low concentrations of 100 parts per million CO in air, (100 ppm). At this exposure we observe consistently, an increase in carboxyhemoglobin (6 %) in blood, indicative of a mild exposure to CO. In our first experiments on function (hippocampus) we find no difference from controls in their learning performance in the Morris Water Maze. “However, slower brainstem conduction time, as measured by the auditory brainstem response, at postnatal day 22 (1.22 milliseconds for controls compared to 1.74 milliseconds for the CO exposed animals, and significantly different, $p > 0001$) suggests a negative effect on myelination of the auditory brainstem pathway. Our biochemical studies show a difference in the ability of the pups to make lipids. In exposed rat pups we find a reduced ability to make the common prominent fatty acid, palmitate; where the reduction is more obvious in cerebellum (by 20%) than it is in whole brain minus cerebellum (by 10%). The capacity to produce palmitate is reduced in liver and kidney, but not in lung.

This year, Hassid et al., (J. Pediatrics 135: p34, 1999) report that prenatal exposure to cigarette smoking is associated with a decrease in arousal in children. They conclude that newborns and infants born to smoking mothers had higher arousal thresholds to auditory challenges than those born to nonsmoking mothers. Our work is relevant to understanding the consequences to neurodevelopment from exposure to cigarette smoke. Although our work is preliminary in scope, the results to date indicate deleterious consequences of a mild exposure to carbon monoxide.

H07

Factors that promote maintenance of postpartum abstinence among women who smoked prior to pregnancy

Quinn, Virginia P.
Kaiser Permanente Southern California

Pregnancy offers a powerful motivation to stop smoking. Approximately half of U.S. women smoking prior to pregnancy quit and remain abstinent through delivery. Unfortunately, significant health gains in pregnancy are eroded in the postpartum period as the majority of quitters return to smoking by six months after delivery. This project is investigating ways to help women maintain cessation after their babies are born.

To guide development of a counseling intervention, we conducted a formative evaluation among new prenatal patients who reported to have stopped smoking for their pregnancy. A 45-minute, semi-structured baseline telephone survey was administered to 53 quitters. Participants reflected the diverse population served by Kaiser Permanente Southern California—two-thirds were white, 15% were Latino, 13% were African-American, and 6% were Asian. Subjects were on average 25 years of age, had completed 13.4 years of education, and half were working full or part-time outside of the home. The survey also obtained information on pregnancy- and smoking-history variables and previously identified correlates of postpartum maintenance. In response to open-ended questioning, 98% of respondents reported it was concern for the health of their unborn baby that motivated them to quit and only 10% also gave their own health as a reason for quitting. Yet, more than half (59%) of the gains women reported as a consequence of not smoking concerned their own improved physical or emotional health status.

Six months after delivery we administered a follow-up interview to 38 (72%) of the women originally interviewed. Multivariate analysis found that prenatal quitters who stopped smoking all at once (i.e., went "cold turkey") compared with those who gradually cut down were much more likely to maintain abstinence. Maintainers also were more likely to be full-time homemakers after delivery and women who during pregnancy were very confident in their ability to stay off cigarettes after their baby was born. In contrast, women who were concerned about their postpartum weight or who had smokers in their social network were at greater risk of returning to smoking.

Findings with implications for intervention include: 1) the differential effects of factors measured during and after pregnancy suggest assessment/intervention is needed before and after delivery; 2) while prenatal cessation is extrinsically motivated by the health of the baby, after quitting most women report intrinsic benefits for their own health and well-being; 3) most women who relapsed after a previous delivery relapsed again and for the same reason that precipitated the previous relapse; and 4) most women intended to quit for good and during pregnancy reported few temptations to smoke. This may explain subjects' low level of engagement in the cognitive and behavioral processes of change which are associated with maintained abstinence.

Results of the formative evaluation were incorporated in a telephone-based postpartum relapse prevention counseling program using motivational interviewing. A randomized trial of the intervention is currently underway. Effective postpartum relapse prevention would make a significant contribution to the health of young women, their newborn infants, and other family members.

I01

Implementation of California AB13: Smoking ban in bars

Bero, Lisa A.; Montini, Theresa
University of California at San Francisco

In January 1995 California AB13, prohibiting smoking in enclosed workplaces, took effect. On January 1, 1998 the smoking ban was extended to protect workers in bars. We are studying the process of implementation and enforcement of the smoking ban in bars by determining the variability in compliance among bar employers; exploring enforcement at the local level; and describing efforts by public organizations to support or undermine the implementation of this labor code. We are conducting in-depth interviews with three sets of respondents: a stratified (by county) random sample of bar owners (target N=123); enforcement officials from each county (target N=58); and activists for or against the smoking ban who were mentioned in the media (target N=30). Transcriptions of the interviews are content analyzed and coded in an ongoing, iterative process to capture recurrent concepts and categories.

To date we have interviewed approximately 41 bar owners, 17 enforcement officials, and 10 activists. Our preliminary analysis indicates that bar owners are most concerned with issues of equity–that all businesses in their area are subject to the same enforcement. Certain conditions facilitate compliance: if other competing bars in the area are smoke-free; if enforcers are active; if the premises is under surveillance for violations that challenge the liquor license (*e.g.*, adult club); if the bar's clientele is predominantly non-smoking (*e.g.*, outdoor sports enthusiasts); if there is an outdoor area where patrons can smoke without leaving the premises; and if bar owners support the labor code and acted to assure staff compliance. Some bar owners engaged in modified compliance, *i.e.*, letting patrons smoke after 10 p.m., during special events, or in a designated area. If other bars in the area allowed smoking, the probability of sustaining compliance was low.

Enforcement officials indicated that, in general, enforcement efforts were slow to be initiated, and typically, over time, a sequence of agencies was delegated enforcement. Enforcement was compromised by local government failing to support enforcement efforts, and organized resistance, such as bar owner phone trees alerting violators of spot-checks. Various enforcement strategies were developed in response to unfolding events. For example, in some areas district court judges ruled that bar owners must adhere to the letter of the law, opening loopholes for them to skirt the spirit of the law. If the option of citing bar owners was incapacitated, local enforcers cited patrons.

To date, only activists who support the smoking ban in bars have agreed to be interviewed. In general, they have worked to coordinate the efforts of various groups, to generate positive publicity for the law, to educate bar owners, and to encourage enforcement.

Compliance with AB 13 (subsequently Labor Code 6404.5) can be broadly conceptualized as a policy aimed at the prevention and cessation of tobacco use among Californians, and specifically conceptualized as a policy to protect workers from involuntary exposure to the harmful effects of ETS. Reduction in exposure to ETS and the consequent reduction in lung cancer and heart disease will only be realized if the law is successfully implemented.

I02

Selling the cigar trend

Bero, L. A.; Malone, R. E., Wenger, L. A., George, A.L., Gruskin, E. - *University of California, San Francisco*

This study aims to better understand the roles of media in shaping the cigar smoking trend, and extends work being conducted as part of a National Cancer Institute study. The recent dramatic increase in cigar use in the United States represents an issue of urgent concern for those working to prevent and decrease tobacco use and the human suffering attributable to tobacco-related diseases. Like cigarette smokers, cigar smokers are at increased risk for a number of diseases, including cancers of the throat and lung. Cigar consumption jumped in 1994 for the first time in twenty years, and has continued to rise. Media and tobacco industry sources often attribute the cigar boom to "internal" factors such as personal resentment of health recommendations, but we theorize that two "external" factors—media coverage and tobacco industry activity— may also have played key roles in creating and sustaining this trend.

The project has the following specific aims:

- Track and compare trends from 1997 through 1999 in cigar advertising, cigar sales, and number of cigar-related newspaper and magazine articles
- Analyze the content of all articles discussing cigars in the five largest circulation US newspapers and all US magazines published from 1997 through 1999
- Analyze the content of all articles discussing cigars in the ten largest-circulation daily California newspapers during the 1986-1999 period
- Analyze cigar advertising and cigar images in a sample of US magazines from 1997 though 1999.
- Examine the consumer and public health community response to media coverage of cigars by analyzing the content, extent, placement, and source of all cigar-related letters-to-the-editor published in California and national newspapers from 1987 through 1999.

To date, we have continued to track trends in cigar advertising, sales, and print coverage; completed a content analysis of newspaper and magazine articles dating from 1987 through 1997; retrieved a sample of cigar advertisements and images drawn from ten high-circulation general magazines and two cigar-focused magazines, identified recurrent themes and conducted preliminary analyses; and retrieved and analyzed a sample of 114 letters-to-the-editor published from 1987 through 1997. At this meeting, we will compare coverage in California and other U.S. newspapers; present findings from our image analyses, and discuss how advertising and images have contributed to the cigar trend.

The mass media's ability to influence social behavior, social change, and the policy agenda itself render them essential elements to consider in developing tobacco policy. The *Healthy People 2000* recommendations on tobacco call for the elimination or severe restriction of "all forms of tobacco product advertising and promotion to which youth younger than age 18 are likely to be exposed." However, cigars remain largely unstudied and unregulated. It is critically important to understand the kinds of pro-cigar messages consumers are receiving from media sources if effective strategies are to be developed to counter them with health information, policy changes and/or counter-advertising.

I03

Smoke-free homes: Factors which support a 'no smoking' rule at home

Burns, David M; Shanks, Thomas G; Gower, Kathryn; Anderson, Christy
University of California, San Diego

Smoke-free homes are an important element in reducing environmental exposure to tobacco smoke. We wanted to test whether work-site smoking bans were associated with smoke-free homes.

Methods: Data from the smoking supplement of the 1992/3 and 1995/6 CPS surveys were analyzed to determine what factors are associated with homes where smoking is not permitted. Analysis was by multivariable logistic regression with all covariates simultaneously in the model. Households are divided into homes where all adults are non-smoking, all adults smoke, and households where there is a mix of smoking and non-smoking adults. Other study factors include working where smoking is not permitted. The average percent of workers in the state of residence who report a full work-site smoking ban was included as an indicator of the social norms in the state with respect to smoking. The presence of children under the age of 13 years was also included. Covariates studied include gender, age, education, income, and ethnicity.

Results: For all smoking status groups, individuals who work where there is a complete smoking ban are significantly more likely to have a smoke-free home compared to individuals who work where smoking is permitted, even when the proportion of smoke-free work sites in the state is included in the analysis. Individuals living in states with a higher proportion of smoke-free work sites are also more likely to have smoke-free homes. Homes with a child under age 13 are more likely to have a smoking ban at home, which is more strongly significant for homes with one or more smoking adults. Hispanic and Asian ethnic groups are two to three times more likely to have smoke-free homes.

Conclusion: Broadening the coverage and effectiveness of work site smoke bans will have the desirable effect of increasing the likelihood of no-smoking rules at home. The indirect effect is reduced exposure of family members to environmental tobacco smoke and less modeling of smoking behavior to children and non-smoking family members.

I04

Tobacco Control Archives: Joe Camel Campaign – Mangini Collection

Butter, Karen A.
University of California, San Francisco

In 1991, R. J. Reynolds (RJR) was publicly charged in the Journal of the American Medical Association (JAMA) with targeting children through its Joe Camel advertising campaign. Rather than terminate the campaign, RJR claimed that it had no idea whether Joe Camel appealed to children. In December 1991, Janet C. Mangini, a San Francisco family law attorney, brought suit to end this campaign, becoming the first person to challenge the tobacco industry for targeting minors with its advertising. The City and County of San Francisco, together with the Cities of Los Angeles and San Jose and ten additional California counties joined the suit, and in December 1997, RJR avoided the litigation process by agreeing to terminate the Joe Camel Campaign. As a result of the settlement, thousands of pages of RJR secret and confidential documents were released to the public.

Building upon the successful model used to digitize the Brown and Williamson Collection, the UCSF Library/Center for Knowledge Management Tobacco Control Archives (TCA) indexed and digitized the approximately 80,000 pages of Joe Camel documents. The documents were released on the TCA website in July 1999, and are searchable and browsable using standardized thesausus terms. Since the release, internet users have accessed the site over 100,000 times.

I05

The role of cigarette prices in different stages of smoking uptake

Emery, Sherry L. and White, Martha M.
UCSD - Cancer Center, University of California, San Diego

Specific Aims: This research project examines adolescent smokers' sensitivity to the price of cigarettes across levels of smoking experience.

Background: There is some agreement in the economics literature that, by raising the price of cigarettes, state and federal excise taxes can play an important role in deterring adolescent smoking. However, this issue is far from resolved. In fact, this result is particularly puzzling in light of the evidence that the majority of adolescent smokers do not buy their cigarettes-and therefore do not experience price. Earlier research has shown that adolescents in the first stages of smoking initiation get their cigarettes from friends, while those whose habit has developed to the level of smoking $\geq$ 1 cigarette/day purchase their cigarettes. Thus, it seems plausible that adolescents' price responsiveness may vary depending on their smoking experience. Previous studies have not had adequate measures of smoking experience to test this hypothesis.

Methods: We used data from the 1993 Teen Attitudes and Practices Surveys (TAPS), a cross-sectional component of a national longitudinal telephone survey that interviewed adolescents whose parents or guardians had responded to the 1989 National Health Interview Survey (NHIS). The 1993 wave included 7,960 adolescents who were interviewed in 1989, plus an additional sample of 4,992 adolescents between the ages of 10 and 15 years old who were not part of the original sample (87% response rate). We used the population sampling unit (PSU) identifiers from the NHIS to identify the adolescents' state of residence, and assigned appropriate cigarette prices and state-level tobacco control policy variables. We conducted separate logistic regression analyses to examine the probability of being a) a current smoker, b) an experimenter, and c) an established smoker (100+ lifetime cigarettes) as a function of cigarette price, tobacco control policies, and relevant demographic and psychosocial variables.

Results: Current smoking status was highly sensitive to the price of cigarettes, with a participation price elasticity of -0.9 (p=0.03). Current smoking status was also significantly influenced by various tobacco control policy variables, such as workplace and school smoking restrictions, tax earmarking, and a tobacco control index, as well as the standard demographic and psycho-social variables. As expected, experimentation was not sensitive cigarette prices (p=0.11) or tobacco control policy variables, but was influenced in similar ways as current smoking status by the other independent variables. Established smoking status was even more sensitive to cigarette prices than current smoking, with a participation price elasticity of -2.04 (p=0.001); the tobacco control, demographic, and psycho-social variables had similar effects in this model.

Conclusions: Our models of current smoking status replicate the results of other researchers. This finding is important because the survey and sampling methodologies used to obtain our data were different from those used in the majority of other econometric analyses of adolescent smoking. Thus, we conclude that higher excise taxes may not deter experimentation, but may deter progression to further smoking. Alternative policy measures may be needed to reach those adolescent smokers who are not deterred by higher cigarette prices. It will be important to validate these results with longitudinal data.

I06

Smokefree bars: Analysis of the response from Long Beach

Lee, Julia A., **Friis, Robert H.**
California State University, Long Beach

A 1995 California Law (AB 13) banned smoking in the workplace, with an exemption for stand-alone bars and also for restaurant bars that had separate ventilation. That exemption was removed, and smoking was banned in all bars beginning January 1, 1998. A prediction of readiness for this policy change in Long Beach was favorable, given the active Tobacco Education Program within the City Health Department and the city's history of voter-approved, tobacco-control ordinances. A 1991 local ordinance banned smoking in enclosed workplaces, including 2/3 of restaurants, but with a complete exemption for bars. Then in April 1994, Long Beach voters approved a stronger ordinance that prohibited smoking in all restaurants and bars, with a partial exemption for 1/3 of both restaurant-bars with their own ventilation and for stand-alone bars. Those partial exemptions for bars in Long Beach were superseded by the 1998 statewide ban on smoking in bars.

We have studied the response to this new tobacco policy in Long Beach using a variety of measures and information sources. Results during the first 18 months of the law's enactment reflected lower satisfaction from stand-alone bars than from restaurant bars. While complaints received by the Health Department about all bars declined over time in 1998, complaints about stand-alone bars continued to outnumber complaints about restaurant bars. Quarterly analysis of taxable sales data for eating and drinking establishments in Long Beach showed a steadily increasing share of total sales throughout the 1990's for eating establishments that served beer and wine. In contrast, numbers of licenses from the Department of Alcoholic Beverage Control (ABC) for stand-alone bars fell throughout the 1990's. (Numbers of all ABC licenses in Long Beach decreased between 1990 and 1995 but then leveled off from 1996 to 1999.) Less satisfaction from stand-alone bars was voiced directly by both bar managers/owners and workers via individual interviews. Personnel from stand-alone bars were less likely to acknowledge positive effects of the law such as less odor on hair and clothes, less eye irritation, and decreased danger of fire. Interestingly, the prevalence of smokers was exceptionally high among both owners/managers and workers from stand-alone bars, especially when compared to 18% smokers from a random sample of Long Beach residents interviewed by telephone. Approval of the smokefree-bars law was reported among only 17% of workers from stand-alone bars, compared to 57% of workers from restaurant-bars and 66% of the residents. Approval of the law among Long Beach residents was associated with a belief that second-hand smoke is unhealthful and the belief that laws and ordinances are effective in reducing smoking in public places.

We will continue to measure changes in the response to the smokefree-bar law in Long Beach through ongoing tracking with these various measures. We will be looking for changes over time in approval and appreciation for the law among bar owners/managers and workers as well as among residents. Results from this study should suggest strategies to increase public acceptance of tobacco control policies, which can reduce exposure to second-hand tobacco smoke.

I07

Modeling the impact of tobacco policy in the evaluation of public health objectives

Ake, Christopher F.; Emery, Sherry L.; Navarro, Ana M.; **Kaplan, Robert M.**
University of California, San Diego

Background: Several states have adopted tobacco excise tax increases, with the dual goals of raising revenue and discouraging smoking. We estimate the impact of the taxes on population health with particular focus on the Latino population of California.

Method: Using a range of cigarette price elasticity estimates specific to Latino smokers, we simulated changes in Latino smoking that would result from a range of actual and proposed cigarette excise tax increases. We associated these projected changes in Latino smoking with changes in health status, using a method that combines morbidity and mortality into a common index of health status: Quality Adjusted Life Years (QALYs). Five sets of estimates were made: 1) Smoking prevalence as a function of price, 2) effects of tobacco use on mortality, 3) Effects of tobacco use on morbidity, 4) integration of prevalence, mortality and morbidity into a model of QALYs, and 5) development of confidence intervals around these estimates using Monte Carlo Simulation. One model estimated the impact of the taxes one year after initiation, and two additional scenarios estimated the impact 75 years into the future (by which time all Latinos in the population would have been subject as adolescents to the tax increase's deterrent effect on smoking).

Results: Under the base case of a $0.50/pack tax, and a Latino smoking prevalence elasticity of –1.0, we estimated that over 2000 Latino QALYs would be saved annually, starting in 1999. Greater benefits will accrue each year, until a steady state relative to population size is reached 75 years after the program is initiated. Higher taxes would produce even greater health benefits.

Conclusions: Tobacco excise taxes may be unique among policy options, in that they may enhance population health status, while increasing tax revenues. Although these revenue increases represent a transfer of income from smokers to non-smokers, we argue that Latino smokers are more responsive to changes in the prices of cigarettes than other smokers, and therefore are not disproportionately shouldering the burden of such a tax, but rather stand to benefit greatly from the health impact.

I08

Simulated effect of tobacco tax variation on Latino health in California

Ake, Christopher F.; **Kaplan, Robert M.;** Navarro, Ana M.; Emery, Sherry L.
University of California, San Diego

Background: Several states have adopted tobacco excise tax increases, with the dual goals of raising revenue and discouraging smoking. We estimate the impact of the taxes on population health with particular focus on the Latino population of California.

Method: Using a range of cigarette price elasticity estimates specific to Latino smokers, we simulated changes in Latino smoking that would result from a range of actual and proposed cigarette excise tax increases. We associated these projected changes in Latino smoking with changes in health status, using a method that combines morbidity and mortality into a common index of health status: Quality Adjusted Life Years (QALYs). Five sets of estimates were made: 1) Smoking prevalence as a function of price, 2) effects of tobacco use on mortality, 3) Effects of tobacco use on morbidity, 4) integration of prevalence, mortality and morbidity into a model of QALYs, and 5) development of confidence intervals around these estimates using Monte Carlo Simulation. One model estimated the impact of the taxes one year after initiation, and two additional scenarios estimated the impact 75 years into the future (by which time all Latinos in the population would have been subject as adolescents to the tax increase's deterrent effect on smoking).

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I09

Reducing minors' access to tobacco: Project CHALK

Landrine. Hope; Klonoff, Elizabeth; Reina-Patton, Astrid
Public Health Foundation and California State University, San Bernardino

Efforts to reduce youth access to tobacco have focused on educating the merchants who sell cigarettes to children despite the laws banning such sales. These interventions have had little or no effect in the short run, and no long term effect on sales. We designed a new intervention to reduce minors' access to tobacco. The intervention focuses—not on the merchant, but—on the community surrounding stores that sell. In a prior study, we found that if an adult customer objected to selling cigarettes to youth, the sale was not made. Hence, in this new intervention, we encourage the community to object to sales of tobacco to youth that they observe.

The design is a multiple baseline design entailing 72 stores. The stores were randomly assigned to three intervention Wave groups (24 stores in each Wave). The intervention consisted of sending community interveners (residents of the neighborhood surrounding the stores) into the neighborhood to distribute flyers daily to adult residents, and to hang posters in stores. The flyers encourage the community to speak up and object when they observe a sale of tobacco to a child. The posters encourage them to do the same, and to think of all youth as their own (e.g., "It takes village to stop youth smoking." "When you see someone selling cigarettes to a child, speak up and object. It could be your kid next time.").

First, Baseline data on minors' rate of access to tobacco was acquired for all 72 store via youth attempts to purchase cigarettes. Next, the intervention was conducted in the Wave 1 (24) stores only and then data on youth access was again collected in all 72 stores after the intervention. The intervention then was conducted in the Wave 2 (24) stores only, and data on youth access collected in all 72 stores after the intervention. Results revealed no decrease (from Baseline) in cigarette sales to youth in the Wave 1 or the Wave 2 stores. The intervention had no effect. The community remained indifferent to youth access to tobacco. Their attitude was, "it's not my child, it's none of my business". At present, the intervention is being conducted in the Wave 3 stores.

I10

Early effects of a tobacco policy prevention trial

Pentz, Mary Ann
University of Southern California

Despite state-wide zero tolerance tobacco policies in schools, adolescents continue to smoke, including on or near school grounds. The tobacco program and policy trial (TOPP) is a prevention trial aimed at improving school tobacco policy effectiveness through changing hypothesized mediators of policy, including actual and perceived enforcement, policy awareness, support, perceived consequences of violation, and norms. Middle schools were randomly assigned to a multi-component policy intervention (3 session policy curriculum for students, school policy review training and process for school staff, awareness training for parents and the parent teacher organization, and policy review interviews with principals), or a control (policy as usual) condition (N=19 schools, 2400 entering 7th grade students).. Data included student surveys, principal interviews, and school records. This study focuses on pre- to six-month post-test data collected on students. Early results show significant effects of the intervention on monthly smoking, policy support, and perceived use norms. Results suggest that the intervention is affecting tobacco use directly, and indirectly through changing policy mediators.

I11

Modeling the relationship between tobacco policy and tobacco use

Pentz, Mary Ann
University of Southern California

The school tobacco program and policy trial (TOPP) is a randomized trial to evaluate the effects of a multi-component tobacco policy intervention on adolescent tobacco use (N=19 schools randomly assigned to program or control; N=2400 7th grade students). As a first step, the relationships between existing tobacco policy (perceived enforcement), predictors of policy effectiveness (perceived consequences of violating school policy, perceived tobacco use norms, policy awareness, and policy support), and tobacco use were modeled on pre-test data using structural equation modeling. Both confirmatory factor analysis (measurement) and structural models produced a good fit with the data (CFI's ranging from .964-.991). As expected, low perceived enforcement was related significantly to tobacco use. Enforcement was positively related to awareness, support, and perceived negative consequences, and negatively related to perceived norms for use. In addition, perceived negative consequences and policy support were negatively related to tobacco use, while policy awareness and perceived use norms were positively related to tobacco use. The constructs evaluated in this study served as the basis for developing the tobacco policy intervention. Results suggest that changing perceived consequences, norms, enforcement, policy awareness, and policy support can have a preventive effect on adolescent tobacco use.

I12

Cost-effectiveness of school-based anti-tobacco education – results from the Tobacco Policy Model

Chen, Laurie L., **Tengs, Tammy O.**, Osgood, Nathaniel D.
Health Priorities Research Group, University of California, Irvine

The Tobacco Policy Model is a computer simulation model capable of predicting the expected short- and long-term public health and economic consequences of any change in tobacco use in California or the United States. In this abstract we describe our work to date analyzing the cost-effectiveness of school-based anti-tobacco education.

The model is designed as a Markovian system dynamics computer simulation model. We obtained secondary data on smoking prevalence and behavior from large national data sets such as the YRBSS, CPS, NHIS, NHANES and BRFSS. Mortality rates and population demographics were derived primarily from US Census projections. We obtained estimates of the direct medical costs of smoking from Hodgson et al (1992) and quality of life from the Quality of Well Being Scale (Kaplan, personal communication). We selected Project Towards No Tobacco (TNT) as the prototype education program based on its documented effectiveness. TNT consists of a 10-day social influences-based curriculum employing multiple teaching modalities in the 7th grade plus two "booster" lessons in the 8th grade. Sussman et al (1995) have reported a >50% reduction in smoking uptake rates in TNT students relative to controls. To estimate the total cost of delivering this educational intervention annually in California and the US, we estimated teacher time, salaries, and the cost of classroom materials. We then used the model to calculate the total net costs and public health outcomes expected with enhanced education efforts nationwide, simulated over timeframes ranging from five to 100 years. Public health outcomes were captured with quality-adjusted life-years (QALYs), a standard measure that simultaneously captures improvements in survival and quality of life. Because some model parameters are uncertain, we simulated a variety of scenarios, varying our estimates of the program's long-term impact on uptake rates, the cost of teacher training, and the assumption of an inter-generational link between maternal smoking and the smoking behavior of adult offspring.

We found that under virtually all scenarios tested, the nationwide implementation of enhanced school-based education improves QALYs and saves money. Further, the program becomes increasingly cost-effective over time. For example, even under the conservative assumption that the program affects uptake rates only during the two years students receive it, delivering the program nationwide for 100 years would cost approximately $5 billion but would save $82 billion in avoided medical costs, resulting in a net savings of $77 billion.

We are now in the process of analyzing anti-tobacco education in California alone. We also will use the model to investigate the cost-effectiveness of other strategies such as anti-tobacco advertising, raising excise taxes, and enhanced cessation efforts in California and the US. We expect that this and future analyses from the Tobacco Policy Model will prove useful to public decision-makers interested in the wise investment of scarce resources.

I13

A longitudinal study of smoking transitions in youth

Gilpin, Elizabeth A. - *University of California, San Diego*

We propose a second follow-up in 1999 of young adults (18-23 years) who were first followed (funded by Robert Wood Johnson Foundation) in 1996 as 15- to 20-year-olds, and first interviewed (baseline) in 1993 as 12- to 17-year olds as part of the California Tobacco Surveys. The main goal of the proposed research is to improve our understanding of what might help prevent people from becoming addicted smokers. Although daily or regular smoking is very unusual before age 15, most eventual regular smokers had their first cigarette before then. Since many adolescents who experiment do not become addicted smokers, it is important to identify what might inhibit or interrupt the smoking uptake process in its later phases, which last into the young adult period.

We hypothesize that cigarette price and smoking restrictions in the workplace and at home might impede the smoking uptake process. These barriers may prevent experimenters from becoming addicted, delay the development of a significant level of cigarette consumption, or even encourage early smoking cessation. In California an additional $0.50/pack excise tax became effective January 1, 1999, and it appears that the tobacco industry will raise the price of cigarettes another $0.45/pack to pay for the recent settlement. This unprecedented rise in cigarette prices after a period of price stagnation provides a unique experimental context; we can compare transition rates in similarly aged cohorts of adolescents who did and did not experience the steep increase in the price of cigarettes. Additionally, as the adolescents from the earlier surveys enter the workforce, they will likely encounter situations when they cannot smoke. Since 1996, nearly all indoor workplaces in California have been smokefree. We will compare transition rates, and cigarette consumption, among those who work in smokefree workplaces, those who attend college and those who work primarily outdoors or who are unemployed. The analyses will adjust for socioeconomic and other known predictors of smoking uptake. Similarly, it will be important to discover whether young adults who lived under home smoking restrictions in their parents' homes, adopt similar home smoking rules when the have a place of their own. We plan to determine whether the smoking behavior of young adults who live in smokefree homes is different from those without such restrictions, again adjusting for other factors, this time including the smoking status of others in the household.

Another goal is to quantify exposure to cigars among young adults, who have shown the greatest proportionate increase in cigar use in recent years. We expect that most cigar use is very occasional and therefore itself not a threat to health, but there is currently no population data that can prove this assertion. What may be of more concern is the role of cigars in fostering cigarette use. Young adult cigar smokers who never became addicted cigarette smokers may turn to cigarettes as a more convenient way to maintain a nicotine addiction they acquired from cigars. Also, former cigarette smokers who smoke cigars may relapse to cigarette smoking for the same reasons. Finally, we will investigate the influence of tobacco advertising and promotions on transition to established smoking, as an extension or our earlier work demonstrating its importance in the transition toward smoking from the earliest stage of being a confirmed never smoker.

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