



Annual Report 2002

from the University of California
to the California State Legislature
on the progress of the
Tobacco-Related Disease Research Program,
established and administered by the University of California
pursuant to Proposition 99, The Tobacco Tax and Health Protection Act of 1988,
Senate Bill 1613 of 1989 and reauthorized pursuant to Assembly Bill 3487 of 1996

Charles L. Gruder, Ph.D.
Executive Director – Special Research Programs
Acting Director – Tobacco-Related Disease Research Program

Michael V. Drake, M.D.
Vice President – Health Affairs

Tobacco-Related Disease Research Program
University of California, Office of the President
300 Lakeside Drive, 6th Floor
Oakland, CA 94612-3550

Phone: 510-987-9870
Fax: 510-835-4740
e-mail: trdrp@ucop.edu
<http://ww.trdrp.org>

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EXECUTIVE SUMMARY

Tobacco consumption in California is at an all-time low due to an effective, comprehensive state tobacco control program and increases in the price of tobacco products and state excise taxes on tobacco. Unfortunately, the need for continued research on tobacco-related disease and tobacco use has not declined with the drop in the number of cigarettes smoked. For decades to come, the state's taxpayers will be paying for the treatment of tobacco-related diseases that are now developing in California's current smokers and in adolescents who are starting to smoke. The Tobacco-Related Disease Research Program (TRDRP) identifies the areas in which there is the greatest need for research, funds research that will address these needs, and disseminates the results of the research to the medical, scientific, and tobacco control communities. TRDRP will continue to be a major contributor to prevention and treatment efforts within the state.

The decline in smoking has resulted in reduced revenue for the state's programs for tobacco-related disease research and tobacco control because they are funded by a portion of the revenue collected from the tobacco excise surtax imposed when California voters passed Proposition 99 in 1988. In 2002, TRDRP was unable to fund 25 research grant applications that had been rated "excellent" by expert peer reviewers because of limited funds. In light of TRDRP's inability to meet all research needs on tobacco-related disease, the program embarked on a strategic planning process to determine achievable goals over the next several years. This process included consultation with TRDRP stakeholders, including researchers, tobacco control experts, and voluntary health organizations. The program intends to put a plan into effect for the 2003 grant cycle.

In the 2002 funding cycle, TRDRP awarded 58 grants for a total of \$20,495,519 to 26 California institutions to address the following research priorities:

- Nicotine addiction and treatment
- Biological research on reducing illness and death from tobacco-related disease
- Health effects of exposure to secondhand smoke
- Epidemiology and surveillance of tobacco-related disease and tobacco use
- Economics and public policy of tobacco-related disease and tobacco use
- Social-behavioral factors in reducing tobacco use.

INTRODUCTION

Tobacco consumption in California is at an all-time low due to an effective, comprehensive state tobacco control program, and to increases in the price of tobacco products and state excise taxes on tobacco. Unfortunately, the need for continued research on tobacco-related disease and tobacco use has not declined with the drop in the number of cigarettes smoked. For decades to come, the state's taxpayers will be paying for the treatment of tobacco-related diseases that are now developing in California's current smokers and in adolescents who are starting to smoke. Research funded by the Tobacco-Related Disease Research Program (TRDRP) has contributed to the success of the state's tobacco control efforts by providing evidence on which to base decisions about programs and policies. TRDRP-funded research has also identified promising new directions for addressing the needs of Californians suffering from tobacco-related diseases. TRDRP identifies areas in which there is the greatest need for research, funds research that will address these needs, and disseminates the results of the research to the medical, scientific, and tobacco control communities. TRDRP will continue to be a major contributor to prevention and treatment efforts within the state.

The decline in smoking has resulted in reduced revenue for the state's programs for tobacco-related disease research and tobacco control because they are funded by a portion of the revenue collected from the tobacco excise surtax imposed when California voters passed Proposition 99 in 1988. In 2002, TRDRP was unable to fund 25 research grant applications that had been rated "excellent" by expert peer reviewers because of limited funds. In light of TRDRP's inability to meet all research needs on tobacco-related disease, the program embarked on a strategic planning process to determine achievable goals over the next several years. This process included consultation with TRDRP stakeholders, including researchers, tobacco control experts, and voluntary health organizations. The program intends to put a plan into effect for the 2003 grant cycle.

TOBACCO-RELATED DISEASE RESEARCH PROGRAM TO DATE

Mission and Goals

TRDRP's mission is to mitigate the impact of tobacco-related illness by funding research that is relevant to issues surrounding tobacco use and tobacco-related disease. The program's goals are consistent with the broader mission of Proposition 99, which is to reduce the human and economic costs of tobacco use by reducing the incidence, prevalence, morbidity, and mortality of tobacco-related disease in California.

TRDRP strives to meet the needs of the research community, the tobacco control community, the health care community, policy makers, and the public by:

- Funding high-quality and innovative research that contributes to the understanding of tobacco use and tobacco-related illnesses and serves California's diverse populations.
- Serving as an information resource for tobacco issues through dissemination of research findings and sponsorship of conferences and symposia.
- Funding research that will lead to more effective disease treatments for California's smokers and former smokers.

- Funding research that will lead to more effective strategies for tobacco use prevention and cessation.

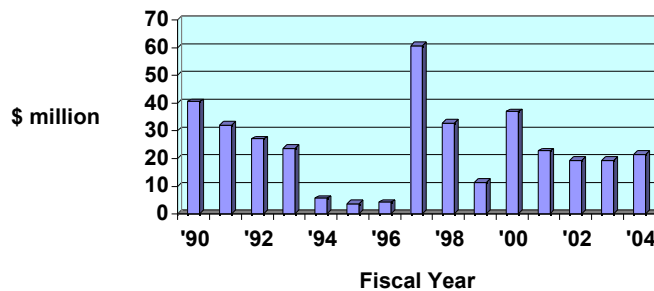
TRDRP strives to meet the needs of the research community by:

- Providing opportunities to researchers to conduct high quality and innovative research that advances tobacco-related science.
- Helping to build the research infrastructure in California that is critical for comprehensive tobacco-related disease research, in part by encouraging investigators to pursue careers in tobacco research through career development grant awards.

Funding History

The primary source of TRDRP funds is the revenue from the tobacco surtax that was established when California voters passed Proposition 99 in 1988. Proposition 99 specified that five percent of this tax revenue be deposited in the Research Account and that it be used for research on tobacco-related disease. Tobacco sales in California have steadily declined since the Proposition 99 tobacco excise surtax went into effect in 1989. Between 1990-91 and 2001-02, TRDRP resources declined from \$26.9 million to \$19.4 million annually. Appropriations from the Research Account to the University of California have shown large fluctuations – from \$40.3 million in 1990 to \$3.65 million in 1995 to \$60.4 million in 1997 (see Figure 1).

Figure 1: Appropriations to TRDRP from Proposition 99 Research Account, 1990-2003



In the past four years, TRDRP’s funding declined due to increased allocations in the state budget from the Research Account to the California Department of Health Service’s California Cancer Registry. In fiscal years 2001, 2002, and 2003, annual appropriations to the Registry were increased from approximately \$1.7 million to approximately \$5 million. The current allocation constitutes more than 40 percent of the Registry’s total budget, compared to 10 to 20 percent in prior years.

Award Funding to Date

Since its inception in 1989 through 2002, TRDRP has awarded investigators at 76 California institutions 962 grants totaling over \$307 million. The grants awarded constituted approximately 24 percent of the 3,989 applications received. The number and dollar amounts funded by subject area are displayed in Table 1.

Table 1: Award Totals by Subject Area

<i>Subject Area</i>	<i>Number</i>	<i>Amount</i>
Cancer	193	\$52,213,543
Cardiovascular Disease	124	\$39,797,170
Epidemiology	119	\$48,221,528
General Biomedical	100	\$27,470,828
Tobacco Use Interventions	105	\$46,485,674
Nicotine Dependence	102	\$30,841,491
Public Health/Policy	96	\$26,428,872
Pulmonary Disease	123	\$35,950,686
<i>Total</i>	<i>962</i>	<i>\$307,409,792</i>

Research Involving Women and Communities of Color

Of TRDRP's 359 active grants, 198 (55 percent) involve human subjects. Of these, 84 (43 percent) involve women subjects and 85 (43 percent) involve subjects from communities of color.

2002 FUNDING CYCLE

Research Priorities

The six research priorities for the 2002 award cycle were:

- Biobehavioral and Nicotine Addiction/Treatment Research
- Biological Research
- Effects of Exposure to Secondhand Smoke
- Epidemiological and Surveillance Research
- Policy/Economics Research
- Socio-Behavioral Research on Tobacco Use

2002 Awards

In 2002, TRDRP awarded 58 grants for approximately \$20.4 million to investigators at 26 California institutions. These awards, constituted 26 percent of the applications reviewed, a slight increase over 24 percent the previous year, largely due to the establishment of a budget cap for research project awards. It was possible for TRDRP to award more money than the amount appropriated in 2002 because unspent funds from grants awarded in previous years were returned and there were savings in administrative expenditures.

Table 2: 2002 Awards by Subject Areas

<i>Subject Area</i>	<i># Awards (%)</i>	<i>Dollars (%)</i>
Health Effects	32 (55)	11,745,369 (58)
Nicotine Dependence	9 (16)	2,541,497 (12)
Interventions/Policy	17 (29)	6,095,850 (30)
Total	58 (100)	20,382,716 (100)

Table 3: 2002 Awards by Type

<i>Award type</i>	<i>Percentage of applications funded</i>	<i># awards/ # applications</i>
Research Project	20.3	(29/143)
IDEA	22.2	(4/18)
Community-Academic	36.4	(4/11)
School-Academic	25.0	(1/4)
New Investigator	19.0	(4/21)
Postdoctoral Fellowship	55.6	(10/18)
Dissertation	66.7	(6/9)

Details of 2002 awards, including abstracts, can be found in TRDRP's Compendium of Awards, which can be accessed on the web at www.trdrp.org, or obtained from the program office (trdrp@ucop.edu or 510-987-9870).

Cornelius Hopper Diversity Award Supplements

The Cornelius Hopper Diversity Award Supplements (CHDAS) are designed to encourage TRDRP-funded principal investigators to mentor individuals who want to pursue careers in research on tobacco use and tobacco-related disease. Qualified applicants for the CHDAS are from groups that are underrepresented among researchers who investigate tobacco use or tobacco-related disease, and/or individuals who will work directly with underrepresented groups that are disproportionately impacted by tobacco use. Five currently funded investigators received supplements to their TRDRP grants for support of new project personnel (see Table 4).

Table 4: CHDAS Summary

CHDAS Trainee	Education	PI	Institution
Andrea Casillas	Post-baccalaureate	Randolph Hastings	Veterans Medical Research Foundation of San Diego
Spring Faller	Post-Masters	Richard Hofstetter	San Diego State University Foundation
Catherine Domier	Graduate	Edythe London	University of California, Los Angeles
Kim Jinsook	Graduate	William McCarthy	University of California, Los Angeles
Pina Maricela	Undergraduate	Ricardo Munoz	University of California, San Francisco

DISSEMINATION OF RESEARCH FINDINGS

In accordance with state statutes, TRDRP continues to disseminate the findings of funded research in a number of ways. The knowledge gained from TRDRP-funded studies is helping to improve the effectiveness of the tobacco control programs supported by the Proposition 99 Health Education Account that are administered by the California Department of Health Services and the California Department of Education. Results of research on tobacco-related disease are also enhancing scientists' understanding of biological mechanisms underlying the cause of tobacco-related disease and pointing the direction to technologies for the earlier detection and more effective treatment of lung disease, heart disease, and cancer.

Scientific Publications

TRDRP-funded investigators have continued to actively disseminate findings from their research in scholarly publications and at scientific conferences. In 2002, funded investigators reported publishing 183 articles in refereed scientific journals, including 149 that had appeared in print and 34 that were accepted for publication and were awaiting appearance in print. These papers appeared in such scientific publications as the *American Journal of Preventive Medicine*; *American Journal of Respiratory and Critical Care Medicine*; *Arteriosclerosis, Thrombosis, and Vascular Biology*; *Journal of Biological Chemistry*; *Journal of Clinical Investigation*; *Journal of Neurochemistry*; *Cancer Research*; *Neuroscience*; *Nicotine and Tobacco Research*; *Psychology of Addictive Behaviors*; and *Tobacco Control*.

Annual Investigator Meeting 2002

Scientific conferences are one of the most effective ways to disseminate recent research findings in a timely manner. TRDRP has held annual conferences at which its funded investigators report their latest findings. The program has expanded the traditional scientific conference model by including tobacco control professionals to give them the opportunity to learn about the latest findings directly from the scientists who are conducting the research.

More than 400 people attended TRDRP's seventh Annual Investigator Meeting (AIM 2002) in San Jose on December 4-5, 2002. The conference theme was "Women and Smoking." AIM 2002 began with the following six workshops:

- "Smoking and Breast Cancer: Is There a Connection?" co-sponsored by the American Cancer Society – California Division, the California Breast Cancer Research Program, and TRDRP.
- "Cardiovascular Disease in Women" sponsored by the American Heart Association Western States Affiliate.
- "Women and Smoking in California" sponsored by the California Department of Health Services' Tobacco Control Section.
- "California's Transdisciplinary Tobacco Use Research Centers" sponsored by the National Cancer Institute.
- "Cutting Edge Issues in Chronic Obstructive Pulmonary Disease (COPD)" sponsored by the American Lung Association of California.
- "Environmental Tobacco Smoke and Adverse Pregnancy Outcomes" co-sponsored by the California Department of Health Services' Tobacco Control Section and TRDRP.

The first day concluded with a Town Hall Meeting on "Harm Reduction," currently a controversial topic in tobacco use cessation. The primary question concerns the development and use of alternative products to cigarettes that carry less health risk.

The second day began with a plenary session on the conference theme, "Women and Smoking," and included stimulating talks by Rosemarie Henson, M.P.H., Director of the Centers for Disease Control and Prevention's Office on Smoking and Health; Cheryl Healton, Ph.D., President and CEO of the American Legacy Foundation; Virginia Ernster, Ph.D., Professor Emerita, University of California, San Francisco; Jill Siegfried, Ph.D., Professor and Co-Director of the Lung Cancer Program, University of Pittsburgh; and Sherri Watson Hyde, Executive Director, National African American Tobacco Prevention Network.

The keynote luncheon speaker, Dr. Diana Bontá, Director of the California Department of Health Services, reported on the California Tobacco Control Program's recent efforts to reduce smoking by women and girls.

Attendees had opportunities on both days to view the scientific posters and discuss research findings with TRDRP-funded investigators. They presented their latest findings on cancer, heart disease, lung disease, nicotine dependence, smoking prevention and cessation, public policy and economics, epidemiology, the health effects of smoking on women and infants, and secondhand smoke exposure.

Newsletter

In 2002, TRDRP published three issues of its newsletter, *Burning Issues*, which contain articles on critical research topics in tobacco-related disease and tobacco use and information about the program and notices of upcoming events. The newsletters are posted on TRDRP's website (www.trdrp.org) and approximately 3,000 hard copies were mailed to program stakeholders.

The 2002 newsletters included articles on "New Tobacco Products" that the tobacco industry is developing and promoting as safer alternatives to cigarettes; "Women and Chronic Obstructive Pulmonary Disease"; the potential implications for research of the new federal regulations developed in response to the Health Insurance Portability and Accountability Act of 1996; "Cigarette Smoking in Prisons"; and tobacco industry research funding.

Website

TRDRP has updated its website (www.trdrp.org) to enable visitors to search research grants, as well as to view all program publications and announcements. The new website should make access easier for potential applicants and other stakeholders.

Conferences

During 2002, TRDRP staff participated in a number of national conferences to stay informed about the latest scientific developments and to network with scientists who are potential applicants or peer reviewers. They made presentations, organized workshops and symposia, and hosted exhibits. TRDRP co-sponsored the inaugural Tobacco Control and Research Summit on smoking in the lesbian, gay and bisexual communities on November 18 in San Francisco. TRDRP staff made several presentations and TRDRP sponsored panel discussions at the National Conference on Tobacco or Health, November 19-21 in San Francisco. TRDRP scientific staff participated in the following annual meetings of professional organizations and specialized conferences: the American Thoracic Society, the Intercultural Cancer Council, the Society for Epidemiologic Research, the International Society for Heart Research, National Institutes of Health Conference on Racial/Ethnic Bias and Health, and New Molecular Approaches for Early Diagnosis and Treatment of Respiratory Diseases.

TRDRP COLLABORATIONS

TRDRP actively participated in tobacco control activities in California, other states, and nationally. The TRDRP Director chaired the executive committee of the Next Generation California Tobacco Control Alliance (NGA), funded primarily by a Smokeless States Grant from the Robert Wood Johnson Foundation. NGA's goal is to reduce tobacco use through statewide implementation of an evidence-based model smoking cessation program throughout California's managed care delivery system. The governor re-appointed the TRDRP Director to the state's Tobacco Education and Research Oversight Committee. TRDRP scientific staff organized presentations at the California Tobacco Control Program's Project Directors Meeting, and participated in a conference on Transcultural Perspectives on Tobacco Use and Health Promotion organized by the University of Southern California's Keck School of Medicine.

TRDRP collaborated with other organizations to share California's experiences and to learn from research efforts in other states and nationwide. TRDRP is an active member of the National Organization of Tobacco Use Research Funders. TRDRP continued to conduct the peer review of research grant applications for the Colorado Tobacco Research Program. The TRDRP Director chaired the Tobacco Control Research Panel for the Louisiana Health Excellence Fund.

2003 FUNDING CYCLE

Budget for 2002-2003

The 2002-03 appropriation to TRDRP from the Proposition 99 Research Account was \$19,434,000, the same amount as in 2001-02. For the second consecutive year, the California Cancer Registry received \$3,200,000 more from the Proposition 99 Research Account than it had received in previous years. As smoking rates continue to decrease over the next few years, all Proposition 99-funded programs, including TRDRP, will need to adapt to reduced revenues. However, the redirection of already diminishing funds is having a detrimental effect on the program's ability to achieve its goals. In light of TRDRP's inability to meet all research needs on tobacco-related disease, the program embarked on a strategic planning process to determine achievable goals over the next several years. This process included consultation with TRDRP stakeholders, including researchers, tobacco control experts, and voluntary health organizations. The program intends to put a plan into effect for the 2003-04 grant cycle.

Research Priorities

The Call for Applications and Application Packets for the 12th annual grant cycle in 2003 were issued in fall 2002. The application submission deadline was January 16, 2003, with funding for new awards slated to begin July 1, 2003.

One goal of research funded by TRDRP is to develop novel methods for tobacco use prevention and cessation. To this end, TRDRP solicited applications for tobacco-related research in biomedical science, neuroscience, social and behavioral science, epidemiology, public health, public policy, and economics. TRDRP invited investigations of the etiology, earlier detection, pathogenesis, diagnosis, and treatment of tobacco-related diseases, and the development, implementation, evaluation, and dissemination of existing or novel approaches to tobacco

control. TRDRP considers for funding all proposals that are relevant to the program's goals. The program encouraged applications to address the following topics identified as high priority for 2003:

- Research that addresses specific California needs with regard to tobacco control among understudied populations in all areas of investigation.
- Sex- and gender-based differences in tobacco interventions and in smoking-related diseases.
- Biomedical research that addresses the health needs of current and/or former smokers, especially translational and clinical studies to develop and validate novel treatments, prevention strategies, validation of early diagnostics, as well as development of more relevant animal models for tobacco-related diseases.
- Examination of the new generation of tobacco products.
- Research focused on prevention of tobacco use across the tobacco control continuum, especially studies of environmental factors that influence initiation of tobacco use, including the role of the tobacco industry.
- Research on tobacco and tobacco smoke constituents, in addition to nicotine, that contribute to tobacco-related diseases and to addiction as well as potentially reinforcing the actions of nicotine.
- Evaluative research that focuses on the impact of state and local public policies and programs, particularly among specific populations.
- Studies examining all aspects of secondhand smoke exposure.

Award Types

- **Research Project Awards** fund investigator-initiated research projects on all aspects of tobacco-related disease and tobacco use. These awards support research that is judged likely to yield valuable outcomes. The projects are judged to be feasible and likely to succeed because they employ sound scientific approaches and offer promising supporting data from preliminary studies.
- **Innovative Developmental and Exploratory Awards (IDEAs)** fund developmental or exploratory research that is not yet sufficiently mature to compete successfully for an individual research award. Although the proposed research might lack adequate pilot data or proven methods, it is creative, intellectually exciting, and shows clear promise to yield findings that could lead to breakthroughs in the field.
- **Research career development awards.** TRDRP offers three award types that are aimed at enhancing the scientific infrastructure for tobacco-related research in California by supporting the development of careers in research. **New Investigator Awards** are aimed at encouraging newly independent investigators to conduct research on tobacco-related issues. **Postdoctoral Fellowship Awards** allow researchers early in their careers to receive training in tobacco-relevant disciplines. **Dissertation Research Awards** provide support for the dissertation research of doctoral candidates who wish to pursue tobacco-related research.
- **Collaborative research awards.** **Community-Academic Research Awards (CARA)** are intended to stimulate and support collaborations between community-based organizations and university-based investigators to perform scientifically rigorous research into tobacco control issues important to California's diverse communities. **School-Academic Research Awards (SARA)** are intended to stimulate and support

collaborations between schools and university-based investigators to perform scientifically rigorous research into tobacco control issues that: 1) are identified as important to schools in the state; 2) are likely to produce results that are meaningful to school-based prevention and intervention efforts; and 3) use methods that are relevant, culturally appropriate, and appropriate in terms defined and accepted by the schools. SARAs are jointly funded by the California Department of Education (CDE) and TRDRP.

Evaluation of Research Grant Applications

Research grant proposals submitted in response to TRDRP's Call for Applications are first screened for relevance to the program's mission. Relevant proposals are assigned to a committee of peer reviewers who are experts in the scientific discipline and subject matter of the proposed research (these committees are known as "study sections"). Peer reviewers are drawn from outside California to minimize actual and apparent conflicts of interest with the applicants. Each study section evaluates applications for their scientific merit. Following state statutes, the evaluation procedure is modeled on the one used by the National Institutes of Health. The study sections' merit ratings are transmitted to TRDRP's Scientific Advisory Committee (see below). The committee uses the scientific merit ratings together with the degree to which a proposal is responsive to funding priorities to make funding recommendations. The awards recommended for funding by the Scientific Advisory Committee represent important and innovative research that promises to advance knowledge needed to improve tobacco control; tobacco use prevention and cessation; protection from secondhand smoke; and prevention, treatment, and diagnosis of tobacco-related disease.

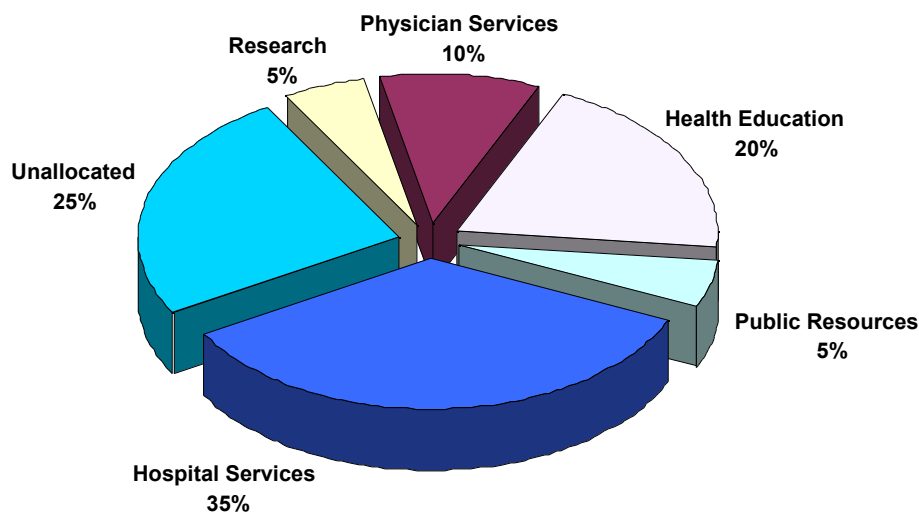
TRDRP HISTORY

Program Administration

TRDRP was established as a result of the passage of Proposition 99 ("The Tobacco Tax and Health Protection Act of 1988") in November 1988. The proposition increased the tax on cigarettes by 25 cents per pack and raised the tax on other tobacco products an equivalent amount. The initiative created the Cigarette and Tobacco Products Surtax Fund, consisting of six accounts in which specific percentages of the revenue are deposited annually (see Figure 2): the Research Account (5 percent), the Health Education Account (20 percent), the Hospital Services Account (35 percent), the Physician Services Account (10 percent), the Public Resources Account (5 percent), and the Unallocated (or General Purposes) Account (25 percent). Collection of the tax began on January 1, 1989.

Proposition 99 specified that the revenues from the Research Account be used to fund research on tobacco-related disease in California. The California Legislature subsequently asked the University of California to establish and administer a research program to facilitate the elimination of smoking in California, and to report annually to the Legislature about the activities of the Program. TRDRP manages all fiscal and programmatic aspects of the tobacco research funding from the Cigarette and Tobacco Products Surtax Fund. As stipulated by the legislation, funding for administrative expenses is limited to five percent of the Research Account. Within the Office of the President at the University of California, TRDRP is one of the Special Research Programs in the Office of the Vice President for Health Affairs.

Figure 2. Distribution of Tobacco Tax Revenue Specified by Proposition 99



Scientific Advisory Committee

In accordance with enabling legislation, a Scientific Advisory Committee advises the University on the administration of TRDRP. Current members represent major California organizations involved in health research. Members are appointed to three-year terms, are not compensated, and may not receive TRDRP funding while serving on the committee. The Committee is charged with recommending the strategic objectives and priorities of TRDRP, and with making final recommendations on grants to be funded based on the established priorities and the scientific merit of the proposals as determined by study section.

TRDRP Coordination with Tobacco Control Programs Funded by the Proposition 99 Health Education Account

TRDRP receives funding from the Proposition 99 Research Account. The California Department of Health Services (DHS) and the California Department of Education (CDE) receive funding from the Proposition 99 Health Education Account. During 2002, TRDRP staff worked with their counterparts from the DHS Tobacco Control Program and the CDE Tobacco Use Prevention Education program to keep abreast of developments in their respective programs, avoid duplication of effort, share expertise, and provide input into the development of each program's goals. To this end, TRDRP conducted a workshop about the Community-Academic Research Awards at the Tobacco Control Program's Project Directors Meeting. The joint funding of School-Academic Research Awards by TRDRP and CDE marks the first time that state agencies have pooled their resources to optimize tobacco control efforts of common interest. TRDRP has had representatives from both DHS and CDE on the Scientific Advisory Committee in order to facilitate coordination between the state's tobacco research efforts, on the one hand, and its community-based and school-based tobacco control efforts, on the other hand.

RESULTS OF FUNDED RESEARCH

The results of research projects that ended in 2002 are summarized below. Abstracts are grouped by subject area.

KEY:

-	TRDRP Grant Number
-	Grant Title
-	Investigator(s)
-	Institution

Abstract Text

Cancer, Heart Disease, and Lung Disease

8RT-0037

Role of Protein Phosphatase 2A in Lung Cancer

Gernot Walter

University of California, San Diego

Smoking causes lung cancer through mutation of genes that are involved in controlling the growth of lung cells. Two classes of genes are important in growth control: (1) Genes that stimulate growth (oncogenes), and (2) genes that inhibit growth (tumor suppressor genes). The former become activated by mutation whereas the latter become inactivated by mutation or deletion. In most cancers, including lung cancer, mutation of both types of genes contributes to the development of cancer.

For many years, our laboratory has investigated the enzyme called protein phosphatase 2A (PP2A). PP2A controls the function of other proteins by removing phosphate residues from the amino acids serine and threonine. PP2A consists of a trimeric complex of a catalytic subunit (C) and two regulatory subunits (A and B). There are two different forms of A (A₁ and A₂), two forms of C (C₁ and C₂), and many different forms of B subunits grouped in families called B, B', B'', and B'''. Both forms of A were recently found to be mutated or deleted in lung cancer and other cancers, suggesting that they play a role as tumor suppressors. Our previous hypothesis was that these mutations affect the capacity of the A subunits to bind certain B subunits and thereby inhibit formation of specific trimeric PP2A complexes that are important in tumor suppression. During the three-year TRDRP funding period we have demonstrated that this hypothesis is indeed correct. Some mutations are highly specific causing the A₁ subunits to be defective in B' binding only, while being normal in B and B'' binding. Similar results were found for the A₂ subunit mutants.

For the future, we propose that expression of A subunit mutants in cancer, which are defective in binding B' subunits, triggers a signal in the cytoplasm that is transmitted to the cell nucleus and

stimulates cell growth. The particular pathway that we believe might be triggered by mutation is called Wnt signaling pathway.

Our findings and the resulting future work are highly relevant to understanding the cause of lung cancer since they are expected to establish a link between A subunit mutations that occur in lung cancer and the Wnt signaling pathway which plays an important role in many cancers. Several A subunit mutants were found in tumor cell lines from smokers, suggesting that smoking may result in a loss of the tumor suppressor function of PP2A. It is conceivable that in the future, based on our studies, drugs can be found that revert the effect of A subunit mutations in lung cancer, i.e. drugs that bind to a mutant A subunit and restore its binding to the B' subunit resulting in normal function of PP2A.

7RT-0026
Suppressor Genes of Lung Cancer
Shi Huang
The Burnham Institute

Diet and smoking are the two major causes of human cancers in the United States with each accounting for approximately 33% of cancer deaths. Lung cancer is strongly linked to smoking and diet, but the mechanisms remain to be established. Inactivation of tumor suppressor genes such as Rb and p53 has been established to play an important role in cancer formation. The RIZ1 gene was isolated based on its ability to bind to the Rb protein. RIZ1 has since been found to be a tumor suppressor belonging to a superfamily of protein/histone methyltransferases. The finding of tumor suppressor function among methyltransferases may significantly advance our understanding of the mechanisms of the diet-cancer relationship.

Significant results from this grant period are the following. RIZ1 was found commonly silenced in lung cancer cell lines. A strong correlation is found between DNA methylation and loss of RIZ1 expression. Consistent with a tumor suppressor role in lung cancer, RIZ1 caused G2/M arrest when it was over expressed in lung cancer cell lines. We also found that the increased RIZ1 protein level in the presence of Rb is correlated with increased mRNA levels. In studying RIZ-Rb interaction, we found that loss of RIZ1 did not rescue the embryo lethality phenotype of Rb mutant mice. Finally, we found that RIZ1 has the suspected protein methyltransferase activities. We further proposed the hypothesis that protein methyltransferases of the RIZ1 class represent a super family of tumor suppressor genes. These enzymes may directly contribute to cancer formation caused by dietary nutrient imbalance. The typical western diet, which is high in meat and low in vegetables, is deficient in methyl donor nutrients (folate, vitamin B12 and B6) and these deficiencies are a major risk factor for lung cancer. Our future research goal is to seek further support for the hypothesis that diet nutrient imbalance causes cancer by directly inactivating the enzyme activity of the protein methyltransferase class of tumor suppressor genes. This could have a great impact in lung cancer prevention.

Introduction. Lung cancer is a direct consequence of smoking and requires a new approach to treatment that will improve efficacy and decrease the deleterious side effects associated with current therapies.

Topic Addressed. This study approaches lung cancer treatment from a new perspective by focusing on the molecular differences between the blood vessels of normal and tumor tissue of the lung to create specific diagnostic and therapeutic probes targeting lung tumors.

Progress Toward Specific Aims. Proteins associated with the cell surface of the lung endothelium of normal and neoplastic tissue have been identified and compared by utilizing 1- and 2-dimensional gel electrophoresis and mass spectrometry. One of these proteins, designated TE3, appears to be expressed only in the lung tumor vasculature and not in normal tissues. Using antibodies to TE3, we have further validated the lung tumor-specificity of TE3 by Western analysis, tissue immunostaining, and in vivo targeting. To better estimate TE3 tumor targeting in vivo, we injected radio-iodinated pTE3 into the tail vein of normal rats and rats bearing lung tumors. After one hour, 25% of the injected dose was detected in the lung tumors (60-fold more than in normal rat lung) with little to no antibody detected in other organs. These results are quite encouraging and indicate that TE3 is exposed on the outside of the cell and is readily accessible by IV injection for lung tumor-specific delivery in vivo. In addition, we have generated a promising antibody, TC004, that appears to be both lung tumor- and caveolae-specific.

Future Directions. Because polyclonal antibodies are not used in treatment, we plan to generate a monoclonal antibody to TE3 to continue our in vivo tumor targeting studies. In addition, we plan to further test tumor-specificity of the TE3 target by whole body imaging using gamma scintigraphy in a rat lung tumor model. We have begun experiments to identify the TC004 antigen. Mass spectrometry analysis of proteins immunoprecipitated by TC004 has indicated that the TC004 antigen is novel. We are continuing to characterize TC004 by tissue expression studies and soon will inject these antibodies intravenously to assess tumor targeting.

Impact. If specific proteins that are only expressed in the blood vessels of lung tumors can be discovered and monoclonal antibodies raised against them, therapeutic targeting of lung tumors may be possible by injection into the bloodstream of drug conjugates (combinations of desired drug [i.e. toxin] and antibodies). Developing the ability to deliver drugs and even genes specifically to tumors while avoiding other tissues will decrease patient suffering from the debilitating and sometimes disfiguring effects of current treatments.

10RT-0016

Analysis of a Lung Tumor Suppressor Gene at 3p21.3

Gerd P. Pfeifer

Beckman Research Institute of the City of Hope

Lung cancers are strongly associated with cigarette smoking. In order to understand how cigarette smoke components cause lung cancer and other smoking-related cancers, it is necessary to understand the sequence of molecular events that leads to formation of a tumor many years or decades later. According to current thinking, a number of genes need to be mutated or functionally disabled before a normal cell loses all normal growth control mechanisms and is brought onto a path of uncontrolled cell division, eventually leading to a tumor. Gene mutation or inactivation has been found in genes such as p53, ras, and p16 at a relatively high frequency in human lung tumors.

However, the most common and earliest chromosomal change in the development of lung tumors occurs on the short arm of chromosome 3, in an area called 3p21.3. At this location, a specific chromosomal piece is often deleted in lung cancer, and sometimes even both copies of this chromosomal material are lost. This phenomenon usually points to the existence of a tumor suppressor gene, which needs to be lost or inactivated completely in order for a tumor to arise.

We have cloned a new gene (named RASSF1) from the common chromosomal deletion area of chromosome 3p21.3 in lung tumors. The gene product has 50% homology to one other protein that interacts with the RAS oncogene product. At this time we know the following about the function of this gene: 1) it is inactivated in a high percentage of human lung cancers (in up to 80% of small cell lung carcinomas); 2) it suppresses cell growth and tumor formation when put back into lung cancer cells; and 3) it can bind to the RAS protein as a dimer with another protein called NORE1, and promote cell death.

We have investigated further how the RASSF1 gene is inactivated in different types of lung cancers and in head and neck tumors. Inactivation of RASSF1 in head and neck cancer is rare. In order to test its presumed function as a tumor suppressor, we have deleted this gene in the mouse and we are in the process to determine if these mice develop cancer, in particular lung cancer.

The study should increase our understanding of the mechanisms of lung cancer initiation. If the causes of smoking-associated cancers are better understood at the molecular level, more efficient early screening procedures and future gene therapy approaches may be developed.

9FT-0183

Siah-family Proteins in Lung Cancer

Shu-ichi Matsuzawa

The Burnham Institute

Defects in the control of cell division occur in essentially all cancers, including those that arise in the aerodigestive tract. The molecular events which underlie the aberrant growth of malignant

cells are only partly understood. Mutations that inactivate the tumor suppressor protein p53 occur commonly in lung cancers and are associated with aggressive clinical behavior of tumors and shorter patient survival. The tumor suppressor p53 has multiple functions in cells, including an ability to induce cell cycle arrest in essentially all types of cells and to trigger apoptosis in some specific cell lineages and cellular contexts. Recently, a mammalian homologue of the *Drosophila seven in absentia (sina)*, called Siah, was reported to be a p53-inducible gene in mammalian cells. However, until now no direct evidence had been obtained demonstrating an effect of Siah on either cell proliferation or survival. In this study, we sought to understand the mechanisms of the intracellular cell growth regulator Siah. We have determined that Siah inhibits cancer cell growth and that it can be induced by some stimuli which cause arrest of tumor cell division. To understand how Siah functions, we identified a novel Siah-interacting protein (SIP) using two-hybrid screens. SIP is a Sgt1-related protein that provides a physical link between Sina/Siah-family proteins and the SCF-complex component Skp1. Furthermore, we have discovered a novel pathway for p53-induced β -catenin degradation involving Siah, SIP and Ebi, a F-box protein which binds β -catenin (Matsuzawa & Reed. *Mol. Cell* 7:915, 2001). A series of protein interactions link genotoxic injury to destruction of β -catenin, thus reducing activity of β -catenin-binding Tcf/LEF transcription factors and potentially contributing to cell cycle arrest in a p53-dependent manner. Thus, Siah, SIP, Skp1, and Ebi collaborate in a novel pathway that controls turnover of β -catenin. The chief hypothesis is that Siah-1 is an important regulator of tumor cell growth, through its effects on ubiquitination and degradation of β -catenin and possibly other target proteins. The results obtained could suggest new strategies for restoring tumor suppressive pathways lost in cancers that have suffered p53 inactivation.

9RT-0050

Recruitment of Leukocytes to Atherosclerosis Lesions In Vivo

Daniel Steinberg

University of California, San Diego

The major cause of death in the United States, both among smokers and among non-smokers, is disease of the coronary arteries leading to heart attacks. The blood vessel disease is called atherosclerosis and it is a progressive degeneration that begins with the entry of white blood cells (monocytes) into the artery wall. The present studies were designed to measure the rate at which monocytes enter the artery wall using a highly sensitive procedure developed in this laboratory over the last few years. The method capitalizes on the sensitivity with which genetic DNA can be amplified using the polymerase chain reaction (PCR). Originally we introduced monocytes from a male donor mouse into female recipients. Because male cells, and only male cells, contain the Y chromosome we could amplify a gene present on that chromosome and totally absent from the DNA in the female recipient. That method worked very nicely and the feasibility, sensitivity and reproducibility of the method were established using that method. It allowed us to measure for the first time the rates at which monocytes enter atherosclerotic lesions in the intact animal, without uncertainties about whether or not in vitro systems behave in a manner that mirrors the situation in the intact animal.

During these studies we showed that one of the drugs currently widely used in the treatment of diabetes (rosiglitazone) could inhibit the progression of atherosclerosis in male mice but we saw no effect in females. It became important to be able to measure the effects of the drug separately in male and female animals, which meant devising a new strategy. Over the past year we have done that, taking advantage of the availability of a strain of mice into which a new gene has been introduced - the gene for a bacterial enzyme, (3-galactosidase. All of the cells in the body of this animal, including of course their blood monocytes, contain about 20 copies of this gene. That makes it possible now to transfer monocytes from either males or females of this strain into normal recipients of either sex and trace the injected cells by using PCR that amplifies the P-galactosidase gene. The sensitivity of this method, because of the 20 copies/cell, is remarkable, allowing us to detect the recruitment of monocytes into the artery wall even before any lesions are visible. If we find that monocyte recruitment is inhibited by rosiglitazone in male mice as well as females, we will need to choose between two hypotheses: 1) that the drug down-regulates the production of a chemoattractant (MCP-1), which has been demonstrated, or 2) that it down-regulates the expression of the receptor for MCP-1, something demonstrated by Dr. O. Quehenberger in our laboratory very recently.

The availability of this new, highly sensitive method should be a powerful general tool for investigators interested in atherosclerosis or in other inflammatory processes.

7RT-0109

Effects of Nicotine on Postinfarction Cardiac Remodeling

Francisco Villareal

University of California, San Diego

Cigarette smoking is a risk factor for heart disease and is associated with excessive mortality. Factors responsible for this excess death rate include diseases such as atherosclerosis and thrombosis. Patients who smoke also suffer from the impairment of tissue wound healing. We propose that inadequate healing of heart tissue affects the outcome of patients who smoke up to the time of their heart attack or beyond. We have modeled in our laboratory how animals with a heart attack who are exposed to nicotine suffer from adverse healing and abnormal growth of the heart. With the purpose of understanding the mechanism by which nicotine induces these alterations we have explored for nicotine modified function of the cells responsible for healing heart tissue. Our data indicates that these cells can respond to nicotine and that nicotine can modestly alter their functions. However, recent data indicates that nicotine may affect healing after a heart attack by impairing the function of the immune system. This distinct possibility will need to be explored in future studies. We have embarked on new studies in rats subjected to a heart attack in order to assess possible drugs that may prevent the undesirable effects of nicotine with excellent results. Briefly, the short-term use of doxycycline after a heart attack reduces the amount of tissue damage. This protective effect appears to be associated with the capacity of doxycycline to limit the amount of damage exerted on the fibrous collagen structure. We have also examined the capacity of adenosine to favorably modify the course of post-infarction cardiac healing and remodeling with positive results. As part of our ongoing studies we have examined humans who suffered heart attacks for altered cardiac growth and function using echocardiography. Results derived from studies of about 30+ patients indicates that smokers who

suffer a heart attack do not show signs of improvement in heart function over the course of 6 months. This is in contrast to nonsmoking patients who show improvement in heart function. Our data also suggest the undesirable enlargement of hearts of patients who smoke and have had a heart attack. Thus, a worsened prognosis may be expected in patients who smoke if inadequate healing occurs in wounded heart tissue. This is because it is essential that heart wounds heal properly in order to avoid developing a severely enlarged and deformed heart. Unfortunately, this adverse outcome is associated with the development of heart failure and even death. A significant impact on TRDRP priority issues is expected from the studies supported under the 4 year tenure of the research award. In the U.S., approximately five million patients suffer heart failure which is the leading cause of hospitalization in Medicare patients, with an associated annual cost estimated at \$50 billion. The studies outlined above focused on the undesirable effects of smoking on patients' recovery from heart attacks. These effects have only begun to be described in animal models of nicotine exposure. If verified by further study, it would be possible to estimate the additional financial costs and preventable loss of life due to smoking after heart attack.

7RT-0018

Determining How Cholesterol-Rich Lp(a) Causes Heart Disease

Robert E. Pitas

The J. David Gladstone Institutes

Smoking increases the risk of heart disease. Although the mechanism is not known with certainty, at least one study suggests that cigarette smoking increases plasma concentrations of lipoprotein(a) [Lp(a)]. High concentrations of low density lipoprotein (LDL) cholesterol are associated with an increased risk of premature heart disease. Lp(a), which consists of apolipoprotein(a) [apo(a)] bound to LDL, appears to be more atherogenic than LDL alone. We hypothesized that the binding of Lp(a) to proteoglycans and plasminogen substrates in the arterial subendothelial space is critical to atherogenesis. To test this hypothesis, we began by generating LDL that binds poorly to artery wall proteoglycans. This proteoglycan-binding-defective LDL caused minimal atherosclerosis when expressed in transgenic mice fed a high-fat, high-cholesterol diet. Dr. Richard Lawn (CV Therapeutics) generated apo(a) that binds poorly to plasminogen substrates and is poorly retained in the artery wall when expressed in transgenic mice. We cloned this mutant apo(a) cDNA and a wild-type apo(a) cDNA into a liver specific vector and injected the resulting constructs into mouse embryos. Analysis of the transgenic mice expressing apo(a) showed robust expression in the liver for both wild-type and mutant apo (a). We bred wild-type and mutant apo(a) mice with mice expressing wild-type LDL or proteoglycan-binding-defective LDL. In mice expressing both LDL and apo(a), most of the LDL was bound to apo(a) and circulated in plasma as Lp(a). These mice are being fed a high-fat, high-cholesterol diet to determine if proteoglycan-binding-defective, fibrin-binding-defective Lp(a) is less atherogenic than normal Lp(a). Our studies will help explain the potent atherogenicity of Lp(a). Specifically, they will determine whether the binding of Lp(a) to proteoglycans and plasminogen substrates in the artery wall is an important first step in atherogenesis. The results will lay the foundation for inhibiting or preventing atherosclerosis with small molecules designed to inhibit the binding of Lp(a) to proteoglycans or plasminogen substrates.

7RT-0032

Lipoprotein Classes and Susceptibility to Atherosclerosis

Stephen G. Young

The J. David Gladstone Institutes

Despite clues regarding smoking's effects on the plasma lipoproteins, many fundamental questions about the relationship between the plasma lipoproteins and atherogenesis remain unanswered. It has not been clear, for example, which classes of lipoproteins are the most atherogenic. Small, cholesterol ester-rich LDL might be the main culprit in atherosclerosis, but some investigators believe that larger VLDL and chylomicron remnant particles might be particularly atherogenic. The principal objective of our project has been to determine if VLDL and LDL differ in their intrinsic ability to promote atherosclerosis. To address this issue, we designed a study to compare the extent of atherosclerosis in two groups of chow-fed mice that have nearly identical plasma cholesterol levels: LDL receptor-deficient apo-B100-only mice ($Ldlr^{-/-}Apob^{100/100}$) (in which nearly all of the cholesterol in the plasma is on small, LDL particles) and apo-E-deficient apo-B100-only mice ($Apoe^{-/-}Apob^{100/100}$) (where nearly all of the cholesterol is carried on VLDL particles). Morphometric analyses of aortic atherosclerotic lesions yielded definitive results. The $Ldlr^{-/-}Apob^{100/100}$ mice had nearly 300% more atherosclerosis than the $Apoe^{-/-}Apob^{100/100}$ mice, even though their total plasma cholesterol levels were identical. This study was published in *The Journal of Clinical Investigation*. Recently, we summarized our studies in an invited review for *Arteriosclerosis, Thrombosis, and Vascular Biology*.

During the past year, we have worked with an animal model for assessing the regression of atherosclerosis and gene expression changes that accompany regression. We hypothesized that both the hypercholesterolemia and the susceptibility to atherosclerosis could be eliminated by switching off hepatic lipoprotein production. To test this hypothesis, we bred "AtheroReversa mice"— $Apoe^{-/-}Apob^{100/100}$ mice that were homozygous for a conditional allele of microsomal triglyceride transfer protein (Mttp) and homozygous for the Mx1-Cre transgene. Expression of Cre from the Mx1-Cre transgene can be induced with injections of polyinosinic-polycytidylic ribonucleic acid (pIpC). Treatment of AtheroReversa mice with pIpC virtually eliminated Mttp expression in the liver (as judged by quantitative RT-PCR assays), abolished lipoprotein secretion by the liver (as judged by transmission electron microscopy), and eliminated LDL cholesterol from the plasma (as judged by FPLC fractionation experiments). Both plasma triglyceride levels and cholesterol levels fell dramatically in pIpC-treated mice, both on chow and high-fat diets. AtheroReversa mice should be ideal for studying regression of atherosclerosis and investigating changes in arterial gene expression that accompany hypercholesterolemia and atherogenesis.

8FT-0150

Effect of Tobacco Smoke on Nitric Oxide Synthesis

Patrick S. Wong

University of California, Davis

Cigarette smoking is associated with an increased risk of cardiovascular disease through increased hypertension and platelet aggregation. Both symptoms may be caused by decreased endogenous nitric oxide (NO) formation. NO has been shown to be an important regulator of blood flow and smooth muscle relaxation. It has been previously shown that cigarette smoking reduces the production of endogenous NO. However, the mechanism of the decrease in NO levels has yet to be determined. Nitric oxide synthase (NOS) is the enzyme responsible for converting L-arginine (one of the 20 essential amino acids) to NO and L-arginine. Previous studies have shown that NOS activity is decreased in the presence of cigarette smoke extract. Also, it has been demonstrated that smokers have lower NOS activity than non-smokers. Although the mechanism of these observations is not known, a possible target includes the formation of adducts of L-arginine, the main substrate for NOS, with components of cigarette smoke. The formation of such aforementioned adducts may lead to decreased L-arginine for NO synthesis or the formation of a compound which inhibits NOS. Both possibilities are supported by studies which show that endothelial dysfunction by cigarette smoke is prevented through dietary L-arginine supplementation.

Experiments in our laboratory have focused on the direct reaction between cigarette smoke extract and L-arginine. In these studies, a solution of L-arginine was exposed to filtered cigarette smoke. We observed the formation of a possible L-arginine adduct. The structure of this adduct was elucidated by ultraviolet and mass spectroscopy, which give information on the structural relationships between atoms in a molecule and basic molecular weights. Based upon these results, we hypothesized that cigarette smoke components react with L-arginine to form an L-arginine adduct. This adduct can then decrease the substrate availability for NOS or even inhibit NOS.

The next step was to identify which of the more than 4,000 components of cigarette smoke may be reacting with L-arginine. We focused on acetaldehyde (a major cigarette smoke component) since the adduct formed between cigarette smoke and L-arginine appears to have a similar molecular weight and ultraviolet spectrum as a possible acetaldehyde-L-arginine compound. Direct synthesis of this adduct with acetaldehyde and L-arginine and further analytical analysis confirms that this complex is definitely formed and acetaldehyde appears to be the major compound involved. The synthesized acetaldehyde-L-arginine adduct was then used in subsequent studies.

The final studies of this project focused on the ability of the acetaldehyde-L-arginine adduct to inhibit NOS in both a purified enzyme system and biological system. In the first study, the acetaldehyde-L-arginine adduct was able to decrease NO synthesis by commercially obtained NOS in a concentration dependent manner. This study demonstrated the ability of this adduct to block NOS activity and revealed the adduct as a potent inhibitor. The final study was to test the ability of the acetaldehyde-L-arginine adduct to inhibit a NOS mediated response in a vascular smooth muscle system similar to the blood vessels in the heart. Incubation of the adduct in

precontracted tissues decreased the tissue response to the biochemical acetylcholine. Acetylcholine mediated relaxation has been shown in previous studies to be a NO mediated response. This in vitro study may provide a model for acetaldehyde (and thus cigarette smoke) induced hypertension.

The significance of information obtained through this study is not only elucidation of the mechanism of smoking-induced cardiovascular disease, but also providing a possible better method of treatment and diagnosis for smokers who have developed cardiovascular disease symptoms through the possibility of L-arginine replacement therapies.

7RT-0051

Programmed Cell Death in Cigarette-Induced Lung Disease

J. Courtney Broadus

University of California, San Francisco

Introduction. This study was designed to show whether beta carotene, a chemopreventive agent, decreased apoptosis and thereby increased DNA or chromosomal damage in cells exposed to asbestos or cigarette smoke extract. The question was raised by several clinical studies of recent years, in which beta-carotene given daily to smokers increased the risk of lung cancer formation. The risk also appeared greater when beta-carotene was given to asbestos-exposed individuals, although the numbers of patients in this category were too small to reach significance.

Progress toward specific aims: Our aims were 1) to determine the major mechanisms by which asbestos/cigarette-smoke extract induces apoptosis in airway epithelial cells, and 2) to determine whether chronic inhibition of apoptosis in cigarette smoke extract-exposed epithelial cells leads to an accumulation of DNA or chromosomal damage. We were able to find support for several key elements of our hypothesis:

- 1) First, beta carotene did significantly inhibit apoptosis in asbestos-exposed cells. Interestingly, this decrease was not associated with antioxidant activity or with a decrease in asbestos uptake by the cells. The decrease in apoptosis led to an increase in the number of cells although the increase was found to be transient, that is, the asbestos-exposed cells failed to proliferate and eventually died by other mechanisms.
- 2) Exposure of the cells to beta carotene was associated with an increase in biomarkers of DNA and chromosomal damage. There was an increase in micronuclei (small fragments of nuclei that are found in the cytoplasm), in aneuploidy (by FISH of chromosome 1 and 9) and in DNA strand breaks. However, we could not show whether the cells with evidence of damage were specifically the same cells that failed to undergo apoptosis. The overall damage found in the cells during the 3-5 days of exposure did not further increase and when beta carotene was not maintained, the damage receded.

Future Direction. Our lab is continuing to study the role of apoptosis in cellular responses to toxic environmental agents and also, more recently, to cancer cell responses to DNA damaging agents such as chemotherapy. Our interest in cigarette smoke will be pursued along with asbestos, as the two agents most associated with environmental damage to human lungs.

Impact. Along with other researchers, we hope that our work will lead to understanding of the complex role of apoptosis in the cellular response to injury. In the case of chemopreventives such as beta-carotene, we hope that our investigation into the inhibition of apoptosis and the increase in DNA/chromosomal damage will provide one possible explanation for the unexpected harm done by beta carotene treatment. As such, future studies may include similar sorts of biologic assays to assess potential harm of long-term supplements.

Smoking Cessation

8RT-0103

Telephone Counseling for Pregnant Smokers

Shu-Hong Zhu

University of California, San Diego

This study proposed to test the effectiveness of telephone counseling as a routine intervention for pregnant smokers in a healthcare setting. The aim was to find out how many pregnant smokers would call a telephone counseling program after they were encouraged to do so by their healthcare providers during their first prenatal visit. It tested the effectiveness of a proactive telephone counseling approach where pregnant smokers who failed to call the program were contacted by counselors (via phone) to encourage them to participate in counseling. The effectiveness of this approach was tested in a randomized controlled design.

The project successfully established recruitment procedures with three large healthcare systems in San Diego, which formed the Partnership for Smoke-Free Families (PSF). Recruitment was extended through the use of a pregnancy brochure and survey to Kaiser Permanente (Northern California) and the Perinatal Care Network, two large groups that were not part of the original proposal. The project developed a specific counseling protocol for pregnant smokers and pilot tested several versions. A specific randomization procedure was piloted and shown to be feasible. This procedure allowed women in the control group to receive counseling if they called back to request it after randomization. A method to collect saliva samples to test nicotine exposure among these women was implemented. This method was added because of concern that proactive recruitment might increase the rate of misreporting of nonsmoking status among pregnant women.

A reactive recruitment contrast group was added to the study from among callers to the California Smokers' Helpline (CSH) who were pregnant and agreed to participate in the study. Randomization was stratified by referral source (PSF, CSH, or pregnancy brochure).

The results obtained to date show the following: a) it is feasible to routinely screen for smoking status during a pregnant woman's first prenatal care visit. PSF screened over 33,000 pregnant women for smoking status in 2 _ years through their healthcare providers. The rate of smokers was originally estimated at 10% but proved to be closer to 7%. Physicians continue to refer about 40 women per month and encourage women to call the Helpline. b) Only a minority of pregnant smokers (<3%) take the initiative to call a counseling program, even though they have been encouraged to do so during their first prenatal care visit. c) Proactive recruitment is a promising method to enroll pregnant smokers in counseling. From 3/99-7/02, 1,739 pregnant smokers were referred by PSF to CSH resulting in many more pregnant smokers receiving services. Although 23% of women refused service and 30% were not reached by phone, over 32% did agree to enroll in counseling and another 12% chose self-help materials. By collaborating with healthcare providers almost half of these pregnant smokers received urgently needed help, suggesting that proactive recruitment is a very promising strategy for intervention.

The effectiveness of the telephone counseling protocol is currently being evaluated. There have been 1,170 clients randomized into the intervention and control groups (N = 826 reactive referrals and N = 344 proactive referrals). Subjects randomized into the intervention receive counseling at a rate of 69%. The median number of counseling calls is three. Contrary to expectations, preliminary data suggest a higher quit attempt rate for the proactively recruited subjects (62%) than for reactively recruited subjects (55%). Although too few evaluations have been conducted thus far to examine success by group or referral source, 21% of all randomized clients have quit for 30 days at the point of the third trimester evaluation call.

9IT-0192

Innovation to Prevent Post-Partum Relapse

Jennifer Haas

University of California, San Francisco

Introduction: Cigarette smoking is the leading cause of preventable illness and death for women in the U.S. Pregnancy is a pivotal event of young adulthood for many women. This is supported by the observation that women are more likely to quit smoking around the time of pregnancy than at any other. Unfortunately women who quit smoking for pregnancy have extremely high rates of relapse post-partum.

Topic addressed: This Innovative Developmental and Exploratory Award (IDEA) was designed to collect pilot data to ultimately design an intervention to prevent post-partum relapse to tobacco use that may incorporate bupropion as well as behavioral interventions.

Progress toward Specific Aims: (1) *To measure the amount of bupropion in the breast milk of women who are lactating but not breastfeeding.* Ten women completed this protocol. We have measured the amount of bupropion and its active metabolites in breast milk, plasma, urine and saliva. We found that the median total amount of bupropion and its active metabolites in breast milk is 193 ng/ ml. Since on average an infant drinks 1 liter of breast milk a day and weighs 4.5 kg, the calculated dosage is 0.04 mg/ day. The standard adult dose for a 60 kg woman is 300 mg day (5 mg/ kg). Therefore the median infant exposure is less than 1/100th of the standard adult dose. This range of exposure is typically considered a safe level of exposure for an infant. In addition we examined the correlation between breast milk, plasma, saliva and urine levels of bupropion to determine whether breast milk levels could be adequately monitored in one of these other body fluids. The correlation between bupropion in breast milk and in urine is 0.88 (p = 0.008). This suggests that levels of bupropion in breast milk could be monitored adequately in urine. (2) *To inventory, categorize and evaluate behavioral smoking cessation and relapse prevention interventions for pregnant and post partum women.* We have identified 11 published articles or funded studies that address smoking cessation and relapse prevention for this target population. We collected materials from the study investigators, and compiled and categorized these materials using a standard profile. (3) *To obtain explicit information about how best to target a multi faceted intervention to prevent post partum relapse to tobacco.* In order to obtain explicit information about how best to target an intervention to prevent post-partum relapse, we proposed a pilot project that would involve interviewing 50 women recruited from two sites.

Based on California estimates, we estimated that it would take approximately 4 months to recruit a sample of 50 women, assuming a 70% response/eligibility rate. After a 6 month period, we were able to identify only 6 eligible women who were willing to participate. For this reason we did not continue with this aim so that resources could be focused on the first two aims.

Future direction: The data collected during this IDEA project will be synthesized to design a trial of a multifaceted intervention to prevent post-partum relapse to tobacco use.

Impact: Pregnancy is an important opportunity to reduce a woman's lifetime exposure to tobacco. The results of this IDEA project should lead to an innovative intervention to prevent post-partum relapse in a multi-ethnic cohort of women.

8RT-0068

Effectiveness of Two Different Regimens in a Smoking Clinic

Scott E. Sherman

University of California, Los Angeles

Many studies have been conducted on the efficacy of different smoking cessation therapies in controlled trials. However, few studies have looked at their effectiveness in everyday clinical practice. This study is an analysis of the effectiveness of bupropion versus bupropion plus nicotine patch in a routine clinical setting at the Sepulveda Veterans Administration Medical Center Smoking Cessation Clinic (SCC). Our primary goals were to compare the two treatment regimens with respect to the percentage of patients able to take the medications, side effects, completion rates of the SCC, six-month abstinence rates, and cost.

This study took place February 25, 2000 through June 29, 2001. During this time, all patients enrolled in the SCC were asked to participate in the study. Consenting patients had the option of agreeing to one or more of the following study components: 1) random assignment to receive bupropion or bupropion plus nicotine patch; 2) completion of the baseline and follow-up survey; and 3) permitting access to their medical records. Of the 708 patient referrals received by the SCC, 388 (55%) attended at least one session of the eight-week long program. Of these, 89% were eligible to receive either of the two treatment regimens. 274 (71%) consented to participate in one or more parts of the study, but only 174 agreed to be randomized to a treatment. Of these, 82 were randomly assigned to the bupropion treatment group and 92 were randomly assigned to the combination group (bupropion plus nicotine patch).

Side effects were reported by 37/67 (55%) of people taking bupropion and 57/81 (70%) of people taking the combination therapy ($p=0.05$). The most commonly reported side effects were dry mouth (8%), insomnia (4%), and constipation (2%). Treatment regimens were changed in 7% of patients that started on bupropion and 14% of patients that started on combination therapy (p -value not significant). Changes in therapy were due mainly to intolerable side effects and patient requests for a different medication.

Two-month smoking abstinence rates were based upon patient self-report and verified by carbon monoxide testing. Among the 174 study patients who were randomly assigned to treatment, 120/174 (69%) successfully completed the 2-month program. The rate of completion was slightly higher among the combination therapy group (38% vs. 27%) although it did not quite reach statistical significance ($p=0.1$).

Six-month smoking abstinence rates were assessed using follow-up telephone interviews. Based on self-reports of the 117 patients who were randomly assigned to treatment and completed the follow-up interviews, 22/52 (42%) of patients who used bupropion and 23/65 (35%) of patients who used combination therapy were abstinent at 6 months. Abstinence was defined as not having smoked at all in the last 30 days prior to the follow-up interview. However, these results were not statistically significant ($p=0.4$).

Our results indicate that combination therapy may be superior to bupropion alone after 2 months but there was no difference in 6-month abstinence rates. However, sample size limitations may have contributed to our inability to find a long term difference.

7RT-0033

Bupropion for Smoking Cessation: a Randomized Trial

Joel A. Simon

University of California, San Francisco

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 bupropion, an antidepressant, to be an effective adjunct for smoking cessation. However, the effectiveness of bupropion has been studied in only a few populations to date.

We conducted a randomized blinded clinical trial at the San Francisco Veterans Administration Medical Center in which smokers received standard treatment including 2 months of the nicotine patch, counseling, and use of self-help literature. In addition, approximately 50% of participants were randomly assigned to receive 7 weeks of bupropion whereas the remaining 50% of participants were randomly assigned to receive a placebo. Neither the participants nor the investigators knew who had been assigned to receive the active drug.

A total of 244 current smokers (86% men) ages 20 to 78 years were enrolled. One hundred and twenty-one participants received bupropion and 123 participants received the placebo. All study participants received 2 months of nicotine replacement therapy using the transdermal patch and 3 months of cognitive-behavioral counseling. During the 7 weeks of treatment with bupropion vs. placebo, there was a non-statistically significant trend toward increased quit rates among participants randomized to bupropion; the self-reported end of-medication treatment quit rates were 64% for the bupropion group and 56% for the placebo group. This trend in self-reported quit rates persisted at 3 months of follow-up, but was not apparent at 6 months and one year of

follow-up. The 12-month quit rates, validated by either saliva cotinine or spousal proxy, were 26% in the bupropion group and 29% in the placebo group and were not statistically different. Based on biochemical validation only, 19% of the bupropion group vs. 25% of the placebo group had quit smoking by one year, again a difference that was not different statistically. There were no serious adverse reactions reported among participants receiving bupropion.

In this randomized blinded smoking cessation clinical trial of mostly veteran participants, the addition of bupropion to treatment with nicotine replacement and counseling did not increase either short-term or long-term quit rates. Bupropion may be an effective treatment to aid in smoking cessation among other populations of Californians, in other settings (e.g., hospitalized smokers), or among smokers who do not or cannot receive nicotine replacement therapy.

6PT-2000

Tobacco Control in Latino Communities – Core

John P. Elder

San Diego State University Foundation

The Tobacco Control in Latino Communities (TCLC) Integrated Research Project (IRP) comprised three interwoven individual projects (IPs), with each providing separate final reports. Two were field interventions that used a community health advisor (CHA) or promotor model to promote smoking cessation (Proyecto Sol) and environmental tobacco smoke (ETS) reduction (Ambiente Fresco), respectively. The third IP studied tobacco taxation in Latino communities and its effect on tobacco consumption. Among its primary aims, the TCLC Core provided support to the two intervention IPs by assisting with program implementation for the promotor-delivered programs. The TCLC core also worked to conduct a process evaluation of the promotor related aspects of the project.

The two IPs and the TCLC core collaboratively developed a paper and pencil measurement tool for process evaluation related to issues affecting both IPs. 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. It measured eight intervention-oriented constructs (i.e., intentions, environmental constraints, anticipated outcomes, perceived normative pressure, self-standards, self-efficacy, emotional reaction, and abilities/skills) drawn from several major theories of behavior change that may predict successful promotores. The instrument also measured 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 individual projects. TCLC core staff also developed a promotor rating scale to be completed by IP staff knowledgeable about promotor performance. Sets of questions were developed to individually assess for each promotor: 1) attitude, initiative, and motivation; 2) personal/interpersonal characteristics; 3) behavioral characteristics and skills; 4) overall performance; 5) training attendance; and 6) intervention attempts and completion of information.

The TCLC core staff assisted the IPs in developing, revising and translating common promotora applications, surveys and training materials and separate training manuals, participant materials and surveys. TCLC core staff also assisted the IPs in training and evaluating four groups of promotores. Positive pre- and 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 Core staff assisted in conducting booster sessions to address them.

Both promotor-based individual projects and the TCLC Core have provided training opportunities to Latino and other health professionals and students regarding the community change agent model and tobacco control research. These studies address potentially effective direct methods for tobacco control in the Latino community primarily through the use of promotores to promote skill building and behavior change procedures for cessation and reduction and prevention of exposure to environmental tobacco smoke.

6PT-2001H

Smoking Cessation in Latinos Using Community Health Advisors

Gregory A. Talavera

San Diego State University

The study recruited Spanish-speaking Latino smokers living in San Diego County. Smokers were randomized (selected by a flip of the coin) into either an intensive group or a comparison group. The intensive group received a culturally sensitive program that promoted smoking cessation and maintenance. This was a 4-month smoking cessation program (that included 4 home visits and 3 phone calls) delivered by community health advisors or promotores. Participants in the comparison group were referred to the Spanish-language California Smoker's Helpline via mailed postcards.

The project trained a total of 17 promotores. Promotores 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 at each home visit and phone call. Once trained, promotores met monthly to brainstorm solutions to problems that arose in the field, support each other's successes, and receive feedback and intervention materials from project staff.

A total of 312 study participants were recruited and recruitment ended September 2000. Demographic data on the 312 enrolled participants indicate that the majority were born in Mexico, about half are women, the average age is 43 years, and most are of a moderately low acculturation level. In terms of smoking characteristics at entry into the study, the average age of initiation was 16 years, 90% consider themselves daily smokers consuming an average of 11-15 cigarettes per day, and 81% have tried to quit in the past.

While initial analyses using the study's 3-month follow-up survey data had shown that the intensive group had more quitters than the comparison group, this finding was no longer present at the 12-month follow-up survey period. When the study was completed, approximately equal numbers of participants in the intensive group (16%) and the comparison group (11%) had quit and this difference was not statistically significant.

We also performed an analysis to identify significant predictors of past-week abstinence at 12 months after the intervention. The initial results show that gender, monthly income, whether participants were born and/or educated in Mexico, other acculturation factors, and whether anyone else in the household smokes did not predict past-week abstinence.

7RT-0135

Smoking Treatment for Substance Abusing Adolescents

Mark G. Myers

University of California, San Diego

Approximately 80% of adolescents treated for alcohol and drug problems also smoke cigarettes. Available evidence suggests that smoking continues at high rates following adolescent substance abuse treatment. The high prevalence and persistence of smoking among substance abusing adolescents identifies these youth as being at high-risk for tobacco-related diseases, and therefore an important target for tobacco intervention. However, little is currently known about how to encourage and assist smoking cessation for substance abusing adolescents. Developing effective smoking treatments for this population is important in order to reduce the long-term health problems associated with tobacco use.

The main goal of this project was to evaluate the effectiveness of a treatment designed to motivate substance abusing teens to cut down and quit cigarette smoking. This study addressed three questions: 1) Do adolescents who receive a smoking intervention during treatment for substance abuse decrease smoking compared with those who do not? 2) Does smoking treatment have any effect on alcohol and drug use following substance abuse treatment? and 3) Will the smoking treatment have different effects for boys and girls?

Fifty-seven adolescents in treatment for substance abuse were recruited to participate in the project. Participants were interviewed four times over an 8-month period. Included in the final analyses were 35 adolescents for whom complete data were available and (for treatment participants) attended at least 3 of the 6 group treatment meetings.

Analyses showed that treatment participants smoked less at the 3-month timepoint than did controls and were significantly more likely to report abstinence than controls at the 3-month follow-up. However, no differences were found at the 6-month timepoint. With regard to the influence of receiving smoking treatment on subsequent alcohol and drug use, no differences were found between treatment and control participants, suggesting that smoking treatment had no detrimental effect on alcohol and drug use. In fact, participants in the treatment condition were more likely to be abstinent from alcohol and drugs than were controls. Finally, preliminary examination found that treatment did not affect girls and boys differently. The small sample size

of the present study requires that these findings be interpreted with caution. The small number of participants made it less likely that a significant effect of treatment would be found, thus the present results should not be interpreted to conclude that smoking treatment was ineffective. Similarly, the small numbers suggest that future studies with larger samples should be conducted to better evaluate whether girls and boys are differently affected by this type of intervention. However, these findings do support the position that smoking treatment does not increase the risk of a return to alcohol and drug use in adolescents receiving substance abuse treatment.

In summary, the present study showed limited support for the effectiveness of smoking treatment for substance abusing adolescents. However, it was demonstrated that this type of intervention can be successfully integrated within existing outpatient substance abuse treatment settings. Future studies of this topic may be strengthened by: a) educating adolescent substance abuse treatment staff regarding the importance of addressing tobacco use; b) having the intervention delivered by treatment staff familiar to the participants; and c) providing longer term education and support for smoking cessation.

7K-0098

Brain Metabolic Changes during Cigarette Craving

Arthur L. Brody

University of California, Los Angeles

Introduction: Craving for cigarettes in smokers attempting to quit has repeatedly been associated with relapse into usage. Current medications to treat dependence on cigarettes, such as the antidepressant bupropion (Zyban) and the nicotine patch or gum, are better than a sugar pill in reducing cigarette craving and relapse in the short term. However, only about 22% of smokers remain cigarette-free after a year or two, even when using these effective treatments. Thus, there is a substantial need for better medications to treat addiction to cigarettes. The goals of the studies performed here were to determine which areas of the brain become more active when smokers are craving cigarettes, and to determine the effect of a known effective treatment, bupropion (Zyban), on both cigarette cue-induced craving and brain activation associated with craving.

Topic Addressed: To study brain activity during craving, positron emission tomography (PET) brain scans were obtained in both smokers and nonsmokers (for comparison) while they were presented with neutral, nature-related videos and (in a separate session) when presented with cigarette-related videos (to induce cigarette craving). We also performed the same protocol in smokers following treatment with bupropion to see if subjects treated with this medication had attenuation of the craving induced by cigarette-related cues and also to determine if brain activation is attenuated in bupropion-treated smokers.

Progress Toward Specific Aims: Results from the first part of this study (comparing smokers with nonsmokers) indicate that several brain regions have changes in activity when smokers are craving cigarettes. Areas of the brain called the anterior cingulate gyrus, prefrontal cortex, and anterior temporal lobe (regions that are involved in anxiety and emotion) became more active

while smokers were exposed to cigarette-related cues. Some of these regions, especially in the prefrontal cortex, correlated with the intensity of cigarette craving. These brain changes were also more strongly related to cigarette craving than to general anxiety.

Results from the second part of the study (comparing untreated smokers with bupropion-treated smokers) indicate that a course of treatment with bupropion attenuates the craving induced by cigarette-related cues. In addition, brain activation (of the anterior cingulate gyrus) in response to cigarette-related cues was attenuated in smokers treated with bupropion.

Future Direction and Impact: In conjunction with other areas of research, these brain imaging findings may offer promising leads to develop better treatments for cigarette craving and addiction to cigarettes. Other researchers are studying brain regions like the anterior cingulate gyrus, anterior temporal lobe, and prefrontal cortex to learn more about the chemicals within these structures and connections with other brain structures. Thus, identifying brain regions that mediate craving may lead to attempts to change these structures through medication or other therapy. In fact, our finding that bupropion attenuates craving and anterior cingulate gyrus activation indicates potential future medication targets for addiction to cigarettes.

Nicotine Dependence and Effects of Nicotine

8RT-0059

Nicotine Effects on Neurological Development

Raju Metherate

University of California, Irvine

A tragic effect of tobacco smoke is its effect on brain development in unborn and newborn babies. Smoking by pregnant mothers results in babies with diminished auditory function, and as these infants age they demonstrate deficits related to higher auditory-cognitive functions (e.g., speech comprehension). This research will determine how exposure to nicotine affects the normal development of the auditory cortex, the highest brain center responsible for hearing.

During the three-year project, we found that chronic nicotine exposure during the second week of life in rats (corresponding to third trimester development in humans) disrupts the functional development of the auditory cortex. This finding implies a period of special sensitivity—a critical period—for the harmful effects of exogenous nicotine on auditory cortex development. We then determined that chronic nicotine exposure affects the expression of genetic material that codes for proteins, N-methyl-D-aspartate receptors, that are important for proper development of brain circuitry. To better study the cellular effects of nicotine exposure on auditory processing, we developed an in vitro preparation containing the final relay centers of the auditory system. The “auditory thalamocortical slice” preparation will enable detailed cellular studies of higher auditory system function and development, including the effects of nicotine. Finally, we have begun experiments that demonstrate significant effects of postnatal nicotine exposure on auditory function in the adult rat. These experiments may provide a link between animal experiments and the deficits in higher auditory functions caused by maternal smoking in humans. The research will benefit the public by increasing our understanding of how nicotine exposure affects brain development and function.

9DT-0115

Nicotine Regulation of the Noradrenergic System

Kathryn O’Leary

University of California, Irvine

It is widely accepted that mothers who smoke during pregnancy are placing their unborn child at risk for a number of cognitive and behavioral deficits, among them attention deficit hyperactivity disorder. Experimentation using animals has indicated that nicotine is the major ingredient in tobacco which can enter the brain and alter its normal functioning. Likewise, nicotine can be transferred through the placenta of a pregnant woman to the brain of the fetus. Exposure to nicotine is particularly problematic for an unborn child because a developing brain is very susceptible to disruption caused by unnatural stimuli, such as can be provided by nicotine. Animals studies have already shown that long-term prenatal nicotine treatment can dysregulate the noradrenergic system, one of the chemical systems which allows neurons to communicate

with one another and send signals throughout the brain. The cerebellum and the hypothalamus are two brain regions that receive input from the noradrenergic system and are the focus of our experiments. Through these studies we have examined the regulation of the noradrenergic system by nicotine, and whether or not it is different for different brain regions. The cerebellum receives noradrenergic information solely from the locus coeruleus (LC) nucleus, while the hypothalamus receives noradrenergic input both from the LC and from the nucleus tractus solitarius (NTS) and the rostral ventrolateral medulla (RVLM). We have characterized nicotine-stimulated norepinephrine (NE) release in the cerebellum and the hypothalamus and have found both developmental and pharmacological differences. In the cerebellum, nicotine stimulates the most NE during the first postnatal week, with a gradual decline to adult levels. In comparison, release in the hypothalamus peaks at postnatal day 1 (P1) at a lower level than the cerebellum, declines until P21 when there is another peak, and then drops down to the lowest level in the adult. We further examined release at P7 and in the adult in both the cerebellum and the hypothalamus by observing the effects of other drugs that stimulate the same receptors as nicotine. These studies indicate that there are different nicotinic acetylcholine receptors (nAChR) stimulating NE release at each age within one brain region, and between regions. Therefore, the consequences of nicotine exposure early in development may differ from those in the adult, and in the cerebellum versus the hypothalamus, because the different receptors produce different effects. Further experiments examined the effects of chronic prenatal nicotine exposure on postnatal nicotine-stimulated NE release in the cerebellum and the hypothalamus. A number of developmental time points were tested in the cerebellum, from P1 through adult. These experiments suggest that early exposure to nicotine does not alter nicotinic regulation of the NE system in the cerebellum at any of the developmental time points tested. Thus far, we have only studied P45 and adult in the hypothalamus, both of which show no alterations in nicotine-stimulated NE release.

8DT-0170

Effects of Nicotine on Brain Development Assessed by 1 H MRS

Christine C. Cloak

Cedars-Sinai Medical Center

Maternal cigarette smoking produces profound health effects including premature labor, low birth weight, stillbirth, and neonatal death. Postnatal growth and behavior also are affected. Despite all the public health warnings many pregnant women still smoke or are placed on nicotine replacement therapies such as the patch. Nicotine is well known as an important component of cigarette smoke and has been implicated in many of the adverse effects of smoking, on fetal development. Although data clearly show that prenatal exposure to nicotine is bad, considerably less is known about the effects of nicotine on the brain during other developmental periods, particularly during puberty. The brain is still maturing rapidly during puberty and most likely it remains vulnerable to the detrimental effects of nicotine during this period as well. We extended our studies to determine if the “window” of vulnerability that exists during very early development extends into adolescence. We used a relatively new imaging procedure called proton magnetic resonance spectroscopy (1 H MRS), which can be performed safely on humans. One of the brain metabolites measured by 1 H MRS is the compound n-acetyl-

aspartate (NAA). In animals with brain damage or humans with neurodegenerative diseases, NAA concentrations are reduced, in a regionally specific manner. The concentration of this brain metabolite reflects the health and viability of neurons.

We hypothesized that developmental nicotine exposure would result in a decrease in NAA concentrations. Our data indicated that NAA concentrations were not reduced in adult offspring exposed to nicotine during development, however other brain metabolites were affected. The primary focus of the study (long-term effects of developmental nicotine exposure) has been completed and written up as the Ph.D. dissertation of Christine Cloak. We are in the process of converting the dissertation into journal articles for publication. Tissue has been collected but not yet analyzed for the progressive developmental effects of nicotine exposure (early developmental time points). Although our hypotheses concerning the long-term effects of nicotine during development on NAA concentrations in the brain were not supported, other measurable metabolites were affected such as glutamate and myo-inositol. Future studies will focus more on these metabolites.

Social/Behavioral Research

8BT-1701

Partnering for Tobacco Control Research among Deaf Youth

Barbara A. Berman and Heidi Kleiger

University of California, Los Angeles

This Pilot CARA is a collaborative effort between researchers at UCLA and the Greater Los Angeles Council on Deafness (GLAD) designed to gain an understanding of tobacco use patterns and unmet tobacco control programming needs among Deaf youth, and to develop intervention strategies that can address these needs. To achieve these ends we proposed to: 1) conduct in-person, semi-structured interviews with 40 male and female Deaf youth with various smoking experiences, examining in depth the findings from the IVQTM study, a TRDRP-funded (6RT-0047) multi-media computer-based survey of this population; 2) forge a statewide tobacco control network of community-based organizations and agencies that work with and serve the Deaf community; and (3) work with this network to define research priorities and strategies, disseminate research findings, and develop a tobacco control intervention for this population for future testing in a scientifically rigorous, randomized controlled trial through a subsequent Full CARA application.

To implement the Pilot CARA, we drew a randomized (by gender and smoking status as identified in the IVQTM survey) sample of 43 subjects; conducted interviewer training; and completed 40 in-depth qualitative signed interviews from among the male and female respondents in our IVQTM study. The videotaped interviews were transcribed into written English; a 10% sample of the interviews was (blindly) re-transcribed to examine consistency. Utilizing well established qualitative data analysis methodology, we identified a start list of concepts, including: respondent knowledge, attitudes, and practices regarding tobacco use; perception of tobacco/anti-tobacco advertisements; exposure to anti-tobacco programming; Deaf self-identity, perceived role models, view of education, activities and future aspirations. This start list was expanded to 32 categories, into which all survey content was preliminarily coded. Themes of interest have emerged based on these steps, and in-depth content analyses of the categories are continuing. Based on initial recommendations from the California Coalition of Agencies Serving the Deaf and Hard-of-Hearing (CCASDHH), our attention has focused on designing and testing a tailored school-based intervention in collaboration with the California School for the Deaf, Fremont [10GT-3101, Pilot SARA/TRDRP). We are currently organizing an ongoing Advisory Committee to review all study findings, to identify community-based strategies that can effectively supplement our school-based effort, and to work with us in disseminating study results statewide.

In encouraging an effective community-academic partnership, this Pilot CARA study allows us to integrate and further our efforts to achieve the goal of tobacco control among Deaf youth, members of a unique, underserved and understudied community, as part of California's effort to reduce tobacco use among young people in every community, statewide.

One of the most notable characteristics of California's population is its cultural diversity. Because California is so culturally diverse, it is important to develop smoking prevention programs that are relevant to adolescents of varying cultural backgrounds. Our previous research had suggested that the influences of peers, parents, and the media on adolescent smoking might vary across cultural groups. We also expected that these influences would change as adolescents and their families acculturated to the U.S. culture, acquiring the values, beliefs, and behaviors of the United States in addition to those of their culture of origin.

The USC Integrated Research Program was undertaken to understand ethnic and cultural diversity in adolescent smoking, and to devise culturally-relevant strategies for prevention programs. This specific project within the IRP focused on understanding the influences of peers, parents, the media, and acculturation on smoking among adolescents of diverse cultural backgrounds living in California. This research has produced several important new research findings:

- Among Hispanic and Asian-American adolescents, acculturation to the U.S. culture is associated with a higher risk of smoking. One explanation of this is that adolescents who are more acculturated are more likely to spend time with friends who smoke, which creates a social norm tolerant of smoking and also increases their access to cigarettes.
- The nature of peer influences appears to vary across ethnic groups. Adolescents from the individualistic U.S. White culture are more likely to rebel against their parents' anti-smoking rules and to smoke as a way of asserting their independence. They tend to do this by associating with smoking peers and then beginning to smoke themselves. Adolescents from more collectivist cultural backgrounds, in contrast, tend not to experience this adolescent rebellion as strongly, and therefore are less likely to seek out rebellious peers and smoke with them.
- Adolescents caught between two cultures might be at extremely high risk of smoking. In particular, multi-ethnic adolescents (those who identify with two or more ethnic groups) appear to be at high risk of smoking initiation.
- A key objective of this study was to measure acculturation among adolescents, and to determine whether acculturation is associated with smoking behavior. However, acculturation turned out to be difficult to measure among 6th-graders, who are still in the process of forming their ethnic identities and also have limited reading comprehension. We developed a short acculturation scale for adolescents, validated it against other measures of acculturation, and administered it to 6th-graders in California. Because the prevalence of smoking was so low in our sample, we did not observe an association between acculturation and smoking. However, we expect that this association might appear as we follow the adolescents into 7th and 8th grade.
- Parental influences on smoking appear to vary across ethnic groups. Parental monitoring (the extent to which parents keep track of their children's activities and whereabouts) was

associated with a lower risk of smoking across ethnic groups. However, emotional attachment with parents appeared to be a protective factor only among Hispanic adolescents.

We plan to continue to follow these adolescents as they progress through middle school and high school, paying careful attention to the predictors of their smoking behavior and the effectiveness of our new culturally-relevant prevention programs. The knowledge gained in this research could be useful in helping educators and policy-makers design and implement smoking prevention programs that will be effective for the diverse California population.

8RT-0034

Ethnic Differences in Cigarette Smoking Dynamics among Youth

Xinguang Chen

University of Southern California

Cultural and ethnic diversity in California makes it necessary and provide a unique opportunity to examine ethnic differences in tobacco use. Information about dynamic changes of adolescent tobacco use by ethnicity is useful for tobacco control planning and decision-making at both state and local levels. Although levels and trends of adolescent cigarette smoking in California in general have been well documented, there is a lack of such information by ethnicity. The primary goals of this project include:

1. Analyzing and comparing levels and secular trends of cigarette smoking among adolescents from the four main ethnic groups in California during 900-2000;
2. Identifying factors associated with ethnic differences in the levels and trends of cigarette smoking; and
3. Testing age-period-cohort models and linear models in analyzing changes in smoking behavior as they are related to tobacco control programs and other factors.

Data used for this analysis were from the California Tobacco Survey (CTS; 1990 to 1993) and the California Youth Tobacco Survey (CYTS; 1994-2000). A total of 28,971 subjects entered the study, 50.5% of them were males, 12,292 (42%) from the CTS and 16,679 (58%) from the CYTS. The mean age (standard deviation) of the subjects was 14.42 (1.68) years with approximately 16-17% in each age group from 12 to 17 years of age. Among the total subjects 49% were non-Hispanic Whites, 35% Hispanic/Latinos, 7% Blacks and 8% Asians, and about 1% with ethnicity not identifiable across data set from various years.

Results from the analysis indicated that 30-day smoking prevalence increased from 1990 to 1997 before it declined, and percentage of never smokers increased from 1990 to 2000. Level of cigarette smoking was the highest among non-Hispanic Whites, the lowest among Blacks and Asian Americans, the median among Latino/Hispanics. Results from the linear modeling analysis indicated that the proportion of never smokers in Hispanic/Latinos increased faster than that for adolescents in other groups during the 1990-2000 period. Findings from the age-period-cohort model analysis indicated that the secular trends of cigarette smoking among adolescents in California were associated with a significant cohort effect, and this effect corresponds with the sustained comprehensive tobacco control in California that began in 1990.

Due to the resignation of the PI of this project to take a new position outside California, the delay in availability of the 2000 CYTS data, and the delay in hiring a qualified statistician for the project, the analysis was not completed by the termination date of the project. The work to be completed includes analyses of factors associated with the secular trends and ethnic differences in adolescents' cigarette smoking, such as pro-smoking media, smoking among important others, opinion/attitudes about smoking, and levels of acculturation. Because the PI has moved out of state, there has been no application for a no-cost time extension of the project to complete the analysis.

9DT-0174

Emotional Intelligence and Smoking

Dennis R. Trinidad

University of Southern California

Introduction: Adolescent tobacco use continues to be a major public health concern. Adolescent smoking prevalence has leveled off after an increase in the early 1990s, but is still higher than at any period of time in the 1980s. New avenues must be explored in order to further increase the effectiveness of today's tobacco use prevention programs. Recent interest in the concept of emotional intelligence (EI) has increased due to the popular media claiming it to be the most important predictor of life success, with some suggesting that EI could account for up to 80% of that variance. Though much popular literature and some scientific literature present claims that are overly optimistic, this exciting field of research continues to grow. Exploration of the relationship between EI and specific health behaviors, such as adolescent smoking, is of particular interest. An understanding of this relationship may help in designing improved targeted smoking prevention programs for adolescents.

Progress Toward Specific Aims: The dissertation based on this research was completed and the specific aims of this project were addressed. Modifications were made to adapt to the lower-than-expected smoking rates, and to make for a more well-rounded dissertation. The greater the adolescent's EI: (a) the more likely the adolescent was to see the negative social consequences of smoking; (b) the greater the adolescent's self-efficacy to refuse offers of cigarettes; and (c) the less likely the adolescent was to intend to smoke in the future. Those with low EI were more likely to intend to smoke if they had low refusal skills or were more hostile, while those with high EI were more likely to intend to smoke if they had previously experimented with cigarettes. The association between emotional intelligence and smoking intentions did not vary significantly across culture/ethnicity, though there was a trend towards significance. The trend suggested that EI might have been more protective against smoking intentions in the next year for White adolescents compared to Asian/Pacific Islander and Hispanic/Latino adolescents. Finally, as EI increased so did perceptions of the social consequences of smoking for those who were acculturated to the U.S. culture.

Future Directions: As the U.S. population becomes more culturally diverse, it becomes increasingly important to identify protective variables and design adolescent smoking prevention programs that will be more effective for adolescents of diverse ethnic and cultural backgrounds.

Those with high EI appear to be more protected against smoking risk factors. Therefore, administration of an EI survey at the baseline data collection phase of a smoking prevention program can help identify higher risk (i.e., low EI) adolescents for targeted, tailored interventions. As adolescent smoking prevention programs evolve, adding novel EI-enhancing components to future prevention programs may lead to increased effectiveness.

Examination of intervention program effects by EI level, or versus a control group, would be informative and may lend support to the current findings. Future research on EI and adolescent smoking would benefit by sampling older adolescents. Future versions of the EI scale used in this dissertation, the MEIS (Mayer, Salovey & Caruso, 1997), can be improved with the addition of even more diverse items within the MEIS, such as pictures of faces, musical selections, and vignettes. Consideration of alternative scoring methods, such as an expert consensus versus the sample consensus, may also diffuse some of the criticisms regarding validity of the MEIS's scoring.

9IT-0234

Cigarette Smoking in HIV-Positive Populations

Gary L. Humfleet

University of California, San Francisco

Given the high smoking rates in HIV-positive populations and the negative effect of cigarette smoking on HIV-related medical conditions, it is important to develop effective smoking treatments for this population. The specific aims of the current project were: to identify the important treatment elements to emphasize in smoking cessation programs for this population; to identify perceived barriers to treatment, preferred treatment elements, and preferred treatment settings; and to use this information to develop effective smoking treatments for HIV-positive cigarette smokers. We successfully achieved these goals.

The first study compared 68 HIV-positive gay male smokers with 67 HIV-negative gay male smokers on measures of nicotine dependence, mood, history of depression, stress, and readiness to quit smoking. We found that HIV-positive and HIV-negative gay male smokers did not differ on measures of nicotine dependence, current mood, and stress. Both HIV-positive and HIV-negative gay men reported high rates of Major Depressive Disorders and high levels of negative mood, particularly in comparison to a sample of male smokers from the general community. Both depression and negative mood are related to smoking treatment failure. We also found that HIV-positive men were significantly more likely to be ready to quit smoking than the sample of HIV-negative men.

We completed focus groups with 92 current smokers and former smokers. Participants identified smoking as a method to deal with stress, and a method for coping with and preventing depression. Many felt that quitting would have no impact on HIV illness. Most HIV-positive smokers in this sample were unaware of specific relationships between smoking and HIV illness. Many report that medical professionals suggest quitting, but offer little support. Many felt that treatments need to be intensive, convenient, and low cost.

Environmental Tobacco Smoke

8KT-0028

Effects of Exposure to Secondhand Smoke

Carlos Iribarren

Kaiser Foundation Research Institute

The overall objective of this study was to examine the health risks associated with non-smokers being exposed to secondhand smoke. The specific aims were to quantify the magnitude of these health risks with particular attention paid to cardiovascular diseases, cancer, and asthma. We were also interested in common health problems such as colds, headaches and hearing loss.

In our work using a large population of men and women who were members of a health plan in the Bay Area, being exposed to smoking by others was indeed related to greater risk of hay fever and asthma, severe headache, accelerated hearing loss with age, and cold/flu symptoms. It was also related with subsequent increased risk of heart disease and stroke, and we found that being exposed to cigarette smoke at home was worse than being exposed outside the home.

We are now studying whether exposure to second hand smoke might increase risk of certain forms of cancer, including lung, upper respiratory tract, stomach, pancreas, bladder, cervical and breast. Our findings may add to the growing knowledge about the dangers of exposure to secondhand smoke.

8RT-0072

Effects of Passive Smoking and Pregnancy

Kenneth W. Turteltaub

Lawrence Livermore National Laboratory

Passive smoking is a form of active smoking. Studies have suggested that second-hand smoke from cigarettes can cause cancer in people who have never actively smoked. The chemicals released from the burning end of the cigarette have not been quantitatively assessed for health effects. Nicotine is a major chemical in cigarette smoke with more of it present in this “side-stream” smoke than in the inhaled main-stream smoke. The aim of this study has been to assess ability of nicotine to reach target tissues (bioavailability) and its ability to bind to DNA and protein at exposure levels equivalent to one cigarette. To measure bioavailability and adducts in specific target tissues, radioactive labeled nicotine (^{14}C -labeled nicotine) was used as a tracer and the samples were analyzed by accelerator mass spectrometry (AMS), a novel and highly sensitive new technique.

To determine if nicotine reached specific tissues, mice were administered [^{14}C]-nicotine by injection at a dose of 125 $\mu\text{g}/\text{kg}$ body weight. Tissues, protein and DNA were analyzed for [^{14}C]-nicotine content between 0 - 48 h post exposure by measuring the ^{14}C in tissues by liquid scintillation counting and in DNA and protein by accelerator mass spectrometry. The result showed that [^{14}C]-nicotine was present in liver, lung, testis, brain and plasma with the highest levels found in the plasma, testis and liver followed by the lung and brain. Peak tissue levels were seen 15-60 minutes after exposure and declined thereafter. This established when nicotine

was present in the tissues of interest. To assess the dose response for nicotine in the tissues, ^{14}C -nicotine was administered to mice over a dose range spanning 75 $\mu\text{g}/\text{kg}$ to 0.5 mg/kg body weight. [^{14}C]-nicotine was measured at all these doses 1 hour following the exposure. The [^{14}C]-nicotine could be quantified in tissue at levels as low as 20 ng of [^{14}C]-nicotine per gram tissue. The greatest amount of nicotine was found in the liver followed by the kidney, lung, heart, and testis at all doses. Studies to assess whether DNA and protein binding was dose dependent revealed measurable adduct levels in protein from the liver, brain and testis at exposure levels of 75 or higher μg per kg body weight. Again, the liver had the highest level of binding, followed by the brain and testis. The nature of the dose responses for both the tissues and protein suggests a non-linear response. This work shows that nicotine is absorbed and distributed to various tissues at low doses. The data further show that nicotine or its metabolites bind to protein and DNA at these low levels. Similar studies with hydroquinone (HQ) implied that it is also bioactivated and binds to liver and bone marrow protein and DNA. HQ treatment resulted in higher protein adducts in bone marrow compared to liver, which suggests that HQ concentrates in the bone marrow.

Further work to build on the present study will pursue the refinement of the dose responses curves in tissues, proteins and DNA. Detailed metabolite analysis should be undertaken to identify the bioavailable forms nicotine and elucidate mechanisms of its action on DNA and protein. Future expansion of the study will compare the effects of ^{14}C -nicotine in female and male mice and in the developmental stages of mice for both protein and DNA damage in various tissues. Comparison of how nicotine is bioactivated at one cigarette equivalent exposure in the pregnant mouse, newborn and adult mice will provide needed data towards the understanding how dose influences bioactivation, and how the biomarkers used to measure chemical effects represent biological damage at low doses.

7RT-0099

Vapor-Phase Organics in ETS: Dynamics and Exposure

Alfred T. Hodgson

Lawrence Berkeley National Laboratory

Environmental tobacco smoke (ETS) is the complex mixture of ambient chemical vapors and particles that result from tobacco consumption. ETS contains the same toxic compounds inhaled directly by smokers, at lower concentrations and in varying proportions. There is strong evidence linking ETS exposure to a variety of health problems, including lung cancer, heart disease, asthma, and impaired respiratory function. Overall, ETS levels in buildings depend upon smoking periodicity and frequency, mixing volume (room size) and ventilation rate. ETS composition also changes over time as individual gases are selectively removed from air by their tendency to sorb (stick) to indoor surfaces. Sorption reduces exposure to some ETS constituents from recently emitted sidestream smoke, but later desorption can cause indirect exposure long after smoking ends. This project aimed to improve our understanding of the effect of sorption processes on ETS composition and exposure under realistic indoor conditions. Emphasis was placed on gas-phase organics in ETS that are established toxic air contaminants.

The main experimental focus of the project was to measure concentrations of representative ETS organic vapors, including the toxics 1,3-butadiene, acrolein, benzene, naphthalene, cresols, and ETS tracer compounds including nicotine and 3-ethenylpyridine, under varied smoking rate, room ventilation and furnishing levels in a simulated indoor environment. We developed a new concept of “exposure-relevant emission factors” (EREFs) to model exposure that includes the effect of initial sorptive losses and re-emission to and from indoor surfaces. EREFs were quantified for 26 ETS organic vapors in a series of 24 three-day experiments conducted in a living-room sized (50 m³) chamber. The chamber initially contained only painted wallboard and aluminum flooring, with carpet and furniture added in subsequent experiments. Cigarettes were machine smoked over three hours, and concentrations were measured during three daily periods that corresponded to active smoking, post-smoking and background. Smoking rate did not affect EREFs, indicating that sorption was directly related to air concentration. However, higher furnishing levels and lower room ventilation rates each substantially increased the amount of sorption, and thus decreased the effective exposure to less volatile toxics such as naphthalene, phenol, cresols, and to ETS tracers, with the largest effect seen for nicotine. These results highlight the importance of using emission factors that are appropriate to a specific environment to estimate exposures and of selecting tracers that adequately mimic the behavior of toxic ETS vapors. The results also indicated that indirect exposure that occurs outside of the presence of active smoking could be significant for some ETS toxics. A subsequent series of three four-week long experiments was conducted in the same room to investigate the effect of sorption processes on potential exposures when smoking occurs habitually on a daily basis. For sorbing compounds, concentrations during nonsmoking periods rose from day to day over the first one to two weeks. Once a steady cycle was obtained, potential indirect exposures during nonsmoking periods to compounds such as naphthalene and cresols were estimated to be about one-half of the total daily exposures. The experiments also demonstrated that ventilation rate is important with respect to the magnitude of indirect exposures. At two air changes per hour, the compounds are quickly removed from the room. As a result, overall exposures are lower and the majority of exposure to many ETS toxics occurs during active smoking.

This work made significant advancements in ETS exposure analysis methods and provided extensive emission factor and vapor-phase concentration data for a wide range of ETS toxics and tracers under realistic building conditions. These methods and data should enable researchers to more accurately assess risks from exposures to ETS organic vapors. The results also may help to direct public policy with respect to reducing ETS exposures.

Tobacco Control Policy

7RT-0180

Implementation of California AB13: Smoking Ban in Bars

Lisa A. Bero

University of California, San Francisco

In 1995, California AB13, prohibiting smoking in enclosed workplaces, took effect. In 1998 the smoking ban was extended to bars. We studied the 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 activist efforts to support implementation. We interviewed three sets of respondents: a random sample of bar owners (to date N=60); enforcement officials (to date N=23); and tobacco control activists (to date N=11). We conducted a content analysis of transcriptions of interviews and coded them.

When the workplace smoking ban went into effect in 1995, most employers voluntarily complied. The bar owners we interviewed claimed they were in compliance, but it became clear that they did not mean 100% compliance 100% of the time. Often they made exceptions, such as letting patrons smoke after 10 p.m., during special events, or in designated areas of the bar. The consequences of non-compliance were minimal: fines were relatively low and enforcement lax.

Many localities had not been faced with the task of having to enforce this state law until it was extended to bars. Local government often lacked resources for enforcement. Enforcement efforts were slow to be initiated, and typically, over time, the responsibility for enforcement was passed from one local agency to another.

This resulted in an important role for tobacco control activists from private non-profit organizations. These voluntary organizations generated public support for the law. Volunteers intervened and repaired breakdowns in the enforcement process, i.e.: initiating a writ of mandamus against a city for failure to enforce; educating and counseling district attorneys and judges to ensure their follow-through in prosecuting labor code violators; encouraging local district attorneys to file unfair business practices charges against non-compliant bars; and case finding to recruit individuals with disabilities for test cases to ensure access of people with sensitivities to secondhand smoke. Those persons who work for Proposition 99-funded programs are prohibited from engaging in enforcement activities; therefore, we were limited to using health education approaches that did not appear to be as effective in facilitating compliance.

Reduction in secondhand smoke and the consequent reductions in cancer and heart disease will only be realized if the smoking ban in bars is successfully implemented. This study has implications for future legislation in terms of:

Compliance: Passage of a law does not assure compliance. Health education models may not work with intransigent entrepreneurs such as bar owners. Activists should address bar owners'

fear that compliance will threaten profit, and raise their awareness of possible financial liability from workers' compensation suits.

Enforcement: The AB13 experience demonstrates a need for tobacco control legislation to clearly delegate and delineate enforcement mechanisms. Even though local control of enforcement appeals to tobacco control activists because of their history of success at the local level, not every locality will support enforcement.

7KT-0193

An Economic Analysis of Factors Influencing Teen Smoking

Sherry Emery

University of California, San Diego

The objective of this research project was to examine public policy issues related to adolescent smoking uptake. We analyzed the extent to which cigarette prices, anti-smoking regulations, and popular attitudes toward smoking affect rates of adolescent smoking initiation.

Although there is some agreement in the economics literature that state and federal excise taxes can play an important role in deterring teen smoking by raising the price of cigarettes, this issue is far from resolved. Research has shown that the price elasticity of demand for cigarettes among adolescents may be more than three times as high as the adult elasticity, and that the strongest impact of price is on adolescent smoking participation (Lewit and Coate, 1981; Chaloupka and Grossman, 1996). However, others have found that the role of price is actually much smaller or insignificant for teens (Wasserman, et al., 1991; Douglas and Hariharan, 1994).

In the first year of the project, our research suggested that there are several reasons why price may not be as important in teens' decisions to smoke as has been suggested so far, and that other factors may be more important than previously reported. First, over 80% of teens who smoke do not buy their own cigarettes; most obtain them from friends or other social sources (Emery, et al., 1999). Second, the price of cigarettes is often highly correlated with anti-tobacco policy variables that are much more difficult to measure; price coefficients may therefore reflect variations which are actually explained by these policy measures.

In the second and third years of this project, we used data from the 1993 cross-sectional component of the national Teen Attitudes and Practices Surveys (TAPS) to examine the extent to which cigarette prices influenced adolescent smoking uptake. We experimented with two model specifications, one with only the standard economic and demographic variables, and one that also included psycho-social variables. In both models, experimentation was not sensitive to cigarette prices or tobacco control policy variables. We found that current and established smoking status and conditional demand were both sensitive to cigarette prices, and the price effect was robust to model specification. Our elasticity estimate for current smoking participation was -0.83 , the conditional demand elasticity for current smokers was -0.87 , and total elasticity -1.70 . For established smoking participation, we estimated elasticity of -1.56 , a conditional demand elasticity of -0.68 , and total elasticity of -2.24 . Our elasticity estimates for current smokers are consistent with others in the literature. Ours is the first study to specifically

examine price elasticity among established adolescent smokers; our results suggest that this group is highly sensitive to cigarette prices. Our preliminary conclusions are that higher excise taxes may not deter experimentation, but appear to play an important role in discouraging smoking participation and may reduce the number of cigarettes smoked and therefore deter progression to established smoking (Emery, et al., 2001).

8RT-0094

Effect of Tobacco Related Policy Change on Smoking Behavior

David M. Burns

University of California, San Diego

Public policy changes with regard to smoking as well as changes in the environment in which the smoker lives and smokes are felt to strongly influence smoking cessation. Evidence from naturally occurring experiments that have examined smoking policy initiatives have shown that changes in smoking behavior can result from workplace smoking restrictions, increases in the cost of cigarettes and physician advice to quit smoking. The purpose of this grant was to use survey data collected in California and nationally to expand our understanding of the relationships between smoking policy changes and smoking behavior changes and to define the time course of these changes.

We used the 1990 and 1996 California Tobacco Survey (CTS) to examine the relationship between machine-measured nicotine yields and the number of cigarettes smoked per day. Using machine-measured nicotine levels for individual cigarette subspecies, we found a significant inverse relationship between the nicotine level of cigarettes and the average number of cigarettes smoked per day. We also used sales-weighted nicotine as a measure of the nicotine yield to examine this relationship. We found that for both survey years, smokers of cigarettes with nicotine levels below 0.95 mg. smoked 20% more cigarettes per day compared to smokers of medium-nicotine cigarettes. However, there was no difference in the mean number of cigarettes smoked per day between smokers of medium and high-nicotine cigarettes.

Analyses of smoking cessation from the 1999 CTS showed having had more education or being African American were associated with a greater likelihood of attempting to make a change in smoking status during the past 12 months. Other characteristics such as being female, older than 44 and smoking more than 4 cigarettes per day were associated with a lower likelihood of attempting to make a change in smoking status during the past 12 months. Being Hispanic or graduating from college or graduate school were associated with a greater likelihood of being a former smoker for 3 or more months at the time of the survey interview.

Analyses of the CTS data also showed significant increases in the proportions of smokers seeing a physician and receiving cessation advice between the 1996 and 1999 surveys. In 1996, smokers receiving advice were more likely to make a change in smoking behavior and to make a quit attempt than smokers who did not receive advice. In 1999, these differences were not observed. A physician's advice to quit was not associated with becoming a former smoker of any length in either survey year.

Currently, we are examining the temporal relationship between the cigarette tax increase in January 1999 in California and the introduction of nicotine replacement therapy (NRT) products over-the-counter with quitting behavior. Preliminary results from these analyses suggest that quitting increased in the months following the tax increase and that the rate of former smokers who used NRT increased in the months following the availability of NRT over-the-counter.

9RT-0048

The Role of Media in Smoking Initiation and Cessation

David M. Burns

University of California, San Diego

Despite claims to the contrary by the tobacco industry, cigarette advertising has targeted underage smokers, African Americans and other racial/ethnic groups, as well as concerned smokers who were contemplating quitting. As a result of this advertising, rates of smoking prevalence increased for adolescents during most of the 1990s, and until recently, smoking prevalence was higher among African-American smokers than among white smokers. It is also likely that the heavy advertising of low-tar cigarettes during the mid-1970s and early 1980s resulted in many smokers switching to low-yield brands rather than quitting smoking.

Using our cigarette advertising database and cigarette sales data, we examined the temporal relationship between the advertising and sales of the top 20 advertised low-tar brands between 1960 and 1996. Results showed that increases in the sales of low-tar cigarettes lagged behind the rise in advertising that featured low-tar themes prior to 1975. After 1975, the sales of these brands increased rapidly following a dramatic increase in advertising. The advertising for stand-alone low-tar brands (Carlton, True, etc.) rose slowly from 1960 to 1975 followed by a steeper rise between 1975 and 1981. Increases in sales of these stand-alone brands lagged behind increases in advertising; however sales increased dramatically after 1975 corresponding to a rapid increase in the advertising of these brands. The sales of low-tar brand extensions such as Marlboro Light and Camel Light also lagged behind increases in advertising for these brands. There was a steady increase in the advertising of low-tar brand extensions between 1969 and 1974, but dramatic increases in sales for these brands did not occur until 1975. The proportion of advertising and sales for low-tar product extensions rose more sharply than the advertising and sales for stand-alone brands, suggesting it may have been easier to get smokers to switch to low-tar extensions than it was to get smokers to try new low-tar brands.

We also examined whether the thematic content of advertisements for mentholated cigarettes popular with African Americans (e.g., Newport and Kool) differed from the thematic content of advertisements for non-mentholated brands popular with whites (e.g., Marlboro, Camel, and Winston). Analysis of the thematic data revealed that advertisements with people themes (themes that describe people or activities pictured in the advertisement) were more likely to appear in ads for cigarette brands preferred by African Americans than those brands preferred by whites. Themes related to smoker's economic concerns (i.e., the price of cigarettes) were more likely to appear in ads for brands preferred by African Americans; however, themes related to the

smoker's health were no more likely to appear in ads favored by African Americans than those preferred by whites. The results of this study suggest that the cigarette companies are using different advertising themes for white and African-American smokers. Future analysis will include the comparison of these same themes for cigarette advertising in magazines popular with African Americans.

9RT-0135
Proposition 99 Archives
Karen Butter
University of California, San Francisco

The University of California at San Francisco (UCSF) Library/Center for Knowledge Management achieved the objectives of its grant to improve access to primary source material in tobacco control.

In the spring of 2001, we hired a project archivist with specific experience in moving image formats as well as in paper and other media. With an emphasis on the Proposition 99 campaign and subsequent implementation, the archivist collected and processed 65.3 linear feet (space on a shelf) of documents, videotapes, audiotapes, CDs, DVDs and artifacts. The project archivist renewed and initiated contacts with agencies and individuals involved in tobacco-control projects throughout the State of California, as well as those in other states with appropriate records.

In the fall of 2001, the archivist conducted an audit of all collections in the Tobacco Control Archives, listing the status of processing and suggesting priorities, and also surveyed a 100-linear-foot collection for possible future acquisition. Although records of the Proposition 10 campaign were not acquired as a discrete collection, since the campaign materials are still tied up in litigation, much content regarding this campaign and the proposition's implementation was found in other acquisitions and existing collections. Implementing organizations were contacted, with some success in acquisition. All materials acquired were at least partially processed, and 10 of the 76 collections in the archives were newly described on the Online Archive of California (<http://www.oac.cdlib.org/>), a Web-based resource that provides guides to materials held in libraries, museums, archives, and other institutions throughout California.

In the course of the project, the Tobacco Control Archives acquired (through donations and purchases) 124 videotapes, mainly from the California Media Campaign. The project archivist oversaw the transfer of all videotaped content in the Tobacco Control Archives (including 138 videotapes previously in the collections, plus the new acquisitions) to a master format for preservation, with access copies made and the originals preserved for the future. Subsequently, the archivist conducted a complete inventory of the videotape collection to ensure its integrity before shipping the master and original copies off site for safekeeping. With the encouragement of the principal investigator and the TRDRP research administrator, the archivist produced a 25-minute video sampler ("Video Rainbow") both for presentation at the December 2001 TRDRP conference and for the use of researchers.

Staff continue to respond to questions about the collection and tobacco control issues in general. During this period a Frequently-Asked Question (FAQ) section was added to the TCA page on the UCSF Web site. Email, telephone, and in-person research assistance were provided to researchers, students, academics, attorneys, and the general public, as well as to health and tobacco-control professionals. The scope of inquiries was international, and assistance was provided in four languages.

Health Effects of Tobacco Use

9RT-0169

Nicotine Effects on Renal Function in Experimental Diabetes

Francis B. Gabbai

University of California, San Diego

Introduction: Smoking has been recognized as a major risk for the development and the progression of kidney disease in patients with both insulin-dependent and non insulin-dependent diabetes mellitus. As a risk factor, smoking: 1) increases the risk for the presence of protein in the urine; 2) shortens the time interval between onset of diabetes and onset of presence of protein in the urine; and 3) accelerates the rate at which patients lose kidney function and require dialysis in order to live. In spite of the major impact of smoking on progression of diabetic renal disease, very little is known about the mechanism(s) by which smoking accelerates kidney injury.

Topic addressed: These studies were designed to: 1) Investigate the effect of intravenous administration of nicotine (as a surrogate for smoking) on kidney function and to define whether nicotine modifies the pressure within the kidney filtering unit (glomerulus); and 2) Test if these changes in intraglomerular pressure can be prevented with the administration of an agent which blocks the effect of angiotensin II (a major kidney hormone).

Progress toward specific aims: Studies performed during this period of funding evaluated the effect of nicotine administration in glomerular pressure in normal rats and rats with experimental diabetes. The results of the studies demonstrate that the administration of nicotine increases glomerular pressure both in normal rats and rats with experimental diabetes. These changes in glomerular pressure were independent of changes in systemic blood pressure.

To investigate if the changes in glomerular pressure observed during nicotine administration reflect an increase in the activity in of one of the major renal hormones (angiotensin II) a new set of experiments was designed in which both normal rats and rats with experimental diabetes received losartan, an agent capable of blocking the effect of angiotensin II prior to the administration of nicotine. Results of this subset of experiments revealed that administration of losartan prevented the increase in glomerular pressure both in control animals and in animals with experimental diabetes during administration of nicotine. These findings clearly demonstrate that nicotine alters kidney function by increasing angiotensin II. The increase in angiotensin II observed during nicotine administration may be of critical importance to understand the effects of smoking/nicotine on progression of renal disease in diabetes. Previous studies have clearly established that increases in angiotensin II activity constitute a major risk factor for progression of renal disease. Further increases in angiotensin II secondary to smoking/nicotine could provide an important stimulus for progression of renal disease.

Future direction and impact: The importance of our present findings that nicotine increases angiotensin II is that the use of commonly prescribed medication for treatment of hypertension and proteinuria, like angiotensin converting enzyme inhibitors or angiotensin II receptor antagonist, may prevent smoking/nicotine induced renal damage. Based on the limited success

of smoking losartan, the possibility that a rather benign medication could be used to prevent or retard the effect of smoking/nicotine on renal function provides an extremely attractive alternative therapy.

7RT-0015

Role of Nicotine/Nicotinic Receptors in Parkinson's Disease

Maryka Quik

The Parkinson's Institute

Although the use of tobacco products is generally linked to harmful effects on health, an accumulating literature now suggests that smoking is associated with a decreased incidence of Parkinson's disease. Parkinson's disease is a movement disorder characterized by tremor, rigidity, and slowness of movement, as well as memory losses and personality changes. Initially the symptoms are mild, but may progress to a state where the individual is bedridden and totally incapacitated. The incidence of Parkinson's disease is about 1% of the population over 50, with an increased occurrence with advancing age. Parkinson's disease develops because of a degeneration of specific brain areas, the nigrostriatal pathway. Drug therapies currently available only treat the symptoms of this disorder. Since Parkinson's disease progression continues, the drugs become less and less effective with time. For this reason, there is a constant search for drug therapies with the potential to halt the neurodegenerative process. Interestingly, there is a lower incidence of parkinsonism in individuals using tobacco products that may be attributed to the nicotine in tobacco. In addition, anecdotal reports suggest that nicotine may have immediate beneficial effects on movement in Parkinson's disease.

The overall goal of our research is to investigate whether nicotine or selective nicotinic drugs may be useful in Parkinson's disease therapy either for symptomatic treatment or as a long-term, neuroprotective strategy to delay or prevent this debilitating neurological disorder. As an approach to this study, we did experiments to identify the molecules or receptors that are stimulated by nicotine to produce its effects in the brain. We focused on the nigrostriatal pathway because this is the one that degenerates in Parkinson's disease. Our results show that there are many different nicotinic receptors in brain regions that are important for Parkinson's disease, but that receptors containing an $\alpha 6$ protein subunit may be particularly important. Evidence for this stems (1) from our studies showing that $\alpha 6$ nicotinic receptors are very selectively localized to the nigrostriatal pathway and present in only a few other brain regions. Furthermore, our results showed that (2) $\alpha 6$ nicotinic receptors are the first to be affected after nigrostriatal damage. In fact, after moderate nigrostriatal damage, which may be similar to the early stages of Parkinson's disease, the $\alpha 6$ nicotinic receptors were the only ones that were decreased while other nicotinic receptor types were not changed.

These results suggest that the development of drugs directed to nicotinic receptor subtypes containing $\alpha 6$ subunits may represent an important therapeutic strategy for Parkinson's disease, either for symptomatic treatment and/or for long-term neuroprotection. The finding that the $\alpha 6$ receptor subtype is very limited in its CNS localization and not present in the peripheral nervous system would result in optimal beneficial and minimal side effects.

7RT-0039

Carbon Monoxide and the Brain Growth Spurt

John Edmond

University of California, Los Angeles

Carbon monoxide (CO) is produced and accumulates as the result of incomplete combustion, from burning tobacco, vehicle exhaust, and other heat producing systems. CO is particularly dangerous in closed and poorly ventilated environments. Inhaled CO can bind to hemoglobin in red blood cells and decreases their oxygen carrying capacity. CO is known to block respiration within cells. It is produced naturally in mammals and may be a distinctive messenger for nerve cells. Our studies were designed to examine the consequences of exposure to CO during a critical period of growth in the brain when it undergoes profound cell division and the development of an intricate multi-cellular organization. To test our hypothesis that exposure to CO causes developmental impairments in brain, we are examining exposure to CO using our animal model, the artificially reared rat pup. An important age for brain growth and onset of myelin formation takes place after birth within the milk-feeding period for the rat. We have developed controlled conditions to examine the specific consequences of mild CO exposure, rather than examine the gross pervasive damage that can be expected from overdosing with CO. We have discovered that CO at 25 ppm and less in air causes significant auditory deficits. The culmination of our studies indicates there are no differences in the data for many measures we have made in test and control conditions. However, we have identified the auditory brain stem as one region that can be compromised by exposure to 12.5 and 25 ppm CO in air. The most distinctive functional impairment to the auditory system is a significant attenuation of the amplitude of the eighth cranial nerve's action potential. Studies at the cellular level, by histological and immunochemical methods, demonstrate that several components of the auditory system are affected by mild CO exposure. The somata of several neurons in the spiral ganglia in the CO exposed animals are distorted in shape and size. The central process of the eighth nerve exhibits some myelin disorganization. Analysis of the cochlea of CO exposed animals demonstrates a marked decrease of cytochrome oxidase, calcium ATPase, NADPH diaphorase and neurofilament immunoreactivity when compared with controls. In the inferior colliculus, the basal c-Fos immunoreactive cells (most likely neurons) are decreased significantly in number in the CO exposed animals. Our studies indicate that several critical components of the auditory pathway are selectively affected by very mild CO exposure. We believe our studies are significant and can be related to human conditions where there is mild exposure to CO in the air. Attenuation of the amplitude of the eighth cranial nerve's action potential in children, similar to what we find in our rat pup model, might be a link to the disorder auditory neuropathy, in which children have normal otoacoustic emissions but a very poor, or absent, eighth nerve action potentials. These children have auditory processing disorders but often have normal hearing "sensitivity" with pure tone testing. Also they show language delays, because they cannot understand words that are spoken. These children are typically identified as "impaired" later in childhood.

7RT-0160

Vitamin E Kinetics in Smokers

Maret G. Traber

University of California, Davis

Smokers are subjected to oxidative stress and are known to have an increased incidence of diseases associated with oxidative stress. Vitamin E is a potent antioxidant that combats oxidative damage, yet little is known about the requirement for vitamin E by humans. The studies we carried out were to address the following questions: Does cigarette smoking increase utilization of plasma vitamin E in vivo? Are there regulatory mechanisms that increase plasma vitamin E in response to oxidative stress? Is α -tocopherol more susceptible to utilization to α -tocopherol in smokers?

To address these questions, we developed liquid chromatography/mass spectrometry (LC/MS) methodologies to measure stable isotope-labeled vitamin E, both α -tocopherol and β -tocopherol. Additionally, methodologies for measuring vitamin E metabolites in urine and serum were established. These methods allowed us to sensitively measure very small quantities of vitamin E in the plasma of smokers and non-smokers.

Aim 1. Does Cigarette Smoking Increase Vitamin E Turnover?

A trial was carried out in 6 smokers and 5 non-smokers. Subjects consumed 75 mg each d_3 -RRR and d_6 -all rac- α -tocopherol acetates (natural and synthetic vitamin E, respectively) daily for 7 days with a standardized breakfast. Fasting blood samples were drawn up to 28 days. After the week of supplementation, the % d_3 tocopherol (d_3 - α -tocopherol / total α -tocopherol \times 100) were similar in both smokers and non-smokers. Subsequently, there was a trend toward a faster disappearance of the plasma % d_3 α -tocopherol in smokers compared with non-smokers (0.30 ± 0.04 compared with 0.24 ± 0.05 , $p=0.056$). The calculated % d_3 half-lives were 55.6 ± 7.4 h in smokers and 72.1 ± 17.3 h in non-smokers ($p=0.063$). On the last day of the study, the % d_3 in smokers had decreased to $1.4\% \pm 0.3\%$ while it was $2.2\% \pm 0.7\%$ ($p=0.04$) in the non-smokers. These data suggested that smoking increases plasma vitamin E disappearance, but further studies were needed to confirm this finding and to assess its cause. Vitamin E kinetics were then evaluated in older (>50 y) smokers ($n=9$) and non-smokers ($n=6$). A similar protocol was followed with 24-hour urine collections on days -7, 0, & 21. Statistical comparisons indicated that there were no significant differences between smokers and non-smokers. It appears that both smokers and non-smokers prefer RRR- α -tocopherol, the natural form of vitamin E; the synthetic form more readily disappeared from the plasma. Therefore, the possibility that smokers might more aggressively metabolize the synthetic form was tested by measuring deuterium labeled CEHCs, but no differences were found between smokers and non-smokers.

Aim 2. Does Cigarette Smoking Up-Regulate Vitamin E Output by the Liver?

Vitamin E kinetics were evaluated in smokers ($n=9$) and non-smokers ($n=13$). Vitamin E, 50 mg each d_6 -RRR- α -tocopherol and d_2 -RRR- α -tocopherol was consumed with breakfast. Blood was drawn at 0, 3, 6, 9, 12, 24, 36, 48 and 72 h; plasma deuterated and unlabeled tocopherols were measured by LC/MS. Urine was collected from 24 to 48 h for metabolite measurements. Both groups similarly preferred α -tocopherol, suggesting that the oxidative stress of smoking does not increase the activity of the tocopherol transfer protein. These studies suggest that vitamin E

kinetics and metabolism are normal in smokers. It is likely that vitamin E supplements will not improve the health status of smokers.

7RT-0025

Transport and Disposition of Nicotine in the Human Placenta

Deanna L. Kroetz

University of California, San Francisco

Smoking during pregnancy is a major health risk for both the mother and her fetus. Nicotine is one of the major components of cigarettes and has been implicated in the adverse effects associated with smoking by pregnant women. The placenta is one potential barrier for limiting exposure of the fetus to potentially dangerous nicotine levels. We hypothesized that the conversion of nicotine to inactive byproducts (metabolism) within the placenta and the transport of nicotine across the placental membranes are the driving mechanisms which regulate nicotine levels in the fetus. The metabolic and transport capacity of the placenta will therefore be an important determinant of fetal nicotine exposure and therefore toxicity. The overall goal of the proposed studies is to understand the molecular mechanisms by which the placenta regulates nicotine levels. The objectives of these studies were: 1) to characterize the transport and metabolism of nicotine in human placenta, including identification of specific drug metabolizing enzymes and transport proteins involved in these processes; and 2) to determine whether the expression of nicotine metabolizing and transport proteins is altered in the placentas from smokers relative to non-smokers.

A series of studies was carried out to identify the specific transporter proteins that can facilitate the movement of nicotine either from the placenta into the fetus or in the opposite direction. Members of the multidrug resistance family of transporters were not able to influence cellular nicotine movement but the organic cation transporters did have this activity. The organic cation transporters were found in human placental tissue and the level of this protein was not affected by the smoking status of the mother. One of the multidrug resistance transporters, MRP1, did not interact with nicotine but the expression level of this protein was significantly lower in placentas from smoking mothers compared to controls. This suggests that MRP1 might play an important role in transporting other toxic constituents of cigarettes and that downregulation prevents movement of these compounds into the fetal circulation. Metabolism of nicotine occurs at only very low levels in the human placenta and in many cases is below the limit of detection. Interestingly, we found that the use of the nicotine replacement therapy lobeline (and herbal preparations containing even very low levels of lobelia) were potent inhibitors of the organic cation and multidrug resistance transporters. Such inhibition could lead to significant drug interactions in smokers receiving replacement therapy.

Despite the education of pregnant women about the harmful effects of smoking on the fetus, up to 16% of these women continue to smoke throughout their pregnancy. The results from the present studies indicate that the placenta does not provide an effective barrier against fetal exposure to nicotine. Common drug metabolizing enzymes and transport proteins that are found in the placenta do not interact with nicotine. These findings have important implications for the

health and viability of fetuses exposed to nicotine and support the overwhelming medical opinion that smoking cessation should be attempted as early as possible during pregnancy.

8DT-0174

A RNA Biomarker for Tobacco Smoke Toxicity

Richard H. Kimura

University of California, Davis

We have recently documented a conserved and novel stress response in animals as diverse as humans and insects. Short Interspersed Repetitive Element (SINE) RNAs increase as a result of cellular insults. Silkworm Bml SINE RNAs are induced by a variety of stress treatments including aqueous tobacco smoke and one of its constituents, catechol. We further determined that Bml SINE RNAs are induced upon exposure to heat shock, heavy metals, cycloheximide and virus infection making the transient increase observed in silkworm larvae a general stress response. In this same survey, we also reported a concomitant HSP 70 mRNA response to many of the diverse stress treatments studied. Since Bml SINEs are present in approximately thirty thousand copies, and the transcripts are directed by RNA pol III, they represent an unusual class of stress genes. Due to the sensitivity of this response to many stress treatments, SINEs may be developed as a versatile biomarker. However, tobacco products severely injure silkworm larvae presumably due to the nicotine. At the dose required for a SINE RNA response, these larvae are very close to death. Immediately upon injection, the larvae vomit a significant portion (c.c. 50% of more) of their body weight. The increase in SINE RNAs may be a secondary response to the initial effects of the acute toxicity of aqueous cigarette tar injection. To complete my study, I tested whether tobacco products induce SINE RNAs in human cells. Unfortunately, Alu (the human SINE) RNAs are not induced by aqueous cigarette tar in HeLa cells.

8IT-0052

Smoke on Bacterial Interactions with Airway Epithelial Cells

Terry Machen

University of California, Berkeley

Maintenance of an intact epithelial barrier is critical for the ability of the lungs to prevent access of bacteria and other pathogens to the blood and the rest of the cardiovascular system. An imaging microscope was used to test whether tobacco smoke causes the junctions that attach adjacent epithelial cells in the lung airways to come apart, allowing access of bacteria (normally retained in the mucus in the airways) to the blood-facing membranes of the epithelial cells. We first tested for interactions between airway epithelial cells and the common gram negative bacterium *Pseudomonas aeruginosa* (PA) in control condition. PA bind and kill the epithelial cells only after the bacteria have contacted the basal (blood side) surface of the epithelial cells, where the bacteria bind and then secrete a specific toxin directly into the cells. This toxin appears to disrupt the normal functioning of the calcium signaling apparatus in the cells as well as the mitochondria that are used for generating energy. These two events occurred nearly simultaneously, so it was possible that the two functions were both affected by the bacterial

toxin, whose function and structure is presently unknown. Thus, these experiments have already contributed to our understanding of how bacteria damage airway epithelial cells. We also used molecular methods to generate bacteria that exhibit green fluorescence so we could track their binding to the epithelial cells in the imaging microscope. Finally, a so-called gene microarray was used to monitor the activity of about 5,000 genes in the airway epithelial cells during exposure to tobacco-treated solution. This resulted in the altered activity (both up and down regulation) of more than 50 of these genes, many of which also exhibited altered regulation when PA contacted the blood side membranes of the epithelial cells. The gene responses during exposure to these bacteria indicated that inflammatory reactions are triggered most significantly from bacterial contact with the basolateral surface, as may occur when epithelia are damaged by smoke. The next step will be to make the critical test of whether tobacco affects the epithelial cells in a way that allows the bacteria to gain access to the key blood-side membranes, thereby exposing the epithelial cells to damaging and inflammatory effects of bacteria.

8RT-0073

A Novel Strategy for Protecting Neurons from Ischemic Injury

Robert Sapolsky

Stanford University

One of the main adverse consequences of tobacco use is increased risk of hypoxic-ischemic brain disease, including the global ischemia of cardiac arrest, and the focal ischemia of a stroke. There is now sufficient knowledge concerning the biology of the resulting neuron death to design protective interventions. The focus of my laboratory has been to design gene therapy strategies to block neuron death post-insult. The approach involves use of viruses (such as herpes simplex) which preferentially infect neurons. Viral genes that lead to dangerous replication are removed and replaced with potentially neuroprotective genes. This “vector” is then introduced into the brain.

We have explored the protective potential of some 20 genes against various neurological insults, both as modeled in neuronal cell cultures, and in animals. The present work concerns one novel intervention. Following ischemic injury, a subset of neurons that die do so by “apoptosis,” or programmed cell death. Such cellular suicide normally occurs during development, or when the immune system targets an infected cell, and the same pathway is maladaptively triggered in some injured neurons. “Apoptosis-inhibitor” genes exist in mammalian cells, and we have shown that the delivery of one of these (called Bcl-2) can decrease damage.

A main defense of the body against viral infection is to trigger apoptosis in infected cells (killing them before the virus can replicate). As a countermeasure, viruses have evolved an array of anti-apoptotic genes. As one facet of the TRDRP grant, we are exploring the neuroprotective potential of these viral genes. The rationale has been two-fold, beyond merely exploring another potential route of neuroprotection. First, it is given wisdom in virology that these are powerfully effective genes. Second, insofar as we have been using a viral vector system to deliver a *mammalian* anti-apoptotic gene, there may be unrecognized benefits to using a viral system to deliver *viral* anti-apoptotic genes, in terms of increased efficiency or potency.

We initially focused on one called Ksbcl-2; this is found in a herpes virus implicated in causing Kaposi's sarcoma, and is structurally related to the mammalian bcl-2 gene in a way suggesting that it should be even more neuroprotective. We have also expanded our work to include three other viral anti-apoptotic genes ("p35" from baculovirus; "CrmA" from cowpox virus; "gamma 34.5" from herpes simplex virus 1). We have observed: a) Ksbcl-2 decreases the neuron death in a culture model of anoxia. Despite this, it is less protective than anticipated, being no more so than its mammalian counterpart, bcl-2. We are now investigating why this is the case. b) The other genes are protective against culture models of anoxia and excitotoxicity, as well as against models of neuron death in the whole rat. c) Surprisingly, after careful study, we find no evidence that such protection actually involves blocking apoptotic death; instead, these genes block other cell death pathways. d) Preliminary data suggest that, instead, they protect by stabilizing neuronal energetics during insults. Our ongoing studies explore the protective potential and mechanisms of action of these genes.

8RT-0059

Nicotine Effects on Neurological Development

Raju Metherate

University of California, Irvine

A tragic effect of tobacco smoke is its effect on brain development in unborn and newborn babies. Smoking by pregnant mothers results in babies with diminished auditory function, and as these infants age they demonstrate deficits related to higher auditory-cognitive functions (e.g., speech comprehension). This research will determine how exposure to nicotine affects the normal development of the auditory cortex, the highest brain center responsible for hearing.

During the three-year project, we found that chronic nicotine exposure during the second week of life in rats (corresponding to third trimester development in humans) disrupts the functional development of auditory cortex. This finding implies a period of special sensitivity—a critical period—for the harmful effects of exogenous nicotine on auditory cortex development. We then determined that chronic nicotine exposure affects the expression of genetic material that codes for proteins, N-methyl-D-aspartate receptors, that are important for proper development of brain circuitry. To better study the cellular effects of nicotine exposure on auditory processing, we developed an in vitro preparation containing the final relay centers of the auditory system. The "auditory thalamocortical slice" preparation will enable detailed cellular studies of higher auditory system function and development, including the effects of nicotine. Finally, we have begun experiments that demonstrate significant effects of postnatal nicotine exposure on auditory function in the adult rat. These experiments may provide a link between animal experiments and the deficits in higher auditory functions caused by maternal smoking in humans. The research will benefit the public by increasing our understanding of how nicotine exposure affects brain development and function.

8DT-072

Modeling the Relation of Smoking to the Ovarian Functions

Yan Liu

University of California, Davis

The goal of this study is to evaluate anovulation, menstrual cycle characteristics, and urinary estrogen and progesterone metabolite levels as well as their relationships to smoking and other host factors. Standard sociodemographic questions were used in the baseline interview. American Thoracic Society questions were adapted to assess active smoking, and validated questions were used to assess self-reported passive exposure to smoke. The ovarian function outcomes were assessed from prospectively collected daily diary information and assays of daily urine samples for metabolites of sex steroid hormones, for multiple menstrual cycles in a large group of working women.

Algorithms to assess ovulatory status using daily urinary levels of estrogen and progesterone metabolites have been previously developed and applied. Neither of these algorithms is perfect, and under non-clinic-based circumstances, a gold standard is not available. In this study, Bayesian methodology has been used to estimate the unadjusted probability of occurrence of anovulation for smokers and non-smokers, and to evaluate the sensitivity and specificity of various algorithms, as well as to assess risk factors for anovulation, all in the absence of a perfect test. The Bayesian models were accomplished using one cycle randomly selected from each woman. Our results indicated that smokers had a moderately, but not statistically significantly, increased occurrence of anovulation.

We examined the effect of smoking on menstrual function, adjusted for other host factors. The day of ovulation for each ovulatory cycle was determined by using the Waller et al. algorithm. The generalized linear mixed models were used to account for the potential intra-woman correlation of outcomes. Menstrual cycle characteristics were examined as both continuous and dichotomous variables. Adjusted mean cycle length and mean luteal phase length did not significantly vary by smoking status, but interaction of smoking with age was observed for the follicular phase length. Smokers who were aged 35 years or older had a significantly decreased mean follicular phase length of 2.17 days (95% CI -3.97, -0.37). In contrast, follicular phase length was not significantly influenced by smoking in women aged 34 years or younger. Passive smoke exposure was associated with a marginally significantly (significance level = 0.05) greater likelihood of long menstrual cycles and long follicular phases, but current smoking was not significantly associated with any of the dichotomous endpoints we examined.

We modeled the population-averaged curves for the excretion profiles of urinary estrogen and progesterone metabolites as smooth functions of time, based on one randomly selected cycle from each participant. This was accomplished using the semiparametric stochastic mixed model developed by Zhang et al, in which regression parameters were used to model the effects of smoking and other host factors. Our results showed that smokers exhibited a non-significantly less pronounced estrogen metabolite excretion level at the midcycle and in the luteal phase. Smokers also had a significantly more pronounced progesterone metabolite excretion level during the entire menstrual cycle. Women who were passively exposed to smoke did not differ in

either estrogen or progesterone metabolite excretion levels compared with non-smokers not passively exposed.

To conclude, results obtained in this study indicate that smoking, a potentially modifiable risk factor, is associated with ovarian function that may in turn be related to subsequent disease risk in women.

9DT-0046

Relationships between Smoking, Homocysteine, and Folate

Archana J. McEligot

University of California, San Diego

Numerous studies have reported on the association between elevated plasma homocysteine and coronary, cerebral, and peripheral arterial disease. A meta-analysis by Boushey et al. (1995) of 27 studies examining the relationship between homocysteine and heart disease revealed that a 5 μ mol/L increase in homocysteine corresponded to a 60% increased risk of heart disease in men and an 80% increased risk for women. Also, several studies have reported that smokers have higher plasma homocysteine concentrations compared to non-smokers. Increasing consumption of folate, primarily found in dark, green leafy vegetables, has been shown to reduce plasma homocysteine concentrations.

We examined plasma homocysteine and folate concentrations in women smokers participating in a diet intervention trial, which investigated the effect of diet on breast cancer recurrence. Women enrolled in the trial and randomized into the intervention group consumed a diet high in vegetables, fruit, and fiber and low in fat, while the control group was encouraged to follow the National Cancer Institutes dietary guidelines. Plasma homocysteine, folate and other key variables were examined at baseline, and 12 months. For the final sample at baseline, participants were 92 smokers matched on baseline dietary folate intake, age and intervention status to 92 non-smokers. The statistical analysis included independent t-test analysis to compare differences in plasma homocysteine and serum folate concentrations, and key dietary variables between smokers and non-smokers. No significant differences were observed for plasma homocysteine and serum folate concentrations at baseline between smokers and non-smokers. Examination of dietary intakes at baseline showed that smokers consumed significantly less vegetables, fruit and fiber, and consumed significantly more alcohol, caffeine and percent energy from fat compared to non-smokers ($P < 0.05$). However, at follow-up, paired t-test analysis revealed that, similar to non-smokers, smokers in the intervention group significantly increased vegetable, fruit and fiber intakes and decreased percent energy from fat from baseline ($P < 0.05$). To examine whether smokers were at a higher dietary folate requirement we conducted a mean percent change analysis. This analysis suggested that even with a proportional increase in dietary folate intake for smokers and non-smokers in the intervention group, plasma homocysteine concentrations significantly decreased only in non-smokers ($P < 0.05$), but this was not observed in the control group.

For the immediate future, we will focus on refining the analysis and submit the results for publication. The findings from this study suggest that at baseline (pre-randomization) smokers have a less healthful diet compared with non-smokers, but that smokers in a diet intervention trial can adhere to dietary goals and increase fruit and vegetable consumption. The mean percent change analysis for the intervention group suggests that further examination, in a larger study, is required to assess higher dietary folate requirements for smokers compared with non-smokers, which could contribute to the understanding of the mechanism associated with cigarette smoking and increased cardiovascular disease risk.

7RT-0073

Maternal Repair of Tobacco-Induced Sperm Lesions

Andrew J. Wyrobek

Lawrence Livermore National Laboratory

The purpose of this research is to generate new knowledge about molecular and genetic factors in paternal germ cells and zygotes that can increase the risk for chromosomally abnormal pregnancies in couples where the father smokes. We hypothesized that (a) paternal exposure to tobacco smoke induces genetic lesions in sperm that accumulate during the repair-deficient period of spermatogenesis and (b) that genetic deficiencies in maternal DNA repair genes may diminish the zygote's ability to repair sperm lesions leading to higher rates of chromosomally abnormal pregnancies and offspring. Three specific questions were addressed:

1. Sperm lesions accumulate during male post-meiosis after chronic exposures to tobacco smoke constituents and what is the dose dependency for paternally transmitted chromosomal aberrations at first-cleavage (1-C1) metaphase?
2. Are specific maternal DNA repair genes (Ku86, Rad54, Xpa, Xpc, Xrcc1, Ape1, and Tp53) involved in the processing of tobacco-induced sperm lesions into chromosomal aberrations at 1C1?
3. Does the genotype of the female partner (i.e., the egg) change the risk of paternally transmitted chromosomal aberrations after chronic paternal exposure to tobacco-smoke constituents before fertilization?

Analysis of 1-C1 metaphases collected after paternal exposure for 1, 2 or 3 weeks with diepoxybutane (DEB), a constituent of tobacco smoke using a PAINT/DAPI probe combination that detects ~60% of all possible exchanges suggest that the last two weeks before fertilization is the critical time-window for the induction of chromosomal damage in sperm after paternal exposure to DEB. Comparisons between acute and chronic exposures suggest that there is accumulation of DNA lesions in sperm and that the repair deficient window of mouse spermatogenesis may be less than two weeks. We have also investigated the expression pattern of the 7 DNA repair genes before and after fertilization. Cumulus cells and oocytes appear to express certain genes differently. Differences in the expression pattern between oocytes and zygotes were detected by RT-PCR and real time PCR. Rad54 and Tp53 were consistently detected in cumulus-free oocytes and zygotes. Microarray experiments (Affymetrix chips) showed that as many as 200 genes are modulated in the zygotes after paternal exposure to DEB. Finally, experiments with XPA null female mice suggest that the nucleotide excision repair

pathway is not involved in the zygotic processing of DEB-induced sperm lesions into cytogenetic abnormalities. This project represents an important first-step towards understanding the molecular interactions between paternal exposure to tobacco smoke, the induction of genetic lesions in sperm, the DNA repair capacity of the fertilized egg, and the risk of paternally transmitted chromosomal abnormalities. These findings will have important implications for identifying at-risk human pregnancies, especially for couples where the father smokes.