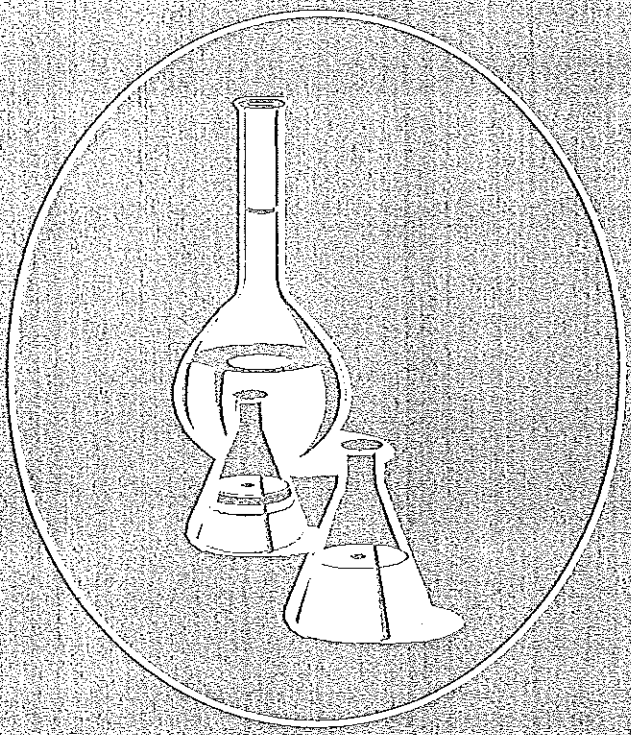
Arizona Disease Control Research Commission



1997-1998 Annual Report

January 1999

ARIZONA DISEASE CONTROL RESEARCH COMMISSION

ANNUAL REPORT
1997-98

Jane Dee Hull, Governor

Henry Reaves, Ph.D., Chairman
Orme Lewis, Jr., Co-chairman

COMMISSION MEMBERS

General Public
Lois Emden
Jose Cardenas, J.D.
Orme Lewis, Jr.

Medical Community
Patricia Moore, Dr.P.H.
John Oakley, M.D.
Eladio Pereira, M.D.

Scientific Research Community
Stan Lindstedt, Ph.D.
Henry Reeves, Ph.D.
Walter Williams, Ph.D., M.D.

Staff
Executive Director: Dawn Schroeder, D.D.S., M.A.
Administrative Services Coordinator: Damika Brock-Jackson
Fiscal Service Specialist II: Dan Powell
Administrative Assistant II: Ismene Quintanilla

1616 West Adams Street, Suite B-25
Phoenix, Arizona 85007
Telephone: 602.542.1028
Fax: 602.542.6380

January 1999

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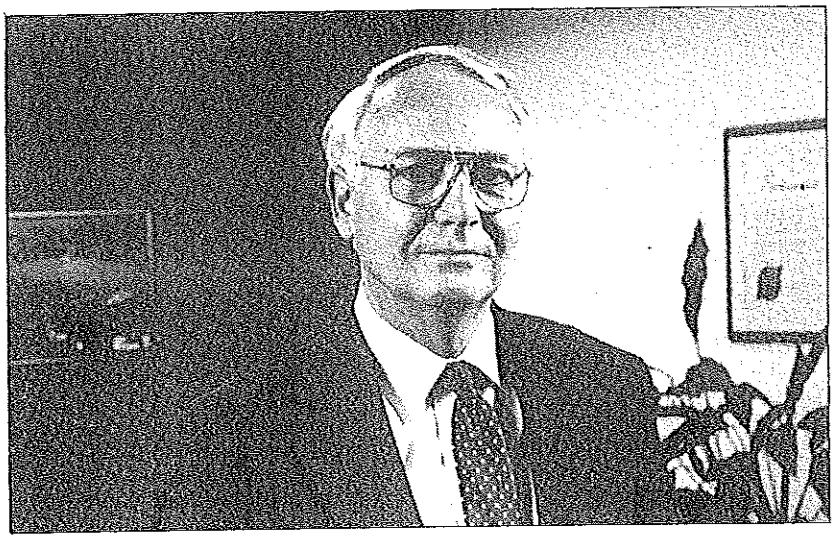


Message from the Chairman

As Chairman of the Arizona Disease Control Research Commission (ADCRC), I am pleased to forward this agency's Fiscal Year 1998 Annual Report. Commission activities contribute to improving the health of Arizonans through scientific research. Established by the Arizona Legislature in 1984, funding comes through the Tobacco Tax Initiative, passed by the voters in 1994. The ADCRC receives five percent of the revenues collected from the tax to fund tobacco-related disease research. Currently, the Commission receives no other funding for its mission of contracting for research into the cause, epidemiology, diagnosis, treatment and prevention of diseases.

Fiscal Year 1998 brought many changes to the Commission. The Legislature passed three revisions to the Commission statutes: 1) the Commission may now elect its own Chairman from amongst the members, a position historically held by the Director of the Arizona Department of Health Services, 2) the Commission may be involved in technology transfer and patent and licensing of discoveries funded with ADCRC monies and 3) a portion of the Tobacco Tax revenues, $10,000,000, was redirected into a four-year Anti-cancer Drug Discovery Program.

The impact of these changes is already being felt. The Commission selected four of its members to serve on a Patent and Licensing Subcommittee to manage technology transfer issues. In the spring of 1998, in partnership with Baylor University, the Commission filed its first patent. The Request for Proposals for the Anti-cancer Drug Discovery Program will be available in late summer and awards will be made in the spring of 1999. This is a much needed program unique to Arizona. As we enter our Sunset Year in FY 1999, we are pleased with the progress that has been made.

The Annual Report is prepared and submitted in January of each year to the Governor, the President of the Senate and the Speaker of the House of Representatives. We appreciate their continued support.

The Commission Members

Nine Commissioners guide the work of the Arizona Disease Control Research Commission. They are appointed by the Governor and confirmed by the Senate. The Commission is divided into three communities, General Public, Medical and Scientific Research. Each community is represented by three Commissioners appointed for three-year terms. Generally, the terms of three members expire each year; Commissioners may be reappointed. The Chairman and Commissioners who served during 1997-98 are presented below.

Henry Reeves, Ph.D., Chairman

Professor Emeritus
Arizona State University

Commissioner Reeves was a Professor of Microbiology at Arizona State University from 1969 to 1993. During that time he also served as chair of the Department of Microbiology from 1970 to 1973 and as Vice President for Research from 1985 to 1991. On leave from Arizona State University, he served as director of the Division of Physiology, Cellular and Molecular Biology at the National Science Foundation from 1976 to 1979. Commissioner Reeves received his B.S. from Franklin and Marshall College and his M.A. and Ph.D. from Vanderbilt University. He was appointed to the Commission by Governor Symington to complete the term of Commissioner James Bloedel in 1995. He was reappointed in 1996 and his term expires in May 1999.

General Public

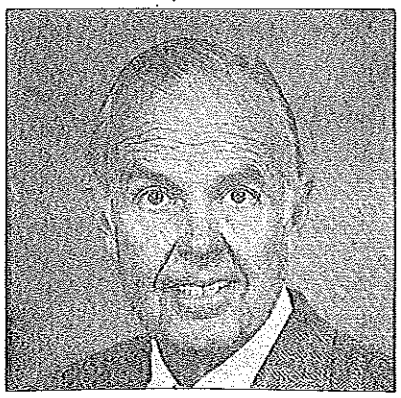
Jose Cardenas, J.D.
Managing Partner, Law Firm of Lewis and Roca, Phoenix

Commissioner Cardenas received a B.A. from the University of Nevada, Las Vegas in 1974 and a J.D. from Stanford University in 1977. He joined the firm of Lewis and Roca in 1978 where he practices primarily in the areas of commercial and civil litigation, intellectual property, and international law. Commissioner Cardenas is a member of the Maricopa County, Arizona and American Bar Associations, the American Law Institute and the Hispanic National Bar Association. He served as president of the Los Abrogados Hispanic Bar Association from 1985 to 1988. Commissioner Cardenas is currently the president of the American-Mexico Commission and is a member of the U.S. Delegation of the NAFTA Advisory Committee on Private Commercial Disputes. He serves as a member of the Minority Council Advisory Committee to Arizona State University President, Lattie Coor. Commissioner Cardenas was appointed to the Commission by Governor Symington in 1996. His term expires in May 1999.

Lois Emden, M.S.
Nutritional Counselor, Paradise Valley

Commissioner Emden received a B.S. in 1963 and an M.S. in Education from Hofstra University in 1967. She is an active participant in the Cancer Awareness Programs sponsored by the Arizona Cancer Center. Commissioner Emden served as an advocate representative for Scientific Peer Review with the 1995 and 1997 Department of Defense Breast Cancer Research Program. She is a Phoenix Art Museum Docent. Commissioner Emden was appointed by Governor Symington in 1994 and reappointed in 1997. Her term will expire in May 2000.

Orme Lewis, Jr., Co-chairman
Managing Director, Select Investments, L.L.C., Phoenix



Commissioner Lewis oversees commercial real estate interests and participates in environmentally sensitive businesses. He currently serves on the governing boards of the Arizona Historical Foundation, Arizona State University Foundation, Phoenix Children's Hospital, and the Polycystic Kidney Research Foundation. He is a former member of the Governor's Regulatory Review Council and the U.S. Advisory Committee on Mining and Mineral Research. Commissioner Lewis was elected to the 23rd and 24th Arizona State Legislatures. He received a B.S. in Economics in 1958 from the University of Arizona. Commissioner Lewis was appointed to the Commission by Governor Symington in 1995 and reappointed by Governor Hull in 1998. His term will expire in May 2001.

Medical Community

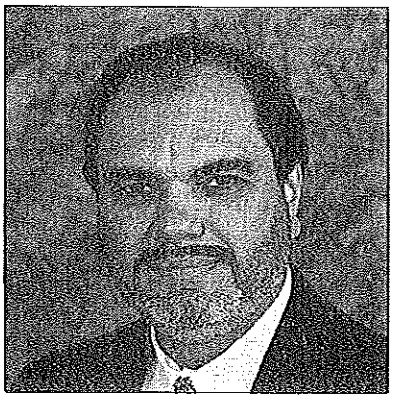
Patricia D. Moore, Dr.P.H., R.N.
Chair, Division of Community Health Nursing
Arizona State University

Commissioner Moore received her doctorate and master's degrees in Public Health from the Johns Hopkins University School of Hygiene and Public Health and a master's degree in Nursing from the Catholic University of America. Commissioner Moore is an Associate Professor and Chair of the Division of Community Health Nursing, Arizona State University. She is a Fellow of the American Academy of Nursing. Appointed to the Commission by Governor Symington in 1993, Commissioner Moore was reassigned to the Medical Community in May of 1994. She was appointed to a second term in 1996 and her term expires in 1999.

John E. Oakley, M.D.
Family Practice, Prescott

Commissioner Oakley received his A.B. from Washington University, St. Louis, and an M.D. from the University of Missouri, College of Medicine. He completed a rotating internship and four-year residency in General Surgery at St. Louis County Hospital. A member of the Arizona Medical Association for 35 years, Commissioner Oakley has also served as president and vice president. For 15 years, he was a preceptor professor for the University of Arizona, College of Medicine. Commissioner Oakley has practiced general medicine and surgery in Prescott since 1962. Commissioner Oakley was appointed by Governor Symington in 1994 and reappointed in 1997. His term expires in May 2000.

Eladio Pereira, M.D., F.A.C.P.
Chief, Internal Medicine
Mariposa Community Health Center, Nogales



Commissioner Pereira received his B.S. in Chemistry from Georgia Tech in 1979. He graduated *Magna Cum Laude* from Emory University School of Medicine in 1983. After completing his Internal Medicine Residency at the same institution, he joined the staff of the Mariposa Community Health Center as a Scholar of the National Health Service. He returned to Emory University in 1990 where he was an Assistant Professor of Medicine and director of the Intensive Care Unit at Grady Memorial Hospital in Atlanta until 1992. He has been a Fellow of the American College of Physicians since 1993. In February of 1998, he was named Chief of the Medical Staff and Clinical Services at Mariposa, overseeing an 11- physician group that provides 60 percent of the medical care in Santa Cruz County. He was appointed to the Commission by Governor Symington in 1995 to complete the term of Commissioner Carlos Gonzales whose term expired in May 1996. Commissioner Pereira was reappointed in 1996. His term expires in May 1999.

Scientific Research Community

Stan Lindstedt, Ph.D.
Professor of Biology
Northern Arizona University

Commissioner Lindstedt received his B.S. in Biology in 1970 from the University of Southern California and his Ph.D. in Zoology from the University of Arizona in 1977. He completed a National Science Foundation Fellowship in the Department of Anatomy, University of Berne, Switzerland in 1981. Commissioner Lindstedt is Treasurer and Steering Committee Member of the American Physiological Society. He is the author of numerous articles and serves as a referee for a number of scientific publications including *Science, Nature* and the *Journal of Applied Physiology*. Commissioner Lindstedt was appointed to the Commission by Governor Symington in 1995 and his term expires in May 1998.

T. Lon Owen, Ph.D.
Professor of Medical Anatomy and Physiology
Northern Arizona University

Commissioner Owen received his B.A. in Zoology from the University of California, Davis, a master's degree in Biology from California State University, Sacramento, and his Ph.D. in Physiology from U. C. Davis in 1972. He was a National Institutes of Health Postdoctoral Fellow at Michigan State University and Visiting Associate Professor in the Pharmacology Department of the University of Arizona College of Medicine. He has chaired the Research Committees of the American Heart Association at both the Arizona Affiliate and Southwestern Regional levels. His publications are in the areas of cardiovascular, aging and environmental physiology. Commissioner Owen was appointed to the Commission in June 1998 by Governor Hull and his term expires in May 2001.

Walter Williams, Ph.D., M.D.
Associate Professor, Department of Radiology
University of Arizona

Commissioner Williams received his B.S. in Chemistry from the University of Missouri in 1963, his Ph.D. in Physical Chemistry from Purdue University in 1968 and his M.D. from Yale University in 1980. Commissioner Williams was a member of the Science Team for the Voyager Spacecraft Missions to Jupiter and Saturn and was a Senior Scientist at the Jet Propulsion Laboratory, California Institute of Technology prior to returning to school to study medicine. From 1985 to 1987, he was a clinical instructor in the Joint Program for Radiology and Nuclear Medicine at Harvard. He has authored numerous publications in the areas of physics and medicine. Commissioner Williams was appointed by Governor Symington in 1994 and reappointed in 1997. His term expires in May 2000.

Commission Staff

Dawn Schroeder, D.D.S., M.A.
Executive Director

Dr. Schroeder received her B.A. in Biology and Psychology from Augustana College in 1969, her D.D.S. with Thesis Honors from State University of New York at Buffalo in 1975 and her M.A. in Health Services Administration from Webster University, St. Louis, Missouri in 1981. She completed a residency in Oral and Maxillo-facial Surgery at the Naval Hospital, Oakland, California in 1985. Dr. Schroeder has held her present position since September of 1992.

Damika Brock-Jackson
Administrative Services Coordinator

Mrs. Brock-Jackson joined the Commission staff in October of 1993. She accepted her current position in October of 1997. Mrs. Brock-Jackson is responsible for purchasing, travel and payroll as well as contract file maintenance. She is also a part-time student at Phoenix College where she is pursuing a management degree.

Daniel Powell
Fiscal Services Specialist II

Mr. Powell has been with the Commission since June of 1994 and accepted his current position in March of 1997. He is responsible for contract expenditures and accounts payable. Mr. Powell spent four years in the U.S. Navy, including a tour of duty aboard the U.S.S. *John F. Kennedy*, prior to joining the Commission staff. He is pursuing a liberal arts degree at Glendale Community College.

Ismene Quintanilla
Clerk-Typist III

Mrs. Quintanilla is the newest member of the staff. She came to the Commission in January 1998. She is the receptionist and is responsible for routine clerical tasks as well as maintaining the mailing and peer reviewer databases. Mrs. Quintanilla is a part-time student at Phoenix College where she is pursuing a degree in accounting.

Summary of 1997-98 Commission Activities

The Commission had 95 contracts with medical and health researchers in Arizona as of July, 1997. Contract summaries are contained in Sections A-C. The section headings list the source of funding and whether the project is in its first, second or third year of funding.

Abstracts for each project, outlining the progress made during the year, are included. Citations for scientific publications and abstracts arising out of the research are also listed. Lay summaries for new awards made in 1998 and scheduled to begin in July 1999 can be found in Section E. These summaries provide an overview of the new research.

Approximately 1059 Requests for Proposals (RFPs) for 1997-98 awards were mailed to potential applicants in August 1996. The amount available for new tobacco-related research contract awards was approximately $2,750,000. In response to the RFP, the Commission received 78 proposals in November 1997. Section D lists the research proposals received in response to this RFP.

In November and December the proposals were sent to a panel of national scientific and medical experts for peer review and evaluation. In January, February and March, the Commission received approximately 230 proposal evaluations prepared by more than 100 out-of-state peer reviewers. Three reviews were sought for each proposal. The proposals and evaluations were distributed to the Commissioners who were formed into three, three-person subcommittees to facilitate the final review process. In April the Commissioners selected 27 award-winning research projects from among the applications submitted. The New Awards are listed in Section D. The number of contracts decreased in FY 1998 with the conclusion of the unrestricted medical research program. During 1998-99, the ADCRC will be managing 85 contracts.

SECTION A

CONTRACTS

TOBACCO-RELATED RESEARCH

YEAR ONE

Emmanuel T. Akporiaye, Ph.D.

University of Arizona
Award Amount FY 1998: $50,000

Active Specific Immunotherapy of Breast Cancer by Inhibition of an Immunosuppressive Cytokine

TGF-β is an immunosuppressive molecule produced by tumor cells that enables the tumor to escape immune cell-mediated destruction. Previously, we demonstrated that insertion of an antisense copy of the TGF-β gene (αsT) into non-metastatic EMT6 murine mammary carcinoma cells reduces both their ability to produce TGF-β and suppress anti-tumor responses. In this study, we show that insertion of the IFN-γ gene, an immune cell activator, into αsT expressing EMT6 cells generates an anti-tumor response greater than EMT6 cells expressing either gene alone. These data suggest that down regulation of TGF-β-mediated immunosuppression combined with IFN-γ-mediated immune augmentation is a useful strategy in treating cancer. We also demonstrated that inserting either gene, alone, into metastatic mammary carcinoma cells, 4T1, reduces their ability to form tumors and metastasize. Studies are ongoing to determine the effect of simultaneous expression of αsT and IFN-γ by 4T1 cells on metastatic spread.

Sherry H. Chow, Ph.D.

University of Arizona
Award Amount FY 1998: $49,999

Pharmacokinetic Modeling of the Anabolites of Nucleoside Analogues

This research program has characterized the tissue disposition kinetics of the active zidovudine metabolites in mice following zidovudine administration. The disposition of the active zidovudine metabolites, after a single dose administration of zidovudine, was found to be different in different tissues. Zidovudine was effectively converted to the active forms in the spleen and bone marrow. Conversion of zidovudine to the active forms was not effective in the lymph nodes and brain. In animals with chronic virus infection, significant changes were observed in the distribution of zidovudine into the lymph nodes and in the phosphorylation of zidovudine in this tissue. The tissue-specific disposition of the active metabolites was similarly observed in animals receiving chronic zidovudine treatment. The inadequate maintenance of the level of active zidovudine metabolites in the lymph nodes and brain has therapeutic implications applicable to Arizona residents, since these two tissues are important targets in treating HIV infection.

William J. Grimes, Ph.D.

University of Arizona
Award Amount FY 1998: $50,000

HLA and p53: An *in vitro* Study of Peptide Immunotherapy in Human Lung Cancer

Our goal is to develop methods to both measure immune responses made against cancer cells of the lung and to develop better antigens that should be able to stimulate an anti-cancer immune response and improve patient outcomes. The protein antigen that we are studying is called p53; it is associated with normal cell growth and behavior. Mutations or alterations in this protein have been the single most important variation found in cancer and are responsible for a large part of the difficulties encountered in treating human lung cancers. We have developed a laboratory method to study the ability of p53 to act as an antigen, or potential cancer vaccine. We stimulate cells of the immune system in the laboratory and then make artificial beads that carry the p53 antigen. The immune cells respond to the beads as if they were actual cancer cells and we can then modify the antigen to try to get a stronger response. These modifications studied in the laboratory should guide our efforts to develop successful anti-tumor immunotherapies against lung cancer, and other forms of cancer.

Kenneth D. Hatch, M.D.

University of Arizona
Award Amount FY 1998: $149,826

HPV, Chlamydia and Cervical Dysplasia Prevalence Among Smokers *vs* Non-Smokers Along the United States-Mexico Border

We have successfully recruited 2193 women, ages 15 years and older, to participate in this binational study of human papillomavirus infection (HPV), chlamydia infection and cervical cancer. Women were recruited from eight different sites in three pairs of communities on the border, cities of Hermosillo and Sonora, Mexico and Tucson, Arizona. To date, we can report on the prevalence of HPV and chlamydia infections. We are currently completing the Pap smear review and are quality-control-checking our data from the risk factor questionnaires we administered. The overall rate of HPV infection along the Arizona-Sonora border was 16.8 percent. No differences in rates of HPV infection were observed by country of residence. In contrast, the rate of chlamydia infections was 4 percent, with significant differences observed by country of residence: 5.2 percent in Sonora and 1.7 percent in Arizona. Over the next year, we will determine the risk factors by country of residence for these infections.

The-Li Huo, M.D., Ph.D.

University of Arizona
Award Amount FY 1998: $50,000

Molecular Mechanism of Hormone-regulated Calcium Transport in Kidney

The goal of this research project is to find out the basis of abnormal calcium metabolism in hypercalcemia, osteoporosis and kidney stone disease. The calcium transport mechanisms may be very abnormal since hypercalcemia is associated with over-production of calcium in bone and under-secretion of calcium in kidney. Osteoporosis and kidney stone disease may be associated with over-secretion (or under-reabsorption) of calcium in the kidney. In the first year of this project, we were in the progress of discovering the most important calcium transporter which is responsible for the kidney calcium reabsorption. We have successfully used the advanced "yeast two hybrid cloning system" techniques. Several candidates of the novel calcium transporters were obtained. Currently, we are in the process of identifying the complete sequences of these candidates. When the sequences are complete and the correct structures are identified, we will be able to study the detailed function and expression of this specific kidney calcium transporter. The results from this research will have significant impact on the development of new drugs which can be used to treat hypercalcemia, osteoporosis and kidney stone disease more effectively.

Douglas F. Lake, Ph.D.

University of Arizona
Award Amount FY 1998: $49,941

Genetic Immunization Against Mutant p53 for Lung Cancer

Tumors are often the result of normal genes developing mutations so they no longer perform their jobs properly. One of these genes is called p53. Mutations in p53 cause tumors in Arizona residents and others throughout the world. Our strategy is to utilize the immune system to attack tumor cells bearing mutant p53. We developed a model in which mouse skin is immunized with tiny gold beads coated with mutant p53 using a "gene gun." Our objectives are to determine how long both mutant p53 DNA and p53 protein remain at the site of immunization and to determine where they migrate after immunization. In this first year of funding, we immunized mice with p53-coated gold beads and were able to track the migration of the plasmid-coated gold beads from skin to secondary lymphoid organs, such as spleen and lymph nodes. In the next phase of our study, we plan to determine the optimal immunization schedule to elicit specific active immunity against p53 and to evaluate the immune cells responsible for attacking tumors bearing mutant p53.

Lynn J. Manseau, Ph.D.

University of Arizona
Award Amount FY 1998: $47,416

Analysis of DRTGEF, A Regulator of rho-type GTPase Signaling

The formation and expansion of tobacco-induced lung cancer is regulated by molecular switches which are called RHO proteins. RHO protein controls various signal transduction events within the cell, inducing whether the cell divides unchecked as in cancer. We have identified a gene in the fruit fly which encodes an RHO activator protein. We call this protein DRTGEF. We are using DRTGEF to ask what happens in the cell if RHO is permanently switched on. We have produced fruit flies with elevated level of the RHO activator protein DRTGEF. The high level of RHO activator protein keeps RHOs turned on and we are analyzing these fruit flies to see the consequences of the elevated level of active RHO protein within the cell.

Furthermore, we have identified the protein that works with our RHO activator gene. This protein is called CDC42. CDC42 is one of the fruit fly RHO proteins. To date, we have fruit flies in hand which have CDC42 protein that does not work. We are studying the effect of RHO activator protein DRTGEF in fruit flies as a model organism. This RHO activator has a close homolog in humans; therefore, we expect that its analysis will be relevant to understanding smoking-induced carcinogenesis in humans and eventually prove useful in targeted drug therapy.

Thomas P. Miller, M.D.

University of Arizona
Award Amount FY 1998: $200,000

Developmental Treatment of Smoking-related Cancers: A Program Project

This program (as founded) focuses on multidisciplinary clinical research to evaluate new drugs and approaches which may ultimately lead to improved treatment and higher cure rates for patients with tobacco-related cancers. Head and neck and lung cancers, tumors caused almost exclusively by tobacco, as well as other tobacco-related cancers (e.g., pancreas cancer) representing a significant percentage of both new cases and deaths from cancer in Arizona each year, are targeted. Benefitting from new knowledge from other Arizona Cancer Center research initiatives, a series of clinical trials is planned. Of particular interest are: 1) strategies to overcome tumor resistance to drug or radiation therapy; 2) drugs with novel mechanisms of action, including those interfering with tumor blood supply; 3) improved drug delivery; and 4) protection of normal tissue during therapy. Progress, during year one, includes completion of four phase I (dose-finding) studies and development of two new phase II (efficacy) studies.

William R. Montfort, Ph.D.

University of Arizona
Award Amount FY 1998: $50,000

Structural Studies of Human Thioredoxin: Target for Anticancer Drug Design

We are studying human thioredoxin, a protein that is overproduced in lung and colon tumors, leading to rapid cell growth. Our goals are: 1) to design specific inhibitors of thioredoxin for use as potential anticancer drugs, 2) to understand the role of thioredoxin in normal and tumorigenic cells and 3) to understand the role of homodimer formation in thioredoxin, a feature of the protein that we discovered. Our approach is to determine the atomic structures of thioredoxin molecules placed in a variety of conditions, including after mutation or after bindings to specific inhibitors, using X-ray crystallography. We also study how the protein behaves biochemically in solution under these same conditions. In year one of this grant, we synthesized and characterized four mutant proteins and determined three thioredoxin crystal structures, providing the basic information necessary to understand how the protein works and how it can be inhibited.

Publications:

Anderson JF, Sanders DAR, Gasdaska JR, Powis G, Montfort WR. Human thioredoxin homodimers: regulation by pH, role of asp 60, and crystal structure of the asp 60–asn mutant. **Biochemistry** 36:13979-13988, 1997.

Sanders DAR. *Thioredoxin homodimers: effects of mutation and oxidation on structure and dimer formation.* Ph.D. Thesis, University of Arizona, 1998.

Montfort WR, Weichsel W. Thymidylate synthase: structure, inhibition, and strained conformations during catalysis. **Pharmacology and Therapeutics** 76:29-43, 1997.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY 1998: $450,000

Preclinical Development of New Anticancer Drugs Necessary to Improving Treatment of Tobacco-related Human Cancer

Again this year, the Arizona Disease Control Research Commission support for the Arizona State University Cancer Research Institute has led to extraordinary progress in the development of new anticancer drugs for improving human cancer treatment. The U.S. National Cancer Institute is now in the process of expanding the clinical evaluation of bryostatin 1 to some forty human cancer clinical trials in as many different collaborative institutions. In addition, nine phase II clinical trials of bryostatin 1 are underway in Canada and another four in England. The initial phase I human clinical trials of dolastatin 10 through the U.S. National Cancer Institute have been completed and the first Phase II trials have begun and will be expanded to twenty or more. Both of these new anticancer drugs required ADCRC assistance for their development to human clinical trials and that requirement continues daily. One of last year's very exciting developments, based on ADCRC support, was the confirmation that our Combretastatin A-4 prodrug is apparently one of the most powerful cancer antiangiogenesis drugs known to date. The first five human cancer clinical trials are scheduled for this fall. Other important advances include the exciting preclinical activity of our auristatin PE against chronic lymphocytic leukemia, non small cell lung cancer and pancreatic carcinoma, as well as with bryostatin 1 combinations. All of these are tobacco-etiology human cancer types. In addition we have discovered a very useful phenstatin prodrug now undergoing development. In summary, the ADCRC support has again led to outstanding research progress.

Publications:

Pettit GR, Xu JP, Hogan F, Williams MD, Doubek DL, Schmidt JM, Cerny RL, Boyd MR. Isolation and structure of the human cancer cell growth inhibitory cyclodepsipeptide dolastatin 16. J Nat Prod 60:752-754, 1997.

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Herald DL, Cascarano GL, Pettit GR, Srirangam JK. Crystal conformation of the cyclic decapeptide phakellistatin 8: comparison with antamanide. J Am Chem Soc 119:6962-6973, 1997.

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Pettit GR, Cichacz ZA, Tan R, Hoard MS, Melody N, Pettit RK. Antineoplastic agents 386. Isolation of sesterstatins 1-3 from the marine sponge *Hyrtios erecta*. **J Nat Prod** 61:13-16, 1998.

Pettit GR, Xu JP, Hogan F, Cerny RL. Antineoplastic agents 369. Isolation and structure of dolastatin 17. **Heterocycles** 47:491-496, 1998.

Pettit GR, Srirangam JK, Barkoczy J, Williams MD, Boyd MR, Hamel E, Pettit RK, Hogan F, Bai R, Chapuis J-C, McAllister S, Schmidt JM. Antineoplastic agents 365. Dolastatin 10 sar probes. **Anti-Cancer Drug Design** 13:243-277, 1998.

Pettit GR, Toki B, Herald DL, Verdier-Pinard P, Boyd MR, Hamel E, Pettit RK. Antineoplastic agents 379. Synthesis of phenstatin phosphate. **J Med Chem** 41:1688-1695, 1998.

Pettit GR, Rhodes MR. Antineoplastic agents 389. New syntheses of the combretastatin a-4 prodrug. **Anti-Cancer Drug Design** 13:183-191, 1998.

Turner T, Jackson WH, Pettit GR, Wells A, Kraft AS. Treatment of human prostate cancer cells with dolastatin 10, a peptide isolated from a marine shell-less mollusc. **Prostate** 34:175-181, 1998.

Roberson RW, Tucker B, Pettit GR. Microtubule depolymerization in *Uromyces appendiculatus* by three new antineoplastic drugs: combretastatin a-4, dolastatin 10, and halichondrin b. **Mycological Research** 102:378-382, 1998.

Garth Powis, Ph.D.

University of Arizona
Award Amount FY 1998: $150,000

Interdisciplinary Basic Science Program Project

Project 1 has shown that the chemopreventive agent, selenium, fed to rats leads to an increase in the levels of the redox enzyme thioredoxin reductase, while a selenium-deficient diet leads to low levels of the enzyme in a variety of tissues, including the lung. Project 2 has developed 3 models with gene alterations relevant to colon cancer that will allow study of nicotine effects on cancer causation. In Project 3, the researchers are developing a sensitive assay for matrilysin secreted into plasma to study the relationship to K-Ras expression. Tumorigenesis studies support the interpretation that DFMO and sulindac act by independent mechanisms. Project 3 is testing whether the expression of matrix metalloproteinases (MMPs) play a functional role in oral squamous cell carcinoma tumor cell invasion and metastasis. New information on the expression of two MMPs, MMP-9 or gelatinase B and MMP-7 or matrilysin in human oral cancer cell lines has been shown.

Scott K. Reaves, Ph.D

University of Arizona
Award Amount FY 1998: $50,000

Regulation of Human Tumor Suppressor p53 Gene Expression By Zinc Status

The human tumor suppressor gene known as p53 is involved in more than half of all human tumors. Because of the involvement of both p53 and zinc deficiency in apoptosis, we have designed experiments to examine how cellular zinc affects the expression of the p53 gene. We have successfully employed three different strategies to deplete cellular zinc in HepG2 cells, a human hepatoblastoma cell line. Data has revealed that both p53 mRNA abundance and nuclear p53 protein levels are affected by cellular zinc status. In addition, the activity of the p53 gene promoter also appeared to be modulated by cellular zinc levels. Thus far our studies have indicated that several aspects of p53 gene expression may be influenced by zinc. Future work will focus on examining how zinc affects the expression and function of p53 to eventually determine optimal zinc levels that may help to reduce the risk of cancer.

William R. Roeske, M.D.

University of Arizona HSC
Award Amount FY 1998: $49,500

Regulation of the Human Delta Opioid Receptor

Smoking has been implicated in lung cancer, which is responsible for 44 deaths per 100,000 people in the period of 1986-1990 in Arizona. Treatment for lung cancer has only a 13 percent five-year survival rate. Lung cancer pain is generally controlled using opioid drugs. Effective opioid drugs, such as morphine and methadone, have a variety of undesirable side effects which include respiratory depression, constipation, nausea, addiction and withdrawal. A new class of opioid drugs, known as the δ-opioid receptor drugs, have recently been developed and studied in animal models. These drugs are effective in pain relief and have fewer side effects. However, these new drugs also show some evidence of drug tolerance in the animal models. Our studies will provide a mechanistic basis to develop strategies for the use of these novel δ-opioid receptor drugs in the treatment of lung cancer pain without the induction of tolerance or side effects associated with the current opioid therapy. Objectives achieved for this year indicate that we can use molecular biology to make the changes in the receptor molecule that will enable us to address these important concerns.

Publications:

Malatynska E, Wang Y, Knapp RJ, Waite S, Calderon S, Rice K, Hruby VJ, Yamamura HI, Roeske WR. Human delta opioid receptor: Functional studies of stably transfected CHO cells following acute and chronic treatment with the selective non-peptidic agonist SNC-80. **J Pharm Exp Ther** 278: 1083-1089, 1996.

Quock RM, Hosohata Y, Knapp RJ, Burkey TH, Hosohata K, Zhang S, Rice KC, Nagase H, Hruby VJ, Porreca F, Roeske WR, Yamamura HI. Relative efficacies of δ-opioid receptor agonists at the cloned human δ-opioid receptor. **Eur J Pharmacol** 326:101-104, 1997.

Varga EV, Stropova D, Rubenzic M, Wang M, Landsman RS, Roeske WR, Yamamura HI. Identification of adenylyl cyclase isoenzymes in CHO and B82 cells by RT-PCR. **Eur J Pharmacol** 348:R1-R2, 1998.

Burkey TH, Ehlert FJ, Hosohata Y, Quock RM, Cowell S, Hosohata K, Varga E, Stropova D, Li X, Slate C, Nagase H, Porreca F, Hruby VJ, Roeske WR, Yamamura HI. The efficacy of δ-opioid receptor selective drugs. **Life Sci** 62:1531-1536, 1997.

Edward B. Skibo, Ph.D.

Arizona State University
Award Amount FY 1998: $37,402

Anti-tumor Agents Targeting Topoisomerase II

The goal of the ADCRC-funded research was the development of new inhibitors of the enzyme topoisomerase II, which plays a role in "unpacking" the chromosomal DNA for replication. A related project was the development of reductive alkylating agents of DNA. Like the topoisomerase II inhibitors, these compounds interfere with the normal function of DNA in the rapidly dividing cancer cell. These agents have the advantage of activation by the cancer cell; in other words, the cancer cell "commits suicide" by converting the drug to the toxic form. These agents are effective against lung cancer and are among the most active under study at the National Cancer Institute. Finally, a comprehensive study of the reaction of known anti-tumor agents with DNA was carried out in order to assist in the development of more effective anti-tumor agents.

Christopher P. Appleton, M.D.

Mayo Clinic, Scottsdale

Award Amount FY 1998: $50,000

Experimental Determinants of Transmitral and Pulmonary Venous Flow: Further Relations to Left Ventricular Filling Pressure

In this first year of study, our goals were to continue to measure the veno-atrial pressure gradients along with left ventricular pressure and relate them to different loading conditions in normal dogs. The work is progressing well now after a delayed start due to personnel changes. Although it has taken several months to get our laboratory up and running, since our restart in May, we have been more successful than ever in recording pulmonary venous pressures reliably. Since all data has not been analyzed, there are no background graphs, diagrams or tables on this first progress report. Similarly, there have been no abstracts or publications. As the work progresses, supporting materials will be included in subsequent progress reports.

Ronald L. Heimark, Ph.D.

University of Arizona

Award Amount FY 1998: $132,713

Mechanism of Vascular Dysfunction in Atherogenesis: Cell-Cell Interactions

Atherosclerosis is a common multifactorial disease, which is the leading cause of death in the United States. This vascular occlusive disease results from a combination of risk factors, such as dietary factors, genetics and smoking. Our overall hypothesis is that both the initiating events and the progression of the disease are characterized by dysfunction in proteins involved in cell-cell organization and communication. To examine this hypothesis, we have developed cell culture conditions for endothelial cells and antibodies to identify changes by factors thought to be involved in the initiation of atherosclerosis in the proteins, which form communicating and adherens junctions. Endothelial cells are normally quiescent in the vessel wall and our studies have examined the role of growth in regulating expression of proteins in the intercellular junction. We also found that a combination cytokines released by inflammatory cells induce a protease cascade, which degrades cytoplasmic proteins anchoring the adherens junction to the cytoskeleton. Thus, we have found that two factors that are thought to initiate atherosclerosis alter expression of proteins present in the intercellular junction. We are determining the functional consequences of these changes.

Yi Ran Wang, Ph.D.

University of Arizona
Award Amount FY 1998: $50,000

Modulation of Transcription by a Frequent Point Mutation in the Human Apolipoprotein A-I Gene Promoter

In 20 percent of the population, a common polymorphism at -78 position of the human apo A-I gene promoter, with a guanine (G) to adenine (A) substitution, is associated with elevated levels of HDL cholesterol and apo A-I. Cigarette smoking abolishes these positive changes. The apo A-I gene promoter activity was determined by transfection of reporter gene constructs into human liver (Hep G2) and intestinal (CaCo-2) cell lines. These constructs contained liver or intestinal specific apo A-I promoter regions, with or without mutation, fused to a reporter gene whose product can be easily measured. The apo A-I gene promoter activity was increased in Hep G2 cells, but not in CaCo-2 cells transfected with apo A-I gene construct. The increase was greater in cells with the mutation than in cells without. Thus, an enhanced apo A-I gene expression in the liver may be responsible for the elevated plasma apo A-I associated with this mutation.

David C. Bloom, Ph.D.

Arizona State University
Award Amount FY 1998: $50,000

Long-term Effects of Nicotine: New Molecular Mechanisms

Fibroblast growth factor 2 (FGF-2) is a protein found in the central nervous system in most mammals. It is thought to play a role in promoting cell growth during development of the nervous system and in maintaining the health of nerves in the adult nervous system. In cancer, it has been shown that FGF-2 is no longer regulated normally. We have shown that nicotine up regulates FGF-2 gene expression and this abnormal FGF-2 expression may be in part responsible for increases in cancers associated with smoking. Our research is pointed towards understanding the mechanism by which nicotine regulates FGF-2 expression in cells derived from the nervous system. We have found a part of the human FGF-2 gene by which nicotine regulates its expression. Our current experiments are focused on finding other proteins that may also be deregulated by nicotine exposure and, thus, may be implicated in smoking-related cancers.

Publication:

Moffet J, Kratz E, Stachowiak MK. Increased tyrosine phosphorylation and novel cis-acting element mediated activation of the fibroblast growth factor-2 gene by nicotine acetylcholine receptor. New mechanism for transsynaptic regulation of cellular development and plasticity. Mol Brain Res 55: 293-305, 1998.

Richard R. Vaillancourt, Ph.D.

University of Arizona
Award Amount FY 1998: $48,510

Intracellular Signal Transduction Pathways Activated by Nicotine

Our research efforts have focused on the identification and characterization of intracellular proteins whose activity is affected by nicotine. This research is important because a better understanding of how these proteins function will help us define the molecular mechanism by which nicotine is an addictive substance. Not only is this research important to Arizonans, it is important to all smokers, as nicotine addiction is the driving force that prevents smokers from being able to stop smoking. It is known that nicotine regulates the activity of an enzyme called tyrosine hydroxylase. This enzyme is critical for the production of dopamine, which is a very important chemical in the brain that drives addictive behavior in people. We have identified two proteins that are important in the regulation of tyrosine hydroxylase. These proteins are called MEKK and PITSLRE α2-1. More importantly, the activity of these proteins also appears to be regulated by nicotine.

Publications:

Fanger GR, Widmann C, Porter AC, Sather S, Johnson GL, Vaillancourt RR. 14-3-3 proteins interact with specific MEK kinases. J Biol Chem 273:3476-3483, 1998.

Porter AC, Vaillancourt RR. Tyrosine kinase receptors and their substrates as oncogenic pathways. Oncogene 17:1343-1352, 1998.

Paul Enright, M.D.

University of Arizona
Award Amount FY 1998: $49,953

Spirometry for the Detection of High Risk Smokers in Southern Arizona Primary Care MD Offices

We sent a questionnaire regarding their smoking cessation practices to all 2833 Arizona primary care physicians. 1267 responded to the survey, with an average age of 48. One-fourth were former smokers and only 3 percent were current smokers. Phoenix physicians were twice as likely to ask their patients about their smoking status. Cardiovascular physicians were 3.5 times more likely to ask than were family practice physicians. About half of physicians provide motivating literature and half refer smoking patients to local smoking cessation resources. We have selected 60 physicians in Tucson to participate in a study, during the next 12 months, to determine if lung function testing improves the smoking cessation rates after a physician has referred a smoking patient to a local smoking cessation resource: Tobacco Freeways.

John Hall, Ph.D.

University of Arizona
Award Amount FY 1998: $89,961

When Gains Go Up In Smoke: Explaining Adolescents' Negative Reactions to Smoking Prevention Campaigns

This grant involves experimental testing of media messages aimed at students. An examination of both pro- and anti-smoking messages are tested as part of this research. Messages were developed, using computer-assisted communication technology to produce CD-ROMs, with manipulations of both pro- and anti-smoking messages. Several different school districts are participating in this research effort and will be given CD-ROMs and suggested curricular materials to develop more effective tobacco education and anti-smoking/tobacco use campaigns. Millions are spent to produce anti-smoking advertisements. Tobacco advertising attempts to present pro-smoking messages. Little is known about how message variables impact attitude and behavior. Year one developed and tested pro- and anti-smoking messages. In year two experimental test of message effectiveness on 4th, 7th, and 10th grade students will be implemented. Because of the methodology supported by ADCRC, funding from two federal agencies in the amount of $1.5 million was obtained.

Li-Wen Lai, Ph.D.

University of Arizona
Award Amount FY 1998: $50,000

Enhancement of Non-Viral Gene Transfer in the Lung

The ultimate goal of this project is to develop a non-viral gene therapy for pulmonary diseases. To enhance gene delivery and prolong gene expression, we tested the effect of polycations (positively charged molecules) on liposome-mediated gene transfer in lung epithelial cell line A549. We have successfully identified polylysine (19 kD) as an efficient enhancer for LipofectAMINE and Lipofectin-mediated gene transfer *in vitro.* The percent increase in transfection efficiency of A549 cells, compared with experiments without polylysine, is 76±22 percent with Lipofectin and 37±16 percent with LipofectAMINE, respectively. Based on the encouraging *in vitro* data, the *in vivo* studies using normal mice (specific aim c) and CAII deficient mice (specific aim d) are currently ongoing. In addition, we have successfully developed an ELISA assay to measure the anti-CAII antibodies in mice after gene therapy. This technology will enable us to assess the side effects of gene therapy. The information obtained from this study will be valuable for guiding the future development of gene therapy for clinical trial in a variety of pulmonary diseases.

Abstract:

Lai L, Inge LJ, Chan DM, Lien YH. Polylysine enhances liposome-mediated gene therapy for renal tubular acidosis in carbonic anhydrase II deficient mice. J Am Soc Nephrol 8:39(2A) American Society of Nephrology, San Antonio, TX, November 1997.

Publication:

Lien YH, Lai L. Respiratory acidosis in carbonic anhydrase II deficient mice. Am J Physiol 274:L301-304, 1998.

Michael D. Lebowitz, Ph.D.

University of Arizona
Award Amount FY 1998: $108,314

Evaluation of the Effects of Smoking on the Development of Chronic Pulmonary and Cardiovascular Diseases in Arizona

This study is a longitudinal follow-up of a community population studied since 1972 whose objectives have been to evaluate cardio-pulmonary morbidity an mortality. The current objectives are to determine the risk factors for such diseases. Recent findings indicate that significant predictors of respiratory and cardiac deaths were: male gender, initial lung function, changes in lung function, weight, smoking and prior disease.

Risk factors were evaluated for changes in an individual's ability to transport oxygen through the lungs to the blood circulation, changes usually associated with significant respiratory diseases (such as emphysema). The negative effects include oxygen deprivation of heart and brain cells. Normally, age and smoking are associated with decrements. We found that negative changes were also related to poor general lung function. In addition, we found that a measure of ability to walk (time-distance related) was related to age and weight; it is also a strong predictor of new heart events.

Patricia B. Hoyer, Ph.D.

University of Arizona
Award Amount FY 1998: $49,990

Mechanisms of Ovarian Follicular Cell Death Initiated by Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAH), contaminants in cigarette smoke, destroy ovarian follicles in laboratory animals and are, therefore, the likely cause of early menopause in women smokers. We are investigating ovotoxicity in rats and mice caused by chronic low-dose exposure to three PAHs (3-MC, DMBA, BaP), because this more closely mimics human exposure. The lowest ovotoxic dose for all three PAHs has been determined in rats and mice. The results demonstrated that mice are more susceptible than rats. Surprisingly, DMBA is the most potent in rats, whereas, 3-MC is the most potent in mice. Our results will allow us to generate an ovotoxic index for each chemical (day and dose causing onset of ovotoxicity). By this mechanism, a predicator of relative ovotoxicity can be made for extrapolation to humans. Our results may intensify interest in altering cigarette smoke contaminants to reduce human risk of early menopause.

Paula D. Johnson, D.V.M., M.S.

University of Arizona
Award Amount FY 1998: $47,954

Cardiac Teratogenicity: The Combined Effects of Exposure to Trichlorethylene and Cigarette Smoke in the Pregnant Sprague-Dawley Rat

This research project is evaluating the effects of inhalation of combined chronic second hand cigarette smoke (SSCS) and trichloroethylene (TCE) in the developing fetal rat heart. The first year was successful in treating pregnant rats with various combinations of SSCS and TCE. The overall project is time-consuming and, at this stage, the hearts are in the process of being evaluated.

Because of the known harm that is caused as a result of SSCS inhalation, and the known effects of TCE as a cardiac teratogen in the fetal rat model, it is suspected that the combination of these two will have a deleterious effect on the developing heart. This is of extreme importance to all Arizonans, not only to those in areas of past or present contamination, but also because of the potential exposure to TCE in drinking water and in the form of inhalation from our cooking, showers, and evaporative coolers.

Pamela J. Kling, M.D.

University of Arizona
Award Amount FY 1998: $46,471

The Effect on Smoking Oxidants, Erythropoietin and Growth Factors in Human Milk

The incidence of premature birth is increased in pregnant smokers. Premature low birthweight infants experience more complications than term infants. All premature infants have a decreased antioxidant status at the same time they are exposed to more oxidants than term infants, promoting a potential toxic imbalance.

Smoking mothers also commonly breast feed their infants. Although discouraged, smoking is not prohibitive to breast feeding. Human mild antioxidant composition is altered with smoking, but it is not known whether the beneficial effects of supplying human milk to premature infants are reversed when the mother smokes cigarettes. Previous studies examined breast fed infants of smokers but these infants were also exposed to passive inhalation of smoke. Therefore, we are analyzing, based on mother's smoking status, the isolated effects of feeding human milk to premature infants in a non-smoking hospital environment.

Publications:

Kling PJ, Sullivan TM, Roberts RA, Philipps AF, Koldovsky O. Human milk as a potential enteral source of erythropoietin, **Pediatric Research** 43:216-221, 1998.

Kling PJ, Roberts RA, Widness JA. Plasma transferrin receptor levels, indices of erythropoiesis and biochemical iron status in healthy term infants, **J Pediatric Hematology/Oncology** 20:309-314, 1998.

Paul St. John, Ph.D.

University of Arizona
Award Amount FY 1998: $120,836

Nicotine Receptors in the Spinal Cord

Nicotinic acetylcholine receptors (nAChR) are complex molecules found on the surfaces of many types of cells throughout the nervous system. nAChR receive and translate signals carried from electrically active nerve cells by the chemical neurotransmitter, acetylcholine. Interaction between acetylcholine and nAChR triggers electrical activity of cells on which nAChR are expressed. Thus, acetylcholine and nAChR are components of molecular switching devices that allow nerve cells to become connected in electrical circuits and information to be transferred across the nervous system. Relevant to the health and welfare of the over one million Arizonans who regularly use tobacco products., nAChR also are principal biological targets of nicotine from tobacco. Nicotine acts acutely as does acetylcholine to activate nAChR and affect nerve cells and circuits, but effects of chronic nicotine exposure, as occurs on habitual use of tobacco products, are less well understood. nAChR in the spinal cord have been postulated to play important roles in physiological functions ranging from sensory processing to control of movement. Chronic use of nicotine alters motor control and pain sensations in smokers. Our understanding is poor about the kinds of spinal cord cells that make nAChR mediate these effects. nAChR exist as a family of similar but distinctive molecules. However, it is not known which of these nAChR subtypes are found on cells in the spinal cord. The project is addressing these research problems.

Work completed so far in this collaborative project confirms that diverse nAChR subtypes are expressed in the mammalian spinal cord. New findings indicate that there actually is more diversity in spinal cord nAChR subtypes than there is in the supra-spinal brain. These findings and anticipated discoveries during the next project period suggest that nicotinic ligands can be developed to selectively affect some of these spinal nAChR subtypes, as they are expressed on unique subsets of spinal neurons, perhaps to beneficially alter processing of pain and other sensory messages and the control of movement.

John J. Marchalonis, Ph.D.

University of Arizona
Award Amount FY 1998: $150,000

Analysis of Autoantibodies to T- Cell Receptors in Rheumatoid Arthritis

Rheumatoid arthritis is an autoimmune disease characterized by chronic systemic inflammation predominantly affecting diarthrodial joints and, frequently, a variety of other organs. The disease affects 1 to 1.5 percent of Americans with a female to male ratio of 3:1. The incidence of the disease is increased to approximately 5 percent in certain tribes of Native Americans and the prevalence in the Tucson area is approximately 5 percent due to the influx of individuals suffering from the disease and the high percentage of Native Americans in the area. Individuals suffering from RA tend to have increased levels of autoantibodies directed against the recognition molecules on their own thymus-derived lymphocytes. We have derived monoclonal antibodies from RA patients. These autoantibodies are predominantly of the immune macroglobulin (IgM) class and react with the combining site region of the T-cell receptor. We propose that the autoantibodies may play a role in immune regulation and that the elevation of these antibodies in RA patients indicates a dysfunction of normal immunological mechanisms. The central question to be addressed is whether these antibodies are essentially the same ones expressed in low levels by healthy individuals in immunoregulation or, whether they represent a distinct disease-related population that uses different immunoglobulin variable region genes. The antibodies were detected using a novel synthetic immunopeptide approach developed here and the monoclonal antibodies differ from classical rheumatoid factors and other characterized IgM autoantibodies. Their presence offers new approaches for diagnosis and potential therapy for this prevalent and crippling autoimmune disease. Adverse lung complications occur in approximately 20 percent of rheumatoid arthritis patients with smoking causing a more rapid progression of lung disease with significant increases in clinical intervention. Since a strong correlation has been found between levels of rheumatoid factor and smoking in males, it will be necessary for us to correlate levels of anti-T-cell receptor and gene usage with sex and smoking status in our patient population.

Publications:

Marchalonis JJ, Schluter SF, Edmundson AB. The T-cell receptor as immunoglobulin: paradigm regained. **Proc Soc Exp Biol Med** 216:303-318, 1997.

Marchalonis JJ, Garza A, Landsperger WJ, Schluter SF, Wang A-C. Binding of human IgG myeloma proteins to autologous T-cell receptor determinants. **Crit Rev Immunol** 17:497-506, 1997.

Judith B. Ulreich, Ph.D.

University of Arizona
Award Amount FY 1998: $102,844

Arizona Liver Transplantation Research: Optimizing Organ Replacement in Tobacco-related Liver Disease

A rat model was used to study whether livers could be protected against damage caused by lack of blood flow (ischemia), as happens during liver transplantation. A chemical, DMSO, given prior to or following ischemia, was shown to protect the organ against loss of viability. DMSO, by preventing cellular damage, prevented the production of heat shock proteins, which cells produce to protect themselves and which are indicative of stress. It also maintained in the liver cells the normal level and activity of cytochrome P450 that could rid the tissue of toxicants. The significance of this research is that it has the potential to provide patients with liver disease a larger pool of donor livers in better condition for transplantation because it allows the use of non-heart-beating donor organs. It may also be effective in maintaining tissue viability and function when given following transplantation so that fewer organs fail to function properly.

Merrie Brucks, Ph.D.

University of Arizona
Award Amount FY 1998: $50,000

The Cumulative Impact of Tobacco Advertising on Young Children's Socialization to Pro-tobacco Attitudes and Behaviors

Substantial progress has been made toward determining which children understand what from tobacco advertisements and when they understand it. Based on extensive presenting, a computer-guided interview procedure has been developed and utilized to gather measures of second and fifth grade children's responses to tobacco advertisements and their beliefs about tobacco use/users. As of June 30, 54 children have been interviewed and school district permissions have been obtained for Fall 1998 to complete additional interviews. Data collected from the 54 interviews have been transformed into appropriate formats for quantitative and qualitative analyses. We have also submitted and, subsequently had accepted, an abstract based on our expectations for analyses currently being conducted on data from the initial 54 interviews and the now completed 200+ interviews.

Evelyn Cesarotti, Ph.D., RN

Arizona State University
Award Amount FY 1998: $63,821

Interventions to Decrease Second-Hand Smoke Exposure in School Children with Asthma

All current research indicates that cigarette smoking and passive exposure may be a major cause of new cases of asthma in children and may contribute to more emergency room visits and hospitalizations. Asthma is also a major cause of school absences in elementary and high schools, accounting for more than 20 million lost school days per year. The research results showed that of the 147 children in this study, 45 percent were exposed to smoking compared with the national average of 25 percent exposed to smoking. However, children with asthma who received the research-developed education and/or counseling programs did have considerably fewer absences and less emergency room visits than those who did not receive the programs. Parents of children with asthma who received counseling on the effect of exposure to smoking on asthma did make changes in smoking behaviors such as smoking less, smoking outside or stopping smoking.

Jenny Chong, Ph.D.

University of Arizona
Award Amount FY 1998: $49,839

Changing the Odds: Research for Prevention

A survey was conducted in 1997 among workers at the Desert Diamond Casino in Tucson, Arizona, to examine whether working in a smoke-filled environment increases smoking initiation among non-smokers and makes quitting harder for those currently smoking. The survey focused on smoking habits prior to and since entering the casino as employees. Results show that the smoking environment did not increase overall smoking prevalence, although, individuals were affected differently depending on their smoking status prior to working in the casino. Proportionately more individuals reduced or gave up smoking after employment at the casino than those who took up smoking. Those who initiated and remained smokers tended to be older than 25 years. Comparisons of the smoking prevalence rates between this population of casino workers with other Arizona populations suggest that the casino population is not representative of the general population.

Theodore M. Dembroski, Ph.D.

University of Arizona
Award Amount FY 1998: $75,491

Biobehavioral Risk Profile of Smokers

A major objective of this research is to use a young adult smoker's own cigarette-induced blood pressure (BP) and heart rate (HR) reactions to enhance a health education effort to promote smoking cessation. Young Arizona smokers (19 and 20-year-olds), were actually shown how their own BP and HR dramatically increased after they smoked a cigarette. The smokers then reviewed health education materials showing how their cardiovascular systems were being damaged by the exaggerated increases. Young adults who were exposed to this combined approach were more likely to agree to quit smoking for 24 hours on their own and then enter a smoking cessation project (76 percent) than those who received only cardiovascular health education alone (56 percent). We believe that the knowledge of one's own cardiovascular reactions to smoking helps overcome the "it won't happen to me" belief that is so prevalent, especially among younger smokers.

SECTION B

CONTRACTS

TOBACCO-RELATED RESEARCH

YEAR TWO

David S. Alberts, M.D.

University of Arizona
Award Amount FY 1998: $146,837

A Study of Genetic Alterations and Recurrence in Colorectal Polyps Associated with Smoking

In Year Two of this project, we have completed the administration of the smoking assessment questionnaire. To date, 550 questionnaires have been completed. All available baseline polyp tissue materials have been reviewed, confirming histopathologic diagnosis. The K-ras analyses of the 735 baseline tissue samples sent to Dr. Matlzman's laboratory in Colorado have been completed. Of the 735 recurrent polyp tissue materials sent for analyses to the Johns Hopkins laboratory, under the direction of Dr. Stan Hamilton, Ph.D., 196 have the K-ras, p53, and DCC analyses completed. In addition, per the QA/QC protocol, 190 K-ras analyses were repeated in the Colorado laboratory and 200 were repeated in the Johns Hopkins laboratory. These analyses will allow for both inter- and intra-laboratory variability to be evaluated. Overall, we have met our time line goals for this complex project and our efforts will continue through the remaining year of this grant to assure the project is completed in a timely manner.

Abstract:

Martinez ME, Maltzman T, Marshall JR, Einspahr J, Reid M, Guillen-Rodriguez J, van Leeuwen B, Ahnen DJ, Alberts DS. Association Between Cigarette Smoking and K-ras Mutation in Colorectal Adenomas; AJE 147(11): S69, 1998.

Publications:

Martinez ME, Reid M, Guillen-Rodriguez J, Marshall JR, Sampliner R, Aiken M, Ritenbaugh C, van Leeuwen B, Mason-Liddil N, Giuliano A, Vargas P, Alberts DS. Design and Baseline Characteristics of Study Participants in the Wheat Bran Fiber Trial, Cancer, Epidemiology, Biomarkers and Prevention, 7: 813-816, 1998.

Paul Consroe, Ph.D.

University of Arizona
Award Amount FY 1998: $49,522

Antiemetic Drug Development for Cancer Treatment

Cannabinoid "agonists," like THC of marijuana, stimulate brain cannabinoid (CB1) receptors to produce desirable (anti-vomiting/analgesia) and undesirable (e.g., amnesia) effects. We discovered that two new cannabinoid drugs, AM630 and SR141716A, have unique effects. First, AM630 can block the binding of cannabinoid agonists to CB1 receptors in mouse and guinea pig brains, but AM630 (like THC) can stimulate CB1 receptors in guinea pig intestines. Next, SR141716A also can block the binding of cannabinoid agonists to mouse and human CB1 receptors. But when given alone, AM630 or SR141716A stimulated the CB1 receptor but they produced an effect (on a CB1 receptor protein) that was opposite to the effect of the cannabinoid agonists given alone. Maybe these drugs have novel therapeutic effects opposite to marijuana (like improving memory)? Mouse, guinea pig and human CB1 receptors are virtually identical and, thus, our findings of opposing effects are important for further research.

Publications:

Hosohata Y, Quock RM, Hosohata K, Makriyannis A, Consroe P, Roeske WR, Yamamura HI. AM630 antagonism of cannabinoid-stimulated gtpγ[35s] binding in the mouse brain. Eur J Pharmacol 321:R1-R3, 1997.

Hosohata K, Quock RM, Hosohata Y, Burkey TH, Makriyannis A, Consroe P, Roeske WR, Yamamura HI. AM630 is a competitive cannabinoid antagonist in the guinea pig brain. Life Sci 61: PL 115-118, 1997.

Landsman RS, Burkey TH, Consroe P, Roeske WR and Yamamura HI. SR141716A is an inverse agonist at the human cannabinoid CB1 receptor. Eur J Pharmacol 334: R1-R2, 1997.

Landsman RS, Makriyannis A, Deng H, Consroe P, Roeske WR, Yamamura HI. AM630 is an inverse agonist at the human cannabinoid CB1 receptor. Life Sci 62: PL 109-113, 1998.

Burkey TH, Quock RM, Consroe P, Ehlert FJ, Hosohata Y, Roeske WR, Yamamura HI. Relative efficacious of cannabinoid receptor agonists in the mouse brain. Eur J Pharmacol 366: 295-298, 1997.

Dominick DeLuca, Ph.D.

University of Arizona
Award Amount FY 1998: $50,000

Organ Culture Approaches for Transplantation of Human Stem Cells

During the first year of this project, we determined the best means to purify the progenitors of T- and B-cells that must be replaced in transplants of patients undergoing intensive chemotherapy for smoking-induced lung cancer. This year, we have used this method to show that the T-cells derived from murine fetal liver donors are qualitatively different from those obtained from the adult bone marrow with respect to the types of T-cells that they produce. We also found that human B-cells can be produced best if their precursors are treated sequentially with the appropriate cytokines. Our organ culture methods allow for a tremendous expansion of cells with the phenotype of the progenitors of all hematopoietic cells. Currently, bone marrow transplantation is the only effective therapy for lung cancer patients, but the low frequency of successful "takes" for these procedures and the limited amount of donor material are major difficulties in treatment. The recent data suggest that we may be able to expand the number of progenitors outside of the body so that there will be more donor material to perform transplants. We have also begun gene therapy studies with the aim of providing a means of inserting genes into the progenitor cells that will aid in rapid growth of these cells in transplant recipients. The development of a reliable means of inserting these genes will greatly aid in rapid reconstitution of T-cell responses in transplant patients. Finally, we have found that nicotine treatment of developing T- and B-cells in organ culture can increase the production of T-cells at low doses and decrease their production at high doses. This result suggests that the immune system of smokers after transplantation may be compromised. It also suggests that since the majority of T-cells in normal individuals are made during fetal and early neonatal life, smoking by mothers puts their unborn children at risk for poor immune response capacity.

Jacquelyn Gervay, Ph.D.

University of Arizona
Award Amount FY 1998: $29,777

The Synthesis of C-glycoside Sulfones as Potential Cancer Therapeutics

The activity of fucosyl and sialyl transferase enzymes has been directly correlated with the metastatic potential of cancer cells. A primary focus of our research is to design and synthesize chemotherapeutics that will inhibit incorporation of α-L-fucose and *N*-acetyl neuraminic acid (NeuAc) into cell surface glycoconjugates. Drugs that block the incorporation of tumor cell markers onto cancer cells may stop the spread of cancer. During the past two years, we have developed unique chemical methods for the synthesis of a new class of compounds having the general structure sugar-CH_2-SO_2-Ch_2-X, that are designed to mimic natural substrates having the general structure sugar-O-PO_2-O-X. Model studies indicated that modifications to the originally proposed synthetic methods would be required since incorporation of the X-group proved problematic. In the past year, two new approaches have been explored. We developed a novel set of precursors based upon glycosyl iodide chemistry that allowed the X-group to be incorporated, albeit, with limited efficiency. More recently, a novel reagent was invented in our group which allows the CH_2-SO_2-Ch_2-X functionality to be incorporated in a one-step reaction. The scope and limitations of these approaches to produce fully functionalized inhibitors are currently under investigation.

Publications:

Gervay J, Hadd MJ. Anionic additions to amoneric iodides: stereoselective synthesis of β C-, *N*- and *O*-glycosides. J Org Chem 62: 6961, 1998.

Gervay J. Glycosyl iodides in organic synthesis: theory and applications, JAL Press, Volume 4, 121-153, 1998.

Gervay J, Nguyen TN, Hadd MJ. Mechanistic studies on the stereoselective synthesis of glycosyl iodides: first characterization of β-glycosyl iodides. Carbohydr Res 300:119, 1997.

Gervay J, Flaherty TM, Holmes D. Studies on the stereoselective synthesis of c-glycoside sulfones as potential glycosyl transferase inhibitor. Tetrahedron 53:16355, 1997.

Anna R. Giuliano, Ph.D.

University of Arizona
Award Amount FY 1998: $126,812

Effects of Smoking on Persistent HPV Infection Among Reproductive Aged Women

For the past two years, we have successfully recruited reproductive aged women into our study of human papillomavirus infection (HPV) and smoking. A total of 818 women have been examined in the first phase of the study. The prevalence of smoking is 36 percent among ages 18-35 who attend a Southern Arizona Planned Parenthood clinic for routine gynecological care. The prevalence of intermediate and high risk HPV infections is 30 percent in this population. Of these women, a total of 189 have participated in our 3-month follow-up clinic and 58 women have participated in our 9-month follow-up clinic. At this point in the study, it appears that smokers have a higher rate of persistent HPV infection compared with non-smokers, however, a larger number of women in the study is needed to confirm this finding.

Arthur F. Gmitro, Ph.D.

University of Arizona
Award Amount FY 1998: $50,000

A Fiber-Optic Confocal Microscope for *in vivo* Imaging

A new type of imaging system has been built for imaging cells inside the human body. This catheter-based microscopic imaging system is intended to aid doctors in the detection and diagnosis of cancer by allowing them to directly visualize cells inside the body. This may obviate the need to extract tissue for biopsy or, at least, aid in the selection of which tissue to biopsy. The basic microscope and fiberoptic catheter system, including catheter optics and mechanical focusing mechanism, have been built and fully characterized. The image quality obtained with the system is excellent and consistent with that predicted by theory. Preliminary results with the imaging of cell cultures and excised tissue samples have demonstrated the basic capability of the system. Experiments are now being conducted to demonstrate the potential of the instrument for *in vivo* imaging.

T. Philip Malan, Jr., Ph.D.

University of Arizona
Award Amount FY 1998: $47,879

Role of c-fos in the Regulation of Neuropathic Pain

Our research focuses on the role of gene regulatory protein Fos and the peptide dynorphin in regulating pain intensity. Both are known, from a great deal of data, to be important in the regulation and, possibly, production of certain types of pain. In the first year of this grant, we showed that blocking of Fos production had a significant effect on inflammatory pain and on normal sensation, but little effect on neuropathic pain (pain caused by injury or disease of nerves). In the second year, we showed that, after inflammation, Fos is important in regulating dynorphin production, but that it has little or no effect in regulating normal dynorphin levels. It appears that the importance of Fos protein may depend on the type of pain and, that in some cases, Fos may exert its effect by stimulating dynorphin production, while in others Fos acts through other mechanisms.

Ana M. Pajor, Ph.D.

University of Arizona
Award Amount FY 1998: $34,640

Cloning and Expression of a Renal Na/Nucleoside Cotransporter

The focus of our research is the way in which nucleoside-based cancer drugs target cells. The drugs are not effective unless they enter the cells and the drugs cross the cell membranes on specific proteins called transporters. We believe that cancer therapy can be improved by designing drugs that are best carried by these transporters. In order to do this, we plan to study the properties of one of the nucleoside transporters using the techniques of molecular biology. After one year of funding, we have achieved the first goal of our project, to clone and sequence the cDNA coding for the pig renal Na/nucleoside cotransporter. This cloned transporter is called pkCNT1 and it is found in the kidney and intestine. This transporter carries pyrimidine nucleosides, such as uridine and thymidine, and interacts with pyrimidine-based drugs including fluoro-uridine, fluoro-deoxyuridine and iodo-uridine. In addition, we have found that the drug gemcitabine, which is used to treat lung cancer, is a potential substrate of this transporter.

Yeh-Shan Peng, Ph.D

University of Arizona
Award Amount FY 1998: $50,000

Effect of Increased Fruit and Vegetable Intake on Plasma Carotenoid Levels and Oxidative DNA Damage in Smokers

The first cycle of the intervention study has been completed. The results were:

1) smokers could increase fruit and vegetable intake from a very early stage (usually the second week) of the intervention;

2) the high intake (5 or more servings/day) of fruits and vegetables was maintained throughout the 6-month intervention;

3) the concentrations of lutein, beta-cryptoxanthin, lycopene, alpha-carotene, beta-carotene and cis-beta-carotene in the plasma of the smokers also increased, on average, by 56 percent, 23 percent, 52 percent, 156 percent, 58 percent, and 63 percent respectively (baseline *vs* 6th month value).

Our procedure for estimating oxidative DNA damage has been successfully modified; the within-person variation over a 3-week period was small, 2.8–9.3 percent. In addition, 14 adult smokers have been recruited for the second cycle of the intervention study. The results, so far, indicate that smokers, like nonsmokers, are capable of increasing fruit and vegetable intake with a resultant increase in the concentrations of carotenoids (potential cancer preventive agents) in the plasma.

William A. Remers, Ph.D.

University of Arizona
Award Amount FY 1998: $86,932

Design of Non-cross Resistant Agents for Lung Cancer

The goal of this project is to discover new compounds that are effective against resistant human lung cancers. To accomplish this goal, we have proposed to synthesize an initial set of 14 compounds and test them against the Lewis lung tumor in mice. From these results, a data base will be created for use in the design of future compounds with improved activity. Active compounds will be further tested against a human lung tumor in mice with deficient immune systems that do not reject the human tumor cells by immunological mechanisms.

To date, the fourteen target compounds have been synthesized on a scale adequate for complete testing and another related compound also has been tested. Results have been obtained for eleven compounds in the Lewis lung tumor assay and six of these compounds have definite effectiveness. One of them gave a 51 percent reduction in tumor growth compared with controls. The results have been entered into the data base, together with the physical and chemical properties of the compounds. One of the active compounds, 6-ethoxyazonafide, was tested against human lung cancer in immuno-deficient mice and found to reduce growth of these tumors by an average of 45 percent.

The compound active against the human lung carcinoma is considered a candidate for a human medicine and plans are being made to obtain preclinical pharmacology on it and introduce it into a Phase I trial. An effective chemical synthesis has been established for its large scale production. Other compounds active against the Lewis lung carcinoma will be tested against the human lung carcinoma in immuno-deficient mice.

Donato Romagnolo, Ph.D.

University of Arizona
Award Amount FY 1998: $51,132

Influences of Tobacco Derivatives on Regulation of Expression of the Breast and Ovarian Cancer Susceptibility Gene BRCA-1

Epidemiological evidence points to tobacco smoking as a possible risk factor in breast carcinogenesis as an early-stage promoter in the process that leads later in life to cell transformation and development of mammary neoplasia. Young women exposed to tobacco smoke during the first years of menarche have a higher risk of developing post-menopausal breast cancer. This risk is increased as the number of smoking relatives increases in the family.

Cigarette smoke contains a complex mixture of compounds some of which, after metabolic activation, may induce DNA damage. In the absence of efficient DNA repair, chromosomal aberrations may initiate carcinogenesis. Our preliminary data provide evidence that a class of tobacco substances, termed polycyclic aromatic hydrocarbons, may compromise the normal production of the BRCA-1 (BR=breast, CA= cancer) protein which is involved in repairing DNA lesions. Loss of BRCA-1 may lead to accumulation of mutations and predispose to the onset of sporadic breast cancer.

Seth D. Rose, Ph.D.

Arizona State University
Award Amount FY 1998: $48,840

Approximately one-third of lung cancers result from the action of a faulty protein that triggers unwanted cell division. To exert its detrimental effect, the faulty protein must be acted upon by a cellular enzyme. We are trying to obstruct that enzyme so that the effects of the faulty protein can be blocked. One prototypic compound was found to potently obstruct the enzyme, reducing its activity to nil within minutes. This led to the design and synthesis of a compound with features tailored to fit into the enzyme-active site for greater selectivity and effectiveness. Two compounds, representing two additional classes of reagents, were submitted to the National Cancer Institute for testing against lung cancer cells grown in culture. Three other compounds, representing two other classes of reagents, were synthesized for testing against the target enzyme, as well as for evaluation of effectiveness against cancer cells grown in cell culture. These studies may lead to the development of effective anticancer agents for the benefit of Arizona residents.

Raymond Taetle, M.D.

University of Arizona
Award Amount FY 1998: $34,067

Transgenic Models for Leukemia and Myelodysplasia (Preleukemia)

This proposal was designed to determine whether a gene, EVI-1, causes acute myelogenous leukemia (AML) or related disorders, myelodysplastic syndromes (MDS). Both AML and MDS are caused by benzene, a carcinogen in cigarette smoke. We have introduced EVI-1 into normal mice so that it is expressed without normal regulation. If this gene predisposes to AML and MDS, the new transgenic mice may develop AML or MDS when challenged with a cancer-causing substance. Two transgenic mice have been generated containing forms of the EVI-1 gene. To perform experiments, the numbers of mice must be expanded and screened for the abnormal gene. The transgenic mice appear normal and have not suffered effects from EVI-1 expression. These mice will be challenged with a hydrocarbon, DMBA, as a benzene substitute and we will determine whether abnormal EVI-1 expression predisposes mice to AML or MDS. From these studies, we will derive a model for AML and MDS induction by benzene in cigarette smoke.

Ann L. Baldwin, Ph.D.

University of Arizona

Award Amount FY 1998: $50,000

What Cellular Mechanisms are Responsible for Histamine-Induced Aerations in Microvascular Permeability

Our overall goal is to determine the mechanisms by which inflammatory agents, such as those produced by smoking, damage the lining of blood vessels (endothelium) and cause subsequent leakage of molecules, such as proteins, from the circulation. In this study, we apply an inflammatory agent, histamine, to a network of small blood vessels in the rat mesentery, a thin sheet of tissue that holds the intestine together, and determine the degree of leakage of albumin, the condition of the cytoskeleton or "scaffolding" of the endothelial cells and the role of white blood cells, that can stick to the endothelial surface, play in causing the leaks. This year, we have demonstrated that histamine causes leaks to form in small veins, but the extent of leakage is limited by the production of nitric oxide that is triggered by histamine. We have also demonstrated that white blood cells do not have to stick to blood vessel walls in order for leaks to occur. Such leakage contributes to cardiovascular diseases. In Arizona, 25 percent of the population smokes, and in 1991, cardiovascular disease was responsible for 38 percent of total deaths.

Abstract:

Valeski JE and Baldwin AL. *In vivo* inhibition of venular leukocyte attachment is associated with decreased histamine leak formation. **Faseb** J 12(4):a28, #161, 1998.

Publications:

Baldwin AL, Thurston G, Al-Naemi H. Inhibition of nitric oxide increases venular permeability and alters endothelial actin cytoskeleton. **Amer J Physiol** 274: H1176-H1784, 1998.

Cheryl A. Dyer, Ph.D.

Northern Arizona University
Award Amount FY 1998: $44,669

The Effect of Nicotine on Ovarian Steroid Hormone Production

Women who smoke become post-menopausal several years sooner than women who do not smoke. Earlier menopause in women is associated with increased incidence of heart disease. Thus, women who smoke lose the natural benefit and protection from heart disease due to decreased ovarian estrogen production. We have been investigating how nicotine in cigarette smoke suppresses the production of ovarian steroid hormones. To examine this question, we use cultured cells from rat ovaries that make steroid hormones and have analyzed the impact of nicotine on their steroid synthesis. Nicotine, at concentrations found in the blood of women who smoke, suppresses the ovarian cell production of the steroid hormone called androstenedione. This particular steroid is essential for the ovary to make estrogen. We have found that nicotine suppresses cultured ovarian cell production of androstenedione because nicotine suppresses the expression of the enzyme required to make androstenedione.

Irwin L. Flink, Ph.D.

University of Arizona
Award Amount FY 1998: $40,271

Cardiac Cell Cycle Progression and Terminal Differentiation

The major goal of this research is to determine the molecular basis explaining the loss of the ability of cardiac muscle cells to undergo mitosis shortly after birth. The retinoblastoma family of proteins is comprised of pRb (retinoblastoma protein), p107 and p130, regulators of cellular proliferation and differentiation, and controllers of cell cycle exit and entry. The activity of these proteins is regulated by a series of phosphorylations that are completed by cyclins and their cognate cyclin-dependent kinases (cdks). In the inactive, under-phosphorylated form, pRb family members interact with the transcriptional factor, E2F/DP1, which is involved in the regulation of DNA synthesis according to the precise timing of a cell cycle clock. We have shown that during early neonatal development, p130 replaces p107 as the principal pRb family member associated with E2F, and pRb becomes less phosphorylated due to the down regulation of cyclins D2 and D3 and cdk 4. Cyclin E, whose activity is required for DNA synthesis at S-phase, is also down regulated. The cdk inhibitors, p21 and p27 were shown to increase at the mRNA and protein levels, which contributes to the overall decrease in activity of several cdks. Taken together, these results describe several changes that block the cardiomyocyte cell cycle during late development. These studies will aid in the development of therapeutic agents that will either replace or modify altered cellular growth genes resulting from the use of tobacco.

Publications:

Flink IL, Oana S, Maitra N, Bahl JJ, Morkin E. Changes in E2F Complexes containing retinoblastoma protein family members and increased cyclin-dependent kinase inhibitor activities during terminal differentiation of cardiomyocyte. J Mol Cel Card 30:563-578, 1998.

Flink IL, Blitz I, Morkin E. Characterization of cellular nucleic acid binding protein from *Xenopus laevis:* expression in all three germ layers during early development. Development Dynamics 211:123-130, 1998.

Joseph Heiserman, Ph.D.

St. Joseph's Hospital
Award Amount FY 1998: $16,154

Clinical Utility of High Performance Gradient

Stroke is a leading cause of death and disability in the United States. Tobacco use increases this risk. Because there is a minor surgical procedure which can reduce the risk of stroke, it is essential to develop a reliable test to identify those individuals who may benefit from this operation. Magnetic resonance angiography (MRA), based on magnetic resonance imaging, can detect these individuals with no risk. This research project evaluated a newly developed method of MRA utilizing high performance gradients. Patients who were undergoing angiography (a diagnostic procedure that, itself, has a risk of stroke) were invited to also undergo MRA using the new technique. These studies were compared to see how closely MRA could duplicate angiography.

High performance gradient MRA duplicated the findings of angiography for mild and moderate stenoses. Vascular loops, which could mimic narrowing using old MRA techniques, were excellently portrayed. Still, severe narrowing often demonstrated signal loss, as seen using older methods. However, this finding occurred at a more severe grade of narrowing than previously, and did not reduce the accuracy for identifying significant narrowing.

In summary, our method improves visualization of vascular structures and pathology, and increases accuracy of MRA for grading vascular narrowing prior to surgery.

Duane Sherrill, Ph.D.

University of Arizona

Award Amount FY 1998: $47,942

Assessment of Genetic Markers Associated with Development of Chronic Obstructive Airway Disease

In the United States, chronic obstructive pulmonary disease (COPD), which includes chronic bronchitis and emphysema, accounts for 5 percent of office visits to physicians and 13 percent of hospitalizations. Cigarette smoking is important in the development of COPD, yet only 15-20 percent of smokers develop clinical disease. In this study, we have addressed the issue of whether susceptibility toward developing COPD, related to smoking tobacco, is an inherited trait. We used pulmonary function measures of forced expired volume in one second (FEV1) and slopes of FEV1 declines with age as markers for susceptibility to COPD, since patients with COPD generally have reduced levels of FEV1 or steeper rates of decline with age. Using FEV1 data from 2620 subjects representing 746 Tucson families, we performed genetics analyses to determine if low levels of FEV1 and change in FEV1 (as determined by regression slopes) are correlated within families and show patterns consistent with Mendelian inheritance. Our results suggest that neither FEV1 or FEV1 slopes conform to a model which assumes a single Mendelian gene. Instead, the analyses suggest that both measures of pulmonary function are most likely determined by environmental factors and/or are influenced by multiple genes.

Ronald J. Lukas, Ph.D.

St. Joseph's Hospital
Award Amount FY 1998: $149,718

Molecular Basis for Nicotine Dependence

Nicotine dependence is thought to drive the habitual use of tobacco products, potentially contributing to a variety of illnesses. The biological targets of nicotine's actions are complex molecules called nicotinic acetylcholine receptors (nAChR), which exist as a large family of subtypes and play critical roles in chemical signaling in the brain and body. An improved understanding about how chronic nicotine exposure affects brain and body function to drive tobacco use requires an improved understanding about how nicotine exposure affects numbers and functions of its own targets, nAChR.

It is well known that acute nicotine exposure stimulates function of nAChR. New findings from this collaborative project confirm that chronic nicotine exposure not only induces increases in numbers but also distinctive, long-lasting losses of function for all nAChR subtypes. Importantly, the latter finding suggests that chronic nicotine exposure disables rather than stimulates chemical signaling mediated by nAChR, that habitual users of tobacco products are characterized by a nervous system with low nAChR signaling and that withdrawal from nicotine might provoke an unpleasant, hyper-stimulation of neuronal activity. Other findings indicate that the depth of functional loss is proportional to the duration of nicotine exposure, implying the existence of heretofore unrealized complexity of nicotine's effects on nAChR. Additional findings demonstrate that different drugs can mimic or block nicotine's effects on nAChR function, but not on nAChR numbers, suggesting strategies to aid smoking cessation and/or to block initiation to tobacco use.

Publications:

Ke L, Eisenhour CE, Bencherif M, Lukas RJ. Effects of chronic nicotine treatment on expression and function of diverse nicotinic receptor subtypes: I. Dose- and time-dependent effects of nicotine treatment. **J Pharmacol Exper Thera** 286: 825-840, 1998.

Lukas RJ. Neuronal nicotinic acetylcholine receptors in *The Nicotinic Acetylcholine Receptor: Current Views and Future Trends.* (FJ Barrantes, Ed.) Springer-Verlag, Berlin/Heidelberg and Landes Publishing Co, Georgetown, Texas: pp. 145-173, 1998.

Lukas RJ. Cell lines as models for studies of nicotinic acetylcholine receptors. In *Neuronal Nicotinic Receptors: Pharmacology and Therapeutic Opportunities.* (SP Americ and JD Brioni, Eds.) Wiley-Liss Inc, New York: pp. 81-97, 1998.

Andrea J. Yool, Ph.D.

University of Arizona
Award Amount FY 1998: $49,793

Influence of Nicotine on Glucose-Sensitive Neurons of the Hypothalamus

The hypothalamus of the brain is important in governing behavior and maintaining body functions. We think that exposure to nicotine alters the ability of hypothalamic neurons to detect sugar (glucose) levels, required for the brain to control feeding behavior, and leads to obesity and related problems. Our research characterizes glucose-detecting neurons to understand the mechanisms that enable this sensing process. We have established techniques for culturing hypothalamic neurons from postnatal rats and are evaluating responses to glucose stimuli using calcium-imaging and electrophysiological recording. A unique subpopulation of neurons respond to glucose by increasing intracellular calcium and changing membrane firing patterns, creating physiologically significant effects appropriate to the known functions of the hypothalamus. Interestingly, more than one pattern of response is observed, suggesting a more complex regulation of feeding behavior than has previously been assumed. Ongoing studies will investigate the consequences of nicotine on the activation of glucose-detecting neurons.

Ian D. Bier, M.S., N.D., L.Ac

Southwest College
Award Amount FY 1998: $49,995

Acupuncture in Smoking Cessation: Randomized, Placebo-controlled Trial

In the second year of the project, the major goals were accomplished. At the 12-month follow-up visit, 70 patients were retained. Data was entered into the computer for interim analysis. The study is on course to follow the patients for 18 months to determine the effectiveness of acupuncture for smoking cessation.

The group that received both true acupuncture and the educational program has maintained a 40 percent cessation rate at 12 months post-baseline, higher then the other groups, ($p<0.05$). In addition, the study design did not include an actual placebo control group due to ethical reasons. Therefore, the combination of acupuncture and education was more effective than education or acupuncture alone, both of which have shown effectiveness above that of placebo in other trials.

For the most recent year of available data (1989), Arizona lost 76,021 productive years of life due to deaths associated with smoking. The economic cost of smoking-related morbidity and mortality in Arizona in 1992, alone, hit $706 million. In 1994, Arizonans spent $896,492,902 in smoking-related costs including health care, morbidity and mortality. Given these statistics, if acupuncture can potentially reduce the number of Arizonans that smoke, it can benefit the state's productivity, economy and the health of its citizens.

Hugh S. Miller, M.D.

University of Arizona
Award Amount FY 1998: $124,963

Reduction in Tobacco Use Among Adolescents Participating in an Incentive-Based Prenatal Care Program

The independent impact of smoking on adolescent pregnancy in Arizona continues to be the focus of our ongoing investigation. Our teen database supports the notion that adolescent pregnancy represents a significant risk for poor perinatal outcome but our data suggest that smoking, while noxious, does not significantly contribute to the poorer perinatal outcome when compared to other variables (adequate nutrition, anemia, appropriate weight gain and good social support mechanisms). The explanation for this may lie in some of the demographic analyses which reveals that Hispanic ethnicity comprises 56 percent of the pregnant adolescents but only 6 percent are smokers. Compare this to white adolescents that represent 30 percent of the pregnant adolescent population and have a smoking prevalence of 32 percent.

In this past year, we implemented the first intervention phase of this project. In order to better assess the value of our smoking cessation program for adolescent mothers, we are also enrolling adult women in this prospective randomized trial so we can determine what the relative benefit is for each group and which components are best suited to each group. A combination of sequential surveys, clinical outcome measures and urinary cotinine levels will enable the evaluation of our program and identify which strategies were the most effective within each patient group. While we complete this phase, we are simultaneously working to establish the final phase of this study which employs incentives to both encourage earlier participation and better compliance with prenatal care and the smoking cessation program.

Dean E. Carter, Ph.D.

University of Arizona

Award Amount FY 1998: $99,194

Synergism Between Smoking and Arsenic Exposure in Lung Injury

In the second year of our project, we established that cigarette smoke-arsenic interaction observed in the first year was not caused by arsenic trioxide alone. The parameters changed were lung glutathione and DNA oxidation. These are indicators of oxidative stress. This is significant because these reactions can be studied in isolated lung preparations. Such preparations are desirable because their use minimizes the number of animals needed. We can study the different compounds (calcium arsenate, arsenic trioxide and arsenic pentoxide), dose levels (maximum effect, minimum effect and no effect), dose durations (acute and subchronic) and observe time parameters more efficiently. In addition, arsenic analyses of the lung tissues showed that the whole-animal inhalation experiments were not delivering the expected arsenic doses. The remainder of the second year was spent in developing the sliced lung technique. Preliminary results indicate that this will be a very useful technique to study lung effects.

Richard L. Friedman, Ph.D.

University of Arizona
Award Amount FY 1998: $50,000

Identification and Characterization of *M. Tuberculosis* Genes Involved in Survival Within Macrophages

Tuberculosis is the most important infectious disease which afflicts mankind. The World Health Organization estimates that one-third of the world's population (1.7 billion people) are infected with tuberculosis and that ten million new cases occur annually. Over three million people die yearly from tuberculosis, the largest single infectious cause of mortality worldwide. For citizens of the state of Arizona, tuberculosis is a serious and rapidly increasing health problem.

The goal of this research is to identify, clone, and characterize genes of *M. tuberculosis* which are required for intracellular survival within macrophages. Using a human macrophage-like cell line, U-937, we have begun to screen both a plasmid and cosmid library containing DNA from virulent *Mycobacterium tuberculosis* in an avirulent strain of mycobacterium (*Mycobacterium smegmatis*). In our screening, we have identified recombinant clones from both the plasmid and cosmid libraries which demonstrate enhanced intracellular survival. One plasmid clone in particular, clone 69, has a four- to six-fold enhanced intracellular survival as compared to vector controls. Further genetic analysis has shown that the enhanced intracellular phenotype of clone 69 is associated with *M. tuberculosis* DNA contained in the recombinant clone. Additional plasmid and cosmid clones, with enhanced intracellular survival, also have been identified and are presently being further analyzed.

Using genetic and molecular biology approaches to study the role of intracellular survival in tuberculosis pathogenesis will expand our understanding and knowledge of the disease process. Study results will lead to the development of improved vaccines and to more effective treatment and prevention of this deadly disease. This will, in turn, reduce the extra risks that smoking citizens of the state of Arizona face when they encounter the agent of tuberculosis in their daily lives.

Michael P. Habib, M.D.

University of Arizona
Award Amount FY 1998: $35, 867

The Effect of Micronutrient Antioxidants on Exhaled Ethane in Cigarette Smokers

Only 15-20 percent of cigarette smokers develop significant lung disease which is costly to Arizona. Antioxidant vitamin ingestion may determine disease susceptibility permitting early intervention. Ethane gas is released in breath during oxidant injury. Our previous work indicates antioxidants (vitamin E, C and β-carotene) reduce ethane and may predict disease susceptibility. Question: Can vitamin C, alone, reduce ethane in and predict smokers at risk for lung disease? We gave vitamin C to 21 smokers for three weeks. Results: Vitamin C had no effect on exhaled breath ethane. Conclusion: Vitamin C, alone, failed to explain changes found in our initial study. This was also the case when vitamin E, alone, was used last year. We next plan to study β-carotene alone. It may be that a combination of all three vitamins is required to demonstrate a large effect on exhaled ethane.

John A. McDonald, Ph.D.

Mayo Clinic, Scottsdale
Award Amount FY 1998: $50,000

Roles of Integrins in Fibronectin Matrix Assembly

Our goal is to understand how living cells create and respond to their surrounding, non-living environments within the body. Cells are surrounded by a complex mixture of molecules called "matrix." This close association is so important that losing the connection with the matrix actually leads to cell death. This is likely one mechanism by which normal cells are prevented from becoming cancerous. Changes in this matrix are also common in respiratory diseases associated with smoking. We are studying the role of molecules on the surface of the cell called "integrins," that help organize the matrix and provide a mechanism to recognize and remove cells that might form cancers. Molecular and cellular experiments are being performed to increase our understanding of exactly how integrins accomplish this.

Publication:

Wu C, Keightley SY, Leung-Hagesteijn C, Radeva G, Coppolingo M, Goicoechea S, McDonald JA, Dedhar S. Integrin-lined protein kinase regulates fibronectin matrix assembly, E-cadherin expression, and tumorigenicity. Journal of Biological Chemistry 273:528-36,1998.

John A. McDonald, Ph.D.

Mayo Clinic, Scottsdale
Award Amount FY 1998: $50,000

Molecular Genetic Analysis of Fibronectin Binding Integrins

All cells (except blood cells) live within or on a complex mixture of molecules called "matrix." This association is so important that cells losing their connections actually die. This mechanism prevents normal cells from becoming cancerous, growing without control and metastasizing or spreading to distant sites. This association must also be repaired when tissue injury or wounding occurs, e.g., in a healing cut. We are studying the role of molecules called "integrins," that help cells recognize and attach to the matrix. We have created artifical genes that program cells in mice to express altered forms of integrins on their surface. The altered integrins can either interfere with the normal integrins, or act as "super integrins," mimicking changes in integrin function thought to be important in cell regulation. Our hope is that by understanding integrin function in living mice, we can learn more about their roles in humans.

I. Glenn Sipes, Ph.D.

University of Arizona
Award Amount FY 1998: $46,705

Determination of the Role of Neutral Endopeptidase in the Development of Small Cell Lung Cancer

Lung cancer is the leading cause of cancer death in the state of Arizona. Small cell lung cancer (SCLC) is a particularly aggressive form of lung cancer which accounts for about 20 percent of all lung cancers. Traditional forms of cancer treatment such as surgery, chemotherapy and radiation are largely ineffective in treating this type of cancer. SCLC is characterized by the expression of autocrine growth factors (AGFs) which are small hormones that stimulate the cell to divide. Another notable feature of SCLC is the expression of the *myb* oncogene, a gene that when inappropriately expressed, leads to the development of cancer. We have recently discovered that the *myb* oncogene activates the expression of AGFs in SCLCs. This finding may provide an explanation for how AGFs are activated in SCLC and may lead to the development of new treatments for SCLC and other types of tumors.

Anne L. Wright, Ph.D.

University of Arizona
Award Amount FY 1998: $113,166

Passive Smoke Exposure, Immunologic Function and Lower Respiratory Tract Illness in Infancy

Lower respiratory tract illnesses (LRIs), such as bronchiolitis, bronchitis, and pneumonia are major causes of infant illness and hospitalization. This project is designed to investigate whether passive smoke exposure alters the immune system response of infants in a fashion that increases their risk of developing LRIs. To this end, we have enrolled 114 infants (59.3 percent of those eligible to participate). Baseline questionnaire data have been obtained on all mothers and the majority of fathers, and blood specimens have been obtained for the majority of subjects. On average, participating mothers have 13 years of school, most (56 percent) are married, 13 percent smoked during pregnancy and 61 percent are Anglo. In part, because of our success in enrolling and following study subjects, we have obtained five years of funding from the National Institutes of Allergy and Infectious Disease. This grant will be used to expand the length of follow-up to five years. Continued long-term success in this project will permit identification of preventable risk factors for this common illness that could have a major impact on the health of infants and on health care costs in Arizona and elsewhere.

Charlene A. McQueen, Ph.D.

University of Arizona
Award Amount FY 1998: $44,319

Genetic Variation in N-Acetyltransferase and the Development Toxicity of Aromatic Amines

An individual's capacity to detoxify chemicals can affect the likelihood of harmful reactions. During pregnancy, the mother and the fetus contribute to this capacity. This project is investigating the development toxicity of 4-aminobiphenyl (4AB), a component of tobacco smoke. 4AB is made more or less harmful in the body by the actions of enzymes called N-acetyltransferases (NAT) and genetic variation in NAT results in different abilities to handle 4AB. Adults are classified as having high or low abilities. In Arizona, about 50 percent of the population has a low ability. Studies funded by this project have shown, in a mouse model, that NATs are present during gestation and that the fetus, as well as the mother, can convert 4AB to potentially toxic products. Future studies are determining if damage to the fetus is related to high or low levels of NAT.

SECTION C

CONTRACTS

TOBACCO-RELATED RESEARCH

YEAR THREE

Ronald L. Heimark, Ph.D.

University of Arizona
Award Amount FY 1998: $31,516

Smoking and Pericytes: Their Roles in Angiogenesis

The greater than 4,000 toxic compounds released in cigarette smoke have two effects on angiogenesis. Smoking inhibits wound repair in skin and results in an increase in various cancers. Angiogenesis is the growth of new blood vessels from existing vessels stimulated by growth factors and is critical for tumor growth and wound healing. The pericyte envelops the endothelial cell lining-capillaries controlling blood flow and inhibiting growth of vessels; the mechanism of action of these cell types in angiogenesis is not understood. The studies in this proposal examined basic mechanisms of pericyte regulation of angiogenesis. We examined the role of angiogenic growth factors, metabolic factors and pericyte cytoskeletal elements in the association of pericytes with capillary fragments, and the regulation of the angiogenic phenotype. The research increased our understanding of pericyte cell-cell interaction with microvessels during angiogenesis and further elucidated signal transduction at the level of the cytoskeleton in normal and disease states.

Bertram L. Jacobs, Ph.D.

Arizona State University
Award Amount FY 1998: $30,000

Regulation of Programed Cell Death in Human Cancer Cells

Our work has been involved in characterizing how a variant of vaccinia virus (VV) can induce human HeLa cancer cells to commit suicide. Since HeLa cells, like most tobacco-induced cancer cells, have mutations that prevent induction of suicide by standard chemotherapeutic agents, we think this virus may be able to overcome one of the main blocks to ridding the body of cancer. The variant of VV that induces suicide in cancer cells has been engineered to remove a gene: the E3L gene. Thus, this gene appears to prevent VV from inducing cancer cells to commit suicide. We have now inserted several well characterized mutants of the E3L gene back into VV to determine how this gene functions by inhibiting the interferon system. These results reinforce the role of this system in fighting cancer.

Mark Nelson, Ph.D.

University of Arizona

Award Amount FY 1998: $29,361

Functional and Clinical Evaluation of thep-16 Protein in Melanoma and Human Lung Cancer

Selenium supplementation has recently been reported to cause a significant reduction in lung and colon cancer incidence. Although the intervention agent, high Se Brewer's Yeast, contained predominately selenomethionine, there is concern as to what form of Se may be active in the yeast formulation. Furthermore, the mechanism of action of selenomethionine as a cancer-preventive agent is not known. We demonstrate that selenomethionine can inhibit cell growth, and induce apoptosis and mitotic alterations in cancer cell lines. Interestingly, normal diploid fibroblasts cell are less sensitive to the anticancer effects of selenomethionine. Selenomethionine can also deplete intracellular polyamine levels. In the rat azoxymethane model of colon carcinogenesis, selenomethionine affected the promotion stages of tumor development. Taken together, these data indicate that at least part of the anticancer effects of high selenium containing yeast can be attributed to selenomethionine. The chemopreventive activity of selenomethionine appears to be mediated through alterations in mitosis and cell loss by apoptosis. Current studies are focused on determining the molecular mechanism by which selenomethionine exerts its anticancer effects. These studies are investigating whether selenomethionine alters the function of the mitotic cyclins in human tumor cells.

Claire M. Payne, Ph.D.

University of Arizona
Award Amount FY 1998: $30,000

Evaluation of Novel Biomarkers for Individuals at Risk for Colon Cancer: Resistance to Apoptosis

The purpose of this study is to evaluate the feasibility of using "resistance to bile salt-induced apoptosis" as a potential biomarker to identify individual Arizona residents at risk for colon cancer. Mucosal biopsies were taken from the lining of the colon of 72 patients during colonoscopy, a procedure used to identify and remove polyps, the precursor lesion to colon cancer. Patients at high risk for colon cancer (HRCC) could be distinguished from those at low risk for colon cancer (LRCC) using this bioassay. Biopsies taken from two additional sites within the colon were used to determine site-to-site variability. The HRCC group showed significantly more variability in apoptosis induction between biopsy sites compared with the LRCC group. The outcome of this study indicates that resistance to apoptosis can be used to assess cancer risk. Future plans are to established key biomarkers that will accurately reflect apoptosis resistance in archived biopsy samples.

Publications:

Payne CM, *et al.* The stress-response proteins poly(ADP-ribose) polymerase and NF-κβ protect against bile salt-induced apoptosis. Cell Death and Differentiation 5:623-636, 1998.

Martinez J, *et al.* Different bile acids exhibit distinct biological effects: The tumor promoter, deoxycholic acid, induces apoptosis and the chemopreventive agent, ursodeoxycholic acid, inhibits cell proliferation. Nutrition and Cancer 31:111-118, 1998.

Henry I. Yamamura, Ph.D.

University of Arizona
Award Amount FY 1998: $29,700

Tobacco, Cancer Pain and Opioids: Determination of the Ligand Binding Domains of the Human Delta Opioid Receptor

Opiates, such as morphine, have been used to manage the pain of tobacco-induced cancers in the state of Arizona. The effects of opioid drugs are mediated through opioid receptors such as mu, delta and kappa receptors. Drugs acting through the delta opioid receptors produce analgesia with minimal side effects. A basis for rational drug design is the pharmacological characterization of the human delta opioid receptor. Molecular biological tools have enabled us to create cell lines expressing the human opioid receptors, to modify the structure of the receptor and to study its effects on receptor function. The sixth and seventh transmembrane domains and the third extracellular loop are the important regions of the human delta opioid receptor for determining delta ligand selectivity. Amino acids, such as Trp 284, Val 296 and Val 297 residues, are important in determining delta opioid ligand specificity.

Abstract:

Li X, Varga EV, Stropova D, Zalewska T, Malatynska E, Knapp RJ, Roeske WR, Yamamura HI. δ-opioid receptor: the third extracellular loop determines naltrindole selectivity. Eur J Pharmacol 300: R1-R2, 1996.

Publications:

Varga EV, Li X, Stropova D, Zalewska T, Landsman RS, Knapp RJ, Malatynska E, Kawaii K, Mizusura A, Nagase H, Calderon SN, Rice K, Hruby VJ, Roeske WR, Yamamura HI. The third extracellular loop of the human δ-opioid receptor determines the selectivity of δ-opioid agonists. Molecular Pharmacology 50: 1619-1624, 1996.

Hosohata K, Burkey TH, Alfaro-Lopez J, Varga E, Hruby VJ, Roeske WR, Yamamura HI. Endomorphin-1 and endomorphin-2 are partial agonists at the human μ-opioid receptor. Eur J Pharmacol 346: 111-114, 1998.

Denise A. Drumm, Ph.D., M.Sc.

St. Joseph's Hospital
Award Amount FY 1998: $6,752

Glycemia Subarachnoid Hemorrhage, Neurobehavioral Outcome in Smokers, Passive Smokers and Nonsmokers

This study examines the interactive effect of glucose and nicotine exposure on cognition and clinical recovery following aneurysmal subarachnoid hemorrhage. Forty-six of the anticipated 50 patients were recruited. Blood and urine samples were obtained for all research participants within 48 hours of rupture. There were 18 males (39 percent) and 28 females (60.9 percent) in this sample. There were no participant deaths associated with rupture and repair of cerebral aneurysms which is likely associated with exclusion of grades IV and V. Actual nicotine exposures were determined by urinary double antibody nicotine metabolites for a continuous measure of nicotine exposure. Nicotine metabolite values of <10 were obtained for nonsmokers. At the time of hemorrhage, nicotine exposures were greater for males than females with respective mean values of 53.25 and 27.39. Analysis of variance indicated this difference to be statistically significant ($p<.09$). Admission and discharge mental status was slightly lower for males than females, although not statistically significant. Length of hospital stay was slightly longer (21.1 days) for males than females (17.5days). Blood glucose levels (BGLs) were higher and more variable in males on most post-operative days. Elevations in BGLs that persisted into the second week of hospitalization were greatest on post-operative days 11 (M=165.23, F=144.94), 12 (M=143.31, F=131.24), and 13 (M=153.5, 127.5) and were higher for males.

Post-operative vasoconstriction (vasospasm) of cerebral arteries was reported for 33 (70 percent) of 41 individuals for whom a determination could be made. The mean nicotine metabolite value for patients who evidenced vasoconstriction was 40.3 compared to a mean value of 21.25 for those who did not experience cerebral vasospasm. Mean nicotine metabolite values for individuals experiencing post-surgical clinical complications was higher (n=31, value=39.0) when compared to individuals for whom complications were not reported (n=10, value=28.20). A determination of cerebral edema was possible for 39 research participants. Evidence of cerebral edema was identified in 24 individuals. Nicotine metabolite values were higher for this group. (41.63) than the group that did not experience cerebral edema (31.60), which in turn increased length of hospital stay by 3 days.

Modeling of the data by means of logistic regression analysis, backward selection method using presence or absence of cerebral vasoconstriction (vasospasm) as the dichotomous outcome variable with nicotine metabolite, post-operative day one blood glucose level and patient sex as the factors entered into the model together indicated a good fit; however, none were found to be

significant alone. This suggests the likelihood of interactive effects. Another logistic regression was run designating presence/absence of clinical complications as the dichotomous dependent variables. Blood glucose values were significant at the $p<.06$ level.

The Pi remained blind to the record during patient evaluations and analysis of preliminary data. Subsequent review of the medical records revealed several factors of potential influence on recovery. An experienced nurse extractor was employed to conduct a systematic review of the medical record for each research participant, taking into account the additional factors for more complete modeling of the data. Preliminary evidence suggests possible influences of other neuroendocrine factors from exogenous and endogenous sources. Due to the small sample size, modeling of these data failed to yield statistically significant results. Although statistical power was low, there were several trends noted that were of interest. Analyses of these data continues.

Elizabeth Krupinski, Ph.D.

University of Arizona

Award Amount FY 1998: $29,800

Interactive Three-dimensional Display for Evaluation of Coronary Artery Disease

The goal of this project was to develop and test new display techniques for three-dimensional myocardial perfusion imaging. The project is important since better display of perfusion data may lead to better evaluation of coronary artery disease (CAD) by the radiologist. CAD is a known risk for smokers and the elderly (a high population of which is in Arizona). The project has two major parts: 1) develop software for displaying 3-D images and 2) evaluate radiologist performance under a variety of viewing conditions. The software phase was successful to a point. It was developed, but a number of very difficult problems could not be overcome to make it clinically useful. Additionally, since the beginning of the grant, commercial software has been made widely available which does exactly what we proposed. With respect to the performance studies, all but the final study (using the display software) were completed successfully.

Eugene Morkin, M.D.

University of Arizona
Award Amount FY 1998: $97,628

Actions of Diiodothyropropionic Acid in Heart Failure: Pharmacology and Cardiac Biochemistry

Heart failure associated with, in many cases, smoking-related coronary artery disease, is a major public health problem in Arizona. Despite advances in treatment, severe heart failure continues to have a 30 percent yearly mortality, which is greater than many forms of cancer. Cellular and biochemical defects in the failing heart are thought to be responsible for its poor performance. New forms of treatment directed toward reversing these abnormalities are needed. The overall objective of this multidisciplinary program is to study the actions and metabolism of 3,5-diiodothyropropionic acid (DITPA), a thyroid hormone analog, in a rabbit model of heart failure after myocardial infarction. Specifically, our objectives are: 1) to characterize the uptake, distribution and rate of elimination of DITPA in animals with heart failure; 2) analyze the effects of DITPA on intracellular calcium transport in the failing heart, which may underlie its ability to improve cardiac performance; 3) determine plasma levels of DITPA and thyroid hormones after administration of the drug. This data is intended to provide the basis of an FDA application for a clinical study of DITPA in heart failure.

Publications:

Pennock GD, Yun DD, Agarwal PG, Spooner PH, Goldman S. Echocardiographic changes after myocardial infarction in a model of left ventricular diastolic dysfunction. Am J Physiol 273:H2018-2029, 1997.

Tomanek RJ, Zimmerman MB, Survarna PR, Morkin E, Pennock GD, Goldman S. A thyroid hormone analog stimulates angiogenesis in the post-infarcted rat heart. J Mol Cell Cardiol 30:923-932, 1998.

Alison Stopeck, M.D.

University of Arizona
Award Amount FY 1998: $29,505

Genetically-modified Endothelial Cells in Vascular Biology

The blood vessel damaging diseases, atherosclerosis and peripheral vascular disease are the major cause of mortality in Arizona. While damaged vessels can be repaired and new vessels (graft) surgically placed, their function is limited by clogging and narrowing of the lumen from abnormal smooth muscle cell growth. Smooth muscle cell growth is controlled by the vessel lining endothelial cells. We have successfully transferred a gene, gamma interferon, into endothelial cells that inhibits smooth muscle cell growth in culture and determined the mechanism by which smooth muscle cell growth is inhibited. We have also established a method whereby endothelial cells are directly isolated, genetically modified to produce a therapeutic gene, gamma interferon, attached to vascular grafts that can then be surgically implanted into people needing new vessels or vessel grafts. The use of genetically-modified endothelial cells in grafts or repaired native vessels may improve graft longevity and vessel function in patients.

James Bloedel, Ph.D.

St. Joseph's Hospital
Award Amount FY 1998: $30,000

Genetic Engineering of Receptors for Nicotine

Nicotinic acetylcholine receptors (nAChR) play important roles in chemical signaling throughout the brain and body . nAChR also are targets of nicotine, which is thought to drive habitual use of tobacco products. An improved understanding of how nicotine affects the brain and body and contributes to habitual use of tobacco products requires an improved understanding about nAChR and their interactions with nicotine.

In this project, we use powerful genetic engineering techniques to introduce nAChR genes into human cells. Thus, we create cells that can make different forms of nAChR for further study. Previously, we mastered two ways to create human cells that now make the simplest possible form of human nAChR, α7-nAChR, which is ideally suited for studies to elucidate basic principles of nAChR structure and function. Using one of these approaches, we can control amounts of human α7-nAChR made by genetically engineered cells. We created mutant forms of α7-nAChR, some of which respond with prolonged signals on interaction with nicotine. Expression of some of these mutant forms of nAChR are toxic to neurons, but not to other cell types, suggesting possible roles in developmentally-related and degenerative neuronal death. One new discovery is the engineering of a truncated, but functional, α7-nAChR, which represents an even simpler model for basic research studies. The mutagenesis findings suggest the possibility that minor genetic differences may affect properties of nAChR and development of the nervous system. Differences across individuals in their nAChR could contribute to individual differences in phenomena, such as sensitivity to nicotine, susceptibility to habitual use of tobacco products and susceptibility of cells that make nAChR to toxic or traumatic injury. We have made initial progress showing that individual differences do, in fact, exist in genes that code for nAChR subunits and in those proteins themselves.

Publications:

Ke L, Eisenhour CE, Bencherif M, Lukas RJ. Effects of chronic nicotine treatment on expression and function of diverse nicotinic receptor subtypes. I. Dose- and time-dependent effects of nicotine treatment. J Pharmacol Exper Thera 286:825-840, 1998.

Lukas RJ. Neuronal nicotinic acetylcholine receptors. in *The Nicotinic Acetylcholine Receptor: Current Views and Future Trends.* (FJ Barrantes, Ed.), Springer-Verlag, Berlin/Heidelberg and Landes Publishing Co., Georgetown, Texas. 145-173, 1998.

Lukas RJ. Cell lines as models for studies of nicotinic acetylcholine receptors. in *Neuronal Nicotinic Receptors: Pharmacology and Therapeutic Opportunities* (SP Arneric, and JD Brioni, Eds.), Wiley-Liss, Inc., New York. 81-97, 1998.

Edward D. French, Ph.D.

University of Arizona

Award Amount FY 1998: $29,504

Marijuana, Nicotine and Dopamine Reward Systems: A Unifying Hypothesis of Dependence

It is now generally recognized by both the scientific community and general public that nicotine-containing products, such as cigarettes, possesses addictive qualities. Considerable basic research has identified the brain neurotransmitter, dopamine, as playing a pivotal role in addiction to many drugs of abuse. Therefore, it seems reasonable to hypothesize that the addictive effects of nicotine are also produced through the activation of dopamine neurotransmission in the central nervous system. Using electrophysiological and behavioral methods, we have found that nicotine is a potent stimulant of dopamine neuronal activity within the ventral tegmental area of both the whole animal and in brain slices. Moreover, the stimulatory effects of nicotine appear to be comprised of both a fast-onset component and a longer lasting phase. Notably, the fast component can be blocked by compounds which antagonize the effects of glutamate, the major excitatory transmitter in the CNS. Through the use of various specific nicotine receptor antagonists, we have also obtained evidence that nicotine-induced activation of dopamine neurons may involve at least two different nicotine receptor subtypes. These data support the hypothesis that nicotine's addictive qualities may reside within its ability to activate brain reward pathways, and that this effect may also involve the release of the neurotransmitter glutamate. By further identifying the receptor subtypes involved in the dopamine neuronal effects of nicotine, we may be able to design selective therapeutic agents for the treatment of nicotine addiction or its withdrawal effects. Moreover, our data also show that the psychoactive ingredient in marijuana, Δ9-THC, also activates dopamine neurons in the midbrain, albeit, by a different mechanism. Thus, the activation of dopamine-containing reward systems appears to be the common final pathway by which nicotine and marijuana express their abuse potential.

Ronald M. Lynch, Ph.D.

University of Arizona
Award Amount FY 1998: $30,000

Regulations of Insulin Secretions form Individual Beta Cells

Diabetes afflicts 9 million Americans and is often associated with obesity. Normally, insulin secretion increases when blood glucose causes changes in blood insulin which then causes cells to become insulin insensitive. Chronic exposure to nicotine also causes insulin insensitivity. Therefore, loss of normal beta cell sensitivity may contribute to changes in body weight after smoking cessation. We have begun studies to determine how glucose and nicotine stimulate insulin secretion. In the past twc years, we have developed probes to measure insulin secretion from single cells on a microscope. In addition, beta cells that do not sense glucose normally have been produced. In the past year, we have focused on evaluating the role that a specific step in glucose metabolism plays in the change in glucose sensing by these cells. Our findings will shed light on alterations in glucose sensing which occur during development of Type II Diabetes and long-term nicotine exposure and withdrawal.

Arnold R. Martin, Ph.D.

University of Arizona
Award Amount FY 1998: $29,405

New Antitubercular Agents: Products and Isosteres of Isoniazid and Pyrazinamide

Twenty-six new isosteric acids related to the standard antitubercular drugs isoniazid (INH) and pyrazinamide (PZA), along with their pivalolyloxymethyl derivatives, were synthesized. Nine of the prepared isosteres and six of the corresponding prodrug derivatives were evaluated for antitubercular activity *in vitro*. The basis for this effort is to design antitubercular drugs that are effective against strains of the tubercle bacillus that are resistant to INH and/or PZA. The concept is to discover new biochemical mechanisms to activate our drugs that differ from those that activate INH and PZA. Resistant strains of *M. tuberculosis* appear to lack the required activation mechanisms for INH and PZA. The emergence of resistant strains is a major factor contributing to the alarming upturn in reported cases of tuberculosis in the state of Arizona, the United States and world wide.

In addition, one hundred and nine (109) 2,4,1-benzodiazaborine derivatives have been prepared. To date, fifty (50) have been evaluated against *Mycobacterium tuberculosis in vitro*. The selection of the 2,4,1-benzodiazaborines as new targets (not part of the original proposal) was based on literature reports of significant general antibacterial activity of the closely related 2,3,1-diazaborines.

As we expected, the isosteric acids related to INH and PZA exhibited only modest activity against *Mycobacterium tuberculosis in vitro*. However, significant activity (equal to 2X the activity of pyrazinamide) was seen for the three POM prodrugs. In the 2,4,1-benzodiazaborine series, the most active compounds tested, thus far, were 16X less active that INH, but 160X more active than PZA. It will be important to determine the cell toxicity of the antitubercular 2,4,1-benzodiazaborines to see if any members of the series show any promise clinically.

Epidemiological studies have established tobacco smoking as a significant risk factor for tuberculosis. This connection has led to the formation of the *Tobacco and Health Committee of the International Union Against Tuberculosis and Lung Disease*. It is, therefore, indeed fitting and appropriate for tobacco tax funds to be utilized for projects seeking new therapies for combating tuberculosis.

Publications:

Davis MC, Ph.D. Dissertation: Chemical synthesis of rationally designed pyridine and pyrazine derivatives and boron compounds for inhibition of *Mycobacterium tuberculosis in vitro*. The University of Arizona, Dissertation Director: Arnold R. Martin, Ph.D., 1997.

Wachter GA, Davis MC, Martin AR, Franzblau SG. Antimycobacterial activity of substituted isosteres of pyridine- and pyrazine-carboxylic acids. J Med Chem 41:2436-2438, 1998.

Davis MC, Franzblau SG, Martin AR. Synthesis and evaluation of benzodiazaborine compounds against *M. tuberculosis* $h_{37}r_v$ *in vitro*. Bioorg Med Chem Lett 8:843-846, 1998.

Y. Howard Lien, Ph.D.

University of Arizona
Award Amount FY 1998: $30,000

Gene Therapy in Carbonic Anhydrase II Deficient Mice: Role of Carbonic Anhydrase in CO2 Exchange and Acid-Base Homeostasis

We investigated the role of carbonic anhydrase II (CA II) on CO_2 exchange in the lung. We found that CA II deficiency is associated with a lower blood pH and [HCO_3^-], and mild CO_2 retention consistent with combined metabolic and respiratory acidosis. When metabolic acidosis was corrected with injection of bicarbonate, CO_2 retention became more profound, indicating that CA II deficient mice, indeed, had severe impairment of removing CO_2. It is most likely that CO_2 retention in these animals is due to CA II deficiency in both red blood cells and type II pneumocytes. The results of intravenous gene therapy using the human CA II gene indicated that successful gene therapy is associated with a significant improvement in CO_2 retention. The human CA II and its effect diminished after one week of gene therapy, a result similar to other studies with liposome-mediated gene therapy. Our studies have enhanced the understanding of mechanisms of CO_2 exchange in the lung and facilitated the development of gene therapy targeted to the respiratory diseases.

Publication:

Lien YH, Lai L. Respiratory acidosis in carbonic anhydrase II deficient mice. Am J Physiol 274:L301-304, 1998.

Nafees Ahmad, Ph.D.

University of Arizona
Award Amount FY 1998: $30,000

Influence of Tobacco Smoking on the Molecular Mechanisms of HIV-1 Transmission from Mother to Infant

Mother-to-infant transmission of HIV-1 is one of major causes of AIDS in children. In addition to viral and host factors, tobacco smoking during pregnancy increases the rate of mother-to-infant transmission of HIV-1 by 3-to-5 fold. Since the state of Arizona has the one of the highest rates of smoking and teen-age pregnancy in the country, smoking will increase the rate of HIV-1 transmission from mothers to infants and convert more HIV-1 infected individuals from asymptomatic to full blown AIDS. To identify and characterize HIV-1 isolates associated with mother-to-infant transmission, we have analyzed the molecular and biological properties of HIV-1 from infected mother-infant pairs following perinatal transmission.

The human immunodeficiency virus type 1 (HIV-1) *vpr* sequences were analyzed from six infected mother-infant pairs following perinatal transmission. We found that 153 of the 166 clones analyzed from uncultured peripheral blood mononuclear cell DNA showed a 92.17 percent frequency of intact *vpr* open reading frames. There was a low degree of heterogeneity of *vpr* genes within mothers, within infants and between epidemiologically linked mother-infant pairs. The distances between *vpr* sequences were greater in epidemiologically unlinked individuals than epidemiologically linked mother-infant pairs. Moreover, the infants' sequences displayed patterns similar to those seen in their mothers. The functional domains essential for *vpr* activity, including virion incorporation, nuclear import and cell cycle arrest/differentiation were highly conserved in most of the sequences. Phylogenetic analyses of 166 mother-infant pairs and 195 other available *vpr* sequences from HIV databases formed distinct clusters for each mother-infant pair and for other *vpr* sequences, and grouped the six mother-infant pairs' sequences with subtype B sequences. A high conservation of intact and functional *vpr* supports the notion that *vpr* play an important role in HIV-1 infection and replication in mother-infant isolates that are involved in perinatal transmission. Furthermore, the biological phenotypes of HIV-1 involved in maternal-fetal transmission were found to be macrophage-tropic and non-syncytium inducing. In conclusion, we should target our preventive strategies on these molecular and biological properties of the virus.

Publications:

Yedavalli VRK, Chappey C, Ahmad N. Maintenance of an intact human immunodeficiency virus type 1 *vpr* gene following mother-to-infant transmission. J Virology 72:6937-6943, 1998.

Yedavalli VRK, Matala E, Chappey C, Ahmad N. Conservation of an intact *vif* gene of HIV-1 during maternal-fetal transmission. J Virology 72:1092-1102, 1998.

Joseph L. Graves, Jr., Ph.D.

Arizona State University West
Award Amount FY 1998: $29,728

Comparative Genetics and Biology of Aging II: Selection Studies and Resistance to Toxic Compounds

Selection for delayed reproduction has been carried our for two years in the five chosen species, *D. melanogaster, D. pseudoobsura, D. hydei, D. arizonae and D. virilis.* Selection for delayed reproduction has been shown to result in both postponed aging and resistance to toxic compounds. Year three examined the physiological performance of these same stocks after laboratory evolution for 61–96 generations for controls and 17–24 generations for experimentals. Year three study confirms the inconsistent relationship between differentiation of these stocks for dessication and starvation resistence, while no statistically different results for ethanol vapor tolerance was recorded. This pattern is in opposition to previous studies of these relations recorded from *D. melanogaster.* In addition, *D. virilis* stocks were shown to be differentiated for patterns of fecundity, but not life span (again, counter to theory). The stocks are currently being further assayed for physiological and genetic alterations resulting from the selection procedure. This study concludes that selection for life history features need not be consistently linked to physiological stress resistance.

Publication:

Graves JL, Chapter 4. General Theories of Aging: Unification and Synthesis. in *Principles of Neural Aging.* pp 35-55. (SF Dani, A Hori, GF Walter, Eds.) Elsevier Press, 1997.

Dianne Lorton, Ph.D.

Sun Health Research Institute
Award Amount FY 1998: $29,657

Neuroimmune Involvement in the Progression of Experimental Arthritis

Rheumatoid arthritis (RA) represents a significant health problem for Arizona residents. Data supports smoking may adversely affect RA by changing sympathetic outflow. Our studies examine whether increased arthritis severity following sympathectomy of fibers supplying immune organs, is due to altered ability of immune cells to process the antigen that induces arthritis. Denervation, during both the time of antigen processing and at the effector phase of the disease, resulted in an increased arthritis severity. Denervation altered immune cell expression of activation markers, altered immune cell distribution and changed immune functions within secondary lymphoid organs of arthritic rats. These data suggest that sympathetic innervation of lymphoid tissue plays a role in both the initiation and effector phase of the disease. Preliminary findings also indicate that there are alterations in spinal cord levels of various neurotransmitters involved in pain pathways with arthritis and following local lymph node 6-OHDA application.

SECTION D

PROPOSALS RECEIVED

TOBACCO-RELATED RESEARCH

FY 1998

Ahmad	University of Arizona	Effect of Tobacco Smoking on HIV-1 Pathogenesis and Disease Progression in Mothers and Infants following Perinatal Transmission	$50000 50,000 50,000
Ampel	University of Arizona	The Effect of Cigarette Smoking on Immune Function and Outcome in Male Patients with Active Coccidioidomycosis in Arizona	$49,995 49,995 49,995
Beach	Sun Health Research Institute	Smoking and Alzheimer's Disease: An Autopsy Study	$36,025 36,025
Bellamy	University of Arizona	Role of Vascular Endothelial Growth Factor in Hematopoietic Malignancies	$50,295 49,448 51,427
Berens	Barrow Neurogical Institute	Human Recombinant Receptor for Nicotine	$49,052 49,387 49,228
Berg	University of Arizona	Optimal Treatment of Prolonged Ventricular Fibrillation in a Swine Model of Prehospital Cardiac Arrest	$50,000 50,000 50,000
Bieber	Arizona State University	Composition of a Unique Receptor for Nicotine	$149,504 149,783 148,608
Bloom	University of Arizona	The Airway Epithelium as a Target for the Anti-inflammatory Action of Inhaled Steroid Therapy	$49,910 49,012 49,920
Bourque	University of Arizona	Detoxification of Carcinogenic PCB'S By Transgenic Tobacco Plants and Algae	$50,000 50,000 50,000
Brinton	Phoenix VA Medical Center	Effects of Cigarette Smoking and its Cessation on HDL Metabolism: Vascular Endothelial Function and Atherosclerosis in Human Subjects	$149,966 149,965 149,965
Brown	University of Arizona	The Effect of Tobacco and its Constituents on Placental Development Differentiation and Immunologic Function	$125,567 109,218 97,240

Brucks	University of Arizona	Young Children's Socialization to Tobacco-Related Attitudes	$147,146 146,706 149,859
Burgoon	University of Arizona	The Smoke Free Work Place: Overcoming Resistance and Implementing Changes in Smoking Behavior	$147,474 144,438 145,524
Burleson	ASU West	Stress and Smoking: Effects of Daily Stress, Nicotine Depedence and Gender on Smoking Behavior	$49,970
Chang	Arizona State University	Defect in DNA Repair: Mechanisms and Consequences	$50,000 50,000 50,000
Chen	University of Arizona	Smoking: Body Composition and Bone Mineral Density in Urban Premenopausal Native American Women	$149,321 141,611
Clark	University of Arizona	The Effect of Selenium on DNA Oxidative Damage in Smokers and Non-Smokers	$124,247 121,061 115,077
Coonrod	Maricopa Medical Foundation	No Mas Cigarros:Smoking Cessation for Latinas	$49,836 48,609
Cress	University of Arizona	Targeting of Lung Cancer Cells with Anti-Adhesive Agents	$48,900 48,900 48,900
Davis	University of Arizona	Nicotine Effects on Blood-Brain Barrier Integrity: Function and Permeability	$48,070 48,290 49,610
Davis	Arizona State University	The Impact of Long-term Cigarrette Smoking on Cognitive Functioning in Middle-aged Men and Women	$48,422 46,776 48,879
de Berge	Behavior Research Center	Arizona's Tobacco Use Reduction Program: Impact on Spanish Speaking Hispanic Women Ages 17-34	$49,353 49,817
DeLuca	University of Arizona	Effects of Nicotine on the Development of Human T Cells	$122,269 127,675 132,661

Drumm	Sun Health Research Institute	Effects of Tobacco Exposure: Coronary Artery Disease and Inflammatory Markers on Cognitive Performance Following CABG in the Elderly	$50,000 50,000 50,000
Eisenberg	New Focus, Inc.	Testing Targeted Tobacco Cessation Approaches for Hispanic Youth	$149,586 144,968 149,687
Erickson	University of Arizona	I QUIT: Smoking Cessation Program for Nurses by Nurses	$49,982 49,986 30,840
Fregosi	University of Arizona	Physiology of Pharyngeal Airway Obstruction	$49,229 45,643 47,881
Gandolfi	University of Arizona	Hepatotoxicity of Smokeless Tobacco Extract	$49,213 49,562 49,883
Graves, Jr.	ASU West	The Impact of Nicotine on the Genetics of Aging: Exposure and Selection for Resistance	$50,000 50,000
Gray	University of Arizona	Tobacco Use Among Young Adults: Quantitative and Qualitative Assessment and Development of Cessation Interventions	$150,000 150,000 150,000
Gunatilaka	University of Arizona	Mechanism-based Discovery of Novel Anti-tumor Agents from Plants	$49,962 47,394 47,515
Hammond, Jr.	ASU West	Cigarette Smoking and Visual Health: Implications for Age-related Macular Degeneration and Cataract	$46,124 49,216
Harris	University of Arizona	Development of High Level Gene Expression Systems for Gene Therapy	$49,772 49,772 49,772
Harris	University of Arizona	Genetic Approaches to Skin Cancer Etiology: Evaluation of Interactions with Environmental Exposure	$148,726 147,909 148,946
Hersh	University of Arizona	Program Project to Develop Novel Gene Therapies for Tobacco-Related Cancers	$150,000 150,000 150,000

Hoppensteadt	Arizona State University	Effects of Nicotine on the GI Tract	$50,000 50,000 50,000
Jacobs	Arizona State University	Specific Induction of dsRNA-mediated Suicide in Lung Cancer Cells	$150,000 150,000 150,000
Katsanis	University of Arizona	Induction of Specific T^{3}-cell Responses Against bcr/abl+ Chronic Myelogenous Leukemia	$50,000 50,000 50,000
Lam	University of Arizona	Development of a Peptide Therapy for Small Cell Lung Cancer	$50,000 50,000 50,000
Lantz	University of Arizona	Effect of Environmental Tobacco Smoke on Lung Growth and Development	$99,989 88,242 90,873
Lax	University of Arizona	Sarcoplasmic Reticulum Gene Expression in Furazolidone-induced Dilated Cardiomyopathy	$50,000 50,000 50,000
Lei	University of Arizona	Alteration of Promoter Activity by a Common Point Mutation at the +83 Position of the Human Apolipoprotein A-I Gene	$50,000 50,000 50,000
Leischow	University of Arizona	Vias a la Salud: Computerized Cancer Risk Reduction Education for Rural Arizonans	$149,988 149,719 147,848
Leischow	University of Arizona	Diverted Youth: Testing a Tobacco Cessation Intervention for Adolescents	$48,564 46,825 48,447
Lien	University of Arizona	Homologous Recombination-Based Gene Therapy for Herediatry Pulmonary Diseases	$50,000 50,000 50,000
Loeb	University of Arizona	Evaluation of Simulator-Based Clinician Training	$23,386
Lopez	University of Arizona	Osteoporosis in Premenopausal Women Smokers Undergoing Chemotherapy for Breast Cancer: A Pilot Study	$147,600 124,628 71,710

Lorton	Sun Health Research Institute	Nicotine-Induced Effects on Disease Development in Adjuvant-Induced Arthritis: Neural -Immune Mechanisms	$50,000 50,000 50,000
Lukas	BNI	Genetic Variations in Receptors for Nicotine	$49,569 49,879 49,697
Mason	Phoenix VA Medical Center	The Effect fo Cigarette Smoking on the Morbidity of Diabetic Foot Ulcerations	$40,425 40,425 29,425
McDonagh	University of Arizona	The Effects of Cigarette Smoke Exposure on the Leukocyte Contribution to Ischemia-Reperfusion Injury in the Heart	$49,223 48,201 49,366
Nagle	University of Arizona	Improved Extracellular Matrix for Treatment of Tobacco-related Occlusive Vascular Disease	$112,204 116,219 118,423
Nelson	University of Arizona	Studies on the Cancer Chemopreventive Effects of Selenium Against Colon Cancer	$50,000 50,000 50,000
Parrish	University of Arizona	Role of Tobacco-derived Cadmium in Prostate Disease	$49,134 49,067 49,520
Payne	University of Arizona	Identification of Individuals at Risk for Colon Cancer Using Selected Stress Response Proteins as Biomakers	$150,000 150,000 150,000
Pennock	Biomedical Research Foundation	Safety and Efficacy of 35-Diiodothyropropionic Acid (DIPTA) in Patients with Congestive Heart Failure	$148,784 140,115
Pettit, R.K.	Arizona State University	Biological Research for Advancing New Anticancer and Anti-infective Drugs to Clinical Trials	$150,000 150,000 150,000
Philipps	University of Arizona	The Effect of Nicotine Patches on Maternal and Fetal Health	$49,748 49,821 48,791

Powis	University of Arizona	Thioredoxin Genetically-modified Mice	$50,000 50,000 50,000
Reems	Blood Systems Foundation	Evaluation of Human Umbilical Cord Blood Hematopoietic Progenitor Cells in a Mouse Transplant Model	$55,000 55,000 55,000
Rider	University of Arizona	Environmental Tobacco Smoke Exposure in Developing Lungs - Effects on Surfactant Metabolism	$49,998 50,000 50,000
Selmin	University of Arizona	Benzo-a-pyrene Alters the Expression of a Novel Membrane-bound Progesterone Receptor	$47,602 49,172 49,734
Shapiro	University of Arizona	Physician-Patient Communication with Heavy Smokers: Comparing Motivational Interviewing with the Prescriptive Approach	$49,998 49,963 48,080
Sherrill	University of Arizona	Assessment of Patterns of Mendelian Inheritance of Addiction to Cigarettes	$49,942 49,903
Shubitz	University of Arizona	Effects of Side Stream Cigarette Smoke on *C. Immitis* Infection in Mice	$129,089 137,292 131,969
Slepian	University of Arizona	Estrogen Modulation of Smooth Muscle Cell Migration: The Role of Altered Integrin Expression and Function	$49,880 49,880 49,880
Sparks	Sun Health Research Institute	The Effect of Nicotine in an Animal Model of Both Cardiovascular Disease and Alzheimer's Disease	$486,019 465,234 464,992
Stopeck	University of Arizona	Targeting Angiogenesis as a Novel Therapy for Lung Cancer	$50,000 50,000 50,000
Taren	University of Arizona	Why Do Women Gain Weight With Smoking Cessation: Understanding the Role of Diet and Metabolism	$144,123 148,463 144,123
Temm	University of Arizona	The Function and Fate of Pericytes Subjected to Nitric Oxide Donors and Nicotine	$50,000 50,000 50,000

Thomas	Arizona State University	Tobacco Use and Youth Sport Participation in Arizona: Preventing Addiction	$49,999 49,587 49,984
Watson	University of Arizona	Exacerbation by Tobacco Smoke of Cytokine Dysregulation in Aging for Cardiotoxicity	$50,000 50,000 50,000
Wessells	University of Arizona	Endothelial Cell-based Gene Therapy to Correct Erectile Dysfunction	$49,511 49,833 49,533
Whitesell	University of Arizona	Microtubule-dependent Intergrin Function as a Therapeutic Target in the Prevention of Restenosis After Angioplasty	$49,142 50,155 50,097
Whitten	University of Arizona	Effects of Vitamin E Dietary Supplementation on Sidestream Cigarette Smoke-Induced Lung Injury	$132,752 126,598 131,618
Winzerling	University of Arizona	The Effects of Chemicals Found in Cigarette Smoke on Iron Metabolism of Lung Cancer Cells	$50,000 50,000 50,000
Wu-Wang	University of Arizona	Effect of Tobacco on the Cellular Functions of Oral Epithelium: Roles of Prostaglandin E2 and Epidermal Growth Factor	$50,000 50,000 50,000
Yamamura	University of Arizona	Adenylyl Cyclase Superactivation after Chronic Opioid Receptor Stimulation	$49,500 49,500 49,500

SECTION E

NEW CONTRACT AWARDS

TOBACCO-RELATED RESEARCH

BEGINNING IN FY 1999

Ampel, M.D., Neil M.

University of Arizona
Award Amount FY 1999: $49,995

The Effect of Cigarette Smoking on Immune Function and Outcome in Male Patients with Active Coccidioidomycosis in Arizona

Coccidioidomycosis, known as "Valley Fever," is an infection caused by the soil-dwelling fungus *Coccidioides immitis*. While common in the desert Southwest, coccidioidomycosis is especially prevalent in Arizona. Over the past decade, coccidioidomycosis has emerged as a major problem in our state. The number of cases reported has nearly tripled from 1990-1995. Moreover, costs are staggering, with 26.8 million spent in 1995 for hospitalization alone. Recent studies conducted in Arizona indicate that older men are most at risk for acquiring active coccidioidomycosis and that cigarette smoking is a significant risk factor for the development of severe infection. The precise role cigarette smoking plays in increasing the risk of coccidioidomycosis is not known. One important factor could be the lack of an appropriate immune response to infection. This response is known to play a critical role in the severity and type of disease that occurs during coccidioidomycosis.

The research proposed will determine the effect of smoking on outcome of coccidioidomycosis among a group of men in Arizona. It will do this in two parts. First, it will compare the specific immune response to infection among actively smoking and non-smoking men with coccidioidomycosis. Second, it will examine the effect of smoking cessation on the severity of illness and on specific immune function on that group of men who were originally smoking at the start of the study. It is hypothesized that smoking will significantly diminish the appropriate immune response to coccidioidomycosis and that cessation of smoking will lead to both improved immune response and improved clinical outcome.

All work will be performed at the Tucson Veterans Affairs Medical Center, a site uniquely positioned to explore these questions. First, this medical center has a specific clinic devoted solely to coccidioidomycosis. All patients who have active coccidioidomycosis in the Medical Center are referred to this clinic for care. Second, the laboratory of the principal investigator has been focused on the human immune response to coccidioidomycosis for the last decade and has been productive in defining the human immune response to this infection. All the assays of immune function proposed for this study are already being performed in this laboratory and results using these methods have been published in the medical literature.

Berens, Ph.D., Michael

Barrow Neurological Institute
Amount Awarded FY 1999: $49,052

Human Recombinant Receptors of Nicotine

Nicotine is a powerful tobacco substance that affects the brains and bodies of an estimated 25 percent of Arizonans, including adolescents. The targets of nicotine action are called nicotine acetylcholine receptors (nAchR). nAchR play important roles in chemical signaling throughout the brain and body, and they usually respond to the natural chemical messenger, acetylcholine. When nAchR are briefly exposed to nicotine, they respond just as they do to acetylcholine. Whereas acetylcholine is quickly eliminated from sites where it acts with nAchR, nicotine can act at those receptors for much longer, producing another spectrum of effects on the brain and body. Nevertheless, our understanding of how nicotine affects the human brain and body and contributes to habitual use of tobacco products is deficient. For example, nAchR exist as diverse subtypes, each having unique physiological roles and anatomic distributions and each being composed of different combinations of genetically distinct building blocks called subunits. Experimental studies examining effects of nicotine at different human nAchR subtypes are scant. From the available information, it is clear that particular nAchR subtypes in humans differ from one another. Moreover, a given kind of human nAchR subtype can differ in minor but physiologically significant ways from the same kind of nAchR subtype found in other mammals or vertebrates. We need an improved understanding of human nAchR subtypes and their sensitivities to tobacco nicotine.

In this project, powerful genetic engineering techniques will be used to introduce human nAchR subunit genes in different combinations into human cell lines. We will create human cells that can make different forms of human nAchR composed of different combinations of subunit building blocks for further study. In previous work, we have perfected techniques for creating cells that make nAchR composed of only one kind of subunit. Now, we will create cells that make more complex forms of nAchR. We will first create cells that can make nAchR composed of $\alpha 4$ and $\beta 2$ subunits. These are the numerically predominant kinds of nAchR in the brain, and they are obvious targets for nicotine actions. Other objectives are to create cells that make nAchR composed as pair-wise combinations of $\alpha 2$, $\alpha 3$, or $\alpha 4$ subunits with $\beta 2$ or $\beta 4$ subunits. Such nAchR may not predominate numerically in the brain, but they may contribute to specific effects of nicotine, in pleasure/reward centers, in learning/memory centers, or in centers of the brain controlling emotions. These cells will be valuable tools for further studies concerning how acute or chronic nicotine exposure affects different kinds of human nAchR, thereby contributing to changes in nervous system function that underlie nicotine dependence.

Bieber, Ph.D., Allan

Arizona State University
Award Amount FY 1999: $149,504

Composition of a Unique Receptor for Nicotine

An understanding of the basis for nicotine dependence and habitual use of tobacco products requires knowledge about the biological targets of nicotine action, called nicotine acetylcholine receptors (nAChR). The nAChR are found throughout the nervous system and exist in several different forms reflecting their composition from different protein components, called subunits. Interactions between nAChR and either nicotine or the natural chemical signaling agent, acetylcholine, alter activity of nerve cells, thereby altering electrical circuit activity in the nervous system. These actions provide a basis for at least some forms of behavior. They contribute to the habitual use of tobacco products by an estimated 25 percent of adult Arizonans. However, the precise composition of several important nAChR subtypes, including those that contain an α7 subunit, is not known. Moreover, it is not known whether nAChR associate with other kinds of cell proteins, which could affect construction or localization within the cell of nAChR. Collectively, these deficiencies in our knowledge about the structure of nAChR and their association with other cell proteins compromise our understanding about how nAChR function is altered by exposure to nicotine. In part, these deficiencies are attributable to a relative paucity of sophisticated techniques to analyze proteins, particularly those like nAChR that reside in cell membranes. This contrasts sharply with the relative abundance of techniques recently developed to analyze genes composed of deoxyribonucleic acids (DNA).

The ultimate and principal objective of this project is to identify proteins that associate with nAChR containing α7 subunits. These nAChR are implicated in several novel and important physiological roles, including effects of nicotine exposure on the nervous system. However, it is not know whether these nAChR contain other subunits and/or associate with other cell proteins to affect construction or localization within the cell of nAChR, thereby affecting chemical signaling mediated by nAChR. Consequently, a practical but critical objective of the project is to develop and refine sophisticated chemical techniques to characterize complex cell membrane proteins such as nAChR and associated proteins. The techniques to be developed and used in the project are predominantly based on an unparalleled process of combined sensitivity, resolving power and flexibility called mass spectrometry (MS). Refinement of MS techniques will be done first in characterization of an abundant form of nAChR from electric fish, and then in characterization of nAChR containing α7 subunits. MS techniques developed during the project will be generally applicable in identification and characterization of many other complex, membrane-associated molecules engaged in chemical signaling, and even toward cloning or related genes. MS techniques will be used during this project to test hypotheses that: 1) nAChR containing α7 subunits are not composed of additional nAChR subunits, but 2) other proteins associated with α7 subunits facilitate assembly of intact nAChR, regulate subcellular localization of nAChR, and modulate coupling of nAChR to other chemical signaling molecules.

Brown, M.D., Mark

University of Arizona
Awarded Amount FY 1999: $124,842

The Effect of Tobacco and Its Constituents on Placental Development, Differentiation and Immunologic Function

Over 20 percent of pregnant patients enrolled in Arizona's pregnancy programs continue to smoke throughout their pregnancy. Women who smoke during their pregnancy are at increased risk for adverse pregnancy outcomes, such as placental abruption, placenta previa, low birthweight (LBW), preterm premature rupture of membranes (PPROM), and preterm labor (PTL) resulting in preterm birth. In Pima County, Arizona, the incidence of low birthweight is 5 percent among non-smokers and 11 percent among smokers. Unfortunately the complications of smoking are not confined to pregnancy, but extend into the neonatal period and even early childhood. The incidence of sudden infant death syndrome (SIDS) is significantly increased among women who smoke during pregnancy, independent of prematurity and neonatal smoke exposure. The combination of low birthweight and prematurity are clearly linked to higher rates of learning disabilities, developmental delay and perinatal morbidity and mortality.

While it is well recognized that tobacco smoke contains over 2,500 chemical constituents, carbon monoxide, nicotine and decreased oxygen availability are considered to be the primary harmful agents. Some of the direct effects these chemicals impose on developing fetus are understood. The effects on the placenta and the specific mechanisms that account for adverse pregnancy outcomes are poorly understood. Tobacco-associated complications of pregnancy are best explained by chronic placental injury. While much has been reported on the effects of smoking in pregnancy, we believe the future lies in better delineation of the specific impact of tobacco smoke on placental development, differentiation and immunologic competence. The interface between the placenta and the uterus is what produces a healthy fetus. Understanding how tobacco and its constituents impact this delicate but important interface is at the center of our research proposal.

The objectives of the proposed research are to determine the effects of tobacco and its constituents on placental development, differentiation, and immunologic function in order to clarify to what extent these changes are responsible for the clinical complications of pregnancy associated with smoking. We hypothesize that: 1) antenatal tobacco exposure changes the placental-uterine interface, 2) antenatal tobacco exposure changes placental-uterine cellular differentiation, 3) antenatal tobacco exposure modifies the immunologic function of the placenta and its uterine partner and 4) antenatal tobacco exposure alters the hormonal and regulatory properties of the placenta producing placental/uterine injury resulting in pregnancies complicated by differing diagnoses (antenatal bleeding, previa, LBW, and prematurity).

Phase 1 of this research will consist of prospective evaluation of placental/uterine cell cultures obtained from pregnancies exposed and unexposed to tobacco. The preliminary observation will provide significant clarification of how tobacco exposure affects the placental-uterine interface, and immunologic competence of the cell lines involved. Phase 2 of this research will seek to further clarify which components of tobacco smoke are responsible for the previously observed changes. Normal term placentas will be exposed to carbon monoxide, nicotine, and reduced levels of oxygen to mimic the elements of tobacco smoke exposure.

Comparison of these two sets of observations will confirm the original observation while isolating which components within tobacco smoke cause the most significant effects. The overall goal of this research is to define the specific impact(s) at the cellular level of tobacco exposure on the placenta-uterine interface and confirm that the toxicity of antenatal smoke exposure is principally mediated through the placenta. These discoveries may lead to more convincing counseling strategies for our pregnant smokers.

Burgoon, Ph.D., Michael

University of Arizona
Award Amount FY 1999: $147,474

The Smoke Free Work Place: Overcoming Resistance and Implementing Changes in Smoking Behavior

Many organizations have been reluctant to make changes in policies concerning when and where employees (and visitors) can smoke on their work site. The University Medical Center (UMC) became a "smoke free work place" with a single designated smoking area in 1997. Prior to this policy change, there were several smoking areas in visible places around the center. In addition, management imposed a differential rate for health insurance for non-smokers with covered dependents who do not smoke that is $20 per month less than for smokers or employees with covered dependent smokers. Enforcement is a priority for UMC; significant negative reactions to these policy changes resulted. This research proposes a multi-faceted three-year program to focus on the difficult task of changing actual *behaviors* (tobacco use) through interventions using different message strategies, computer-assisted decision making and active participation of employees. Moreover, employee morale and attitudes toward policies and employees will be measured throughout. Measurement of actual smoking behavior, enrollment in cessation programs, and direct measures of tobacco use are employed. Different strategies and channels will be used that are most effective with different sub-groups of employees. An econometrics analysis will be completed to determine projected cost savings in health care from this policy change. Also, this set of studies will provide a template for other organizations who desire to create smoke free work environments and reduce tobacco use, while minimizing employee resistance.

The goals of this research are: 1) to reduce the incidence and prevalence of tobacco use in the work place; 2) test different message strategies targeted at specific sub-groups of employees to reduce tobacco use; 3) to use different communication channels (e.g., written messages, closed-circuit television, computer-assisted decision making tools, active employee participation) to educate and motivate employees to reduce tobacco use; 4) test the effects of different interventions on employee morale and attitudes toward the policies; 5) provide an econometrics analysis of the projected health care cost savings resulting from this policy change and 6) provide information for other organizations on how to implement smoke free work environments in the most effective way with the least employee resistance. Specific hypotheses about the communication strategies to affect such goals are derived from *a social influence approach* to smoking cessation that incorporates a variety of scientific theories.

Burleson, Ph.D., Mary H.

Arizona State University West
Amount Award FY 1999: $49,970

Stress and Smoking: Effect of Daily Stress, Nicotine Dependence, and Gender on Smoking Behavior

According to the Surgeon General, smoking remains the single most important preventable cause of disease and death in the United States. Disease processes thought to be linked with smoking include coronary heart disease, atherosclerosis, hypertension, and lung cancer. While smoking, alone, is considered to be a powerful risk factor for various cardiovascular problems, research has shown that psychological stress can exacerbate the impact of smoking on heart rate and blood pressure. Moreover, smoking has been shown to prolong one hormonal component of the stress response that can possibly lead to suppression of the immune response and to damage the memory centers of the brain. Many smokers say that they smoke to relax or relieve tension. If this is true, then smokers could be subjected to the combined effects of stress and smoking quite frequently. Laboratory studies have shown that smokers smoke more often and more deeply when they are asked to complete a stressful task, but the link between stress and smoking in natural settings has not been firmly established. The current study is aimed at examining the link between stress and smoking in daily life. In addition, the study is designed to look at differences between men and women and between addicted and non-addicted smokers in the strength of this link. The findings of this study could improve the design of smoking cessation programs by indicating whether stress in everyday life influences smoking rate or urges to smoke and whether the strength of this influence is different for different groups of smokers. It is estimated that approximately 23 percent of all adult (and 26 percent of young adult) Arizonans smoke cigarettes. Therefore, this research has the potential to directly affect a large proportion of the Arizona population. In addition, because smoking is linked with health problems in the smokers' families and with job and relationship strain, a very large proportion of the population could be helped indirectly by the application of these findings to improve cessation programs.

The main goal of this study is to establish whether increases in daily stressful events lead to higher rates of cigarette smoking in adult smokers. The first hypothesis, which is derived from this main goal, is that daily stressful events will prospectively predict the number of cigarettes smoked. Two competing hypotheses are derived from the second goal. The second goal is to determine whether stress predicts smoking more reliably for dependent (addicted) smokers or for non-dependent (non-addicted) smokers. One hypothesis is that addicted smokers are more likely to respond to stress by smoking than are non-addicted smokers. The competing hypothesis is that because the smoking rates of addicted smokers are generally more stable that those of non-addicted smokers, stress is less likely to influence the smoking rates of addicted smokers than those of non-addicted smokers. The third goal of the study is to determine if the strength of the stress and smoking relationship is stronger for women than it is for men. The third hypothesis to be tested is that women are more likely to smoke in response to stress than are men. The central objective of the project is to determine what factors might lead to more smoking (or a greater desire to smoke) in smokers. This knowledge could prove useful in designing smoking cessation programs that more closely fit the needs of those who want to quit smoking. For example, if women smokers tended to smoke in response to stress more than men, the implication would be that stress management components of cessation programs should be targeted especially toward female clients. This tailoring of programs would allow for more efficient allocation of resources in cessation efforts.

Coonrod, M.D., M.P.H., Dean

Maricopa Medical Foundation
Award Amount FY 1999: $49,836

No Mas Cigarros: Smoking Cessation for Latinas

Smoking cessation is an important part of tobacco control. Efforts that enable smokers to quit may help diminish the substantial burden of smoking-related diseases. These illnesses account for at least one-third of all deaths in women aged 35 to 69 in the U.S. Each year one,600 women in Arizona die of lung or cervical cancer, both of which are related to smoking. The burden of these diseases is disproportionately borne by minority women. For example, cervical cancer is more common in Latino women and when it occurs, is more deadly because it is identified at a more advanced stage. Currently, between 8 and 15 percent of Latino women smoke. However, it is anticipated that, as these women become more acculturated, an increasing percentage will smoke, thus leading to more smoking-related deaths. In light of the sizable population of Latino women in Arizona, it is critical that we identify a means of assisting their efforts to stop smoking.

This pilot study has three objectives:

First, we wish to determine if a culturally competent smoking cessation program is more effective than a standard smoking cessation program. Many have advocated this, based on logic, but the supporting data are lacking.

Second, we will determine the increased benefit of a behavioral program when added to treatment with a newly approved drug for smoking cessation, bupropion. While drug therapy for smoking cessation may be time-efficient, rates of cessation are low (20 percent rate of quitting at 6 month follow-up). We would like to see if a behavioral program can increase this rate.

Third, we will measure the effectiveness of bupropion therapy among Latino women since its efficacy in this group has not been reported.

Cress, Ph.D., Anne E.

University of Arizona
Award Amount FY 1999: $48,900

Targeting of Lung Cancer Cells with Anti-Adhesive Agents

Despite the high response rates resulting form chemotherapy, the majority of small cell lung cancer patients die with chemoresistant tumors. Although the tumor cells are killed in 80 percent of the patients, less than 10 percent survive more than two years. Patients die from highly drug-resistant lung tumors which are no longer killed by the drugs and remain in the lung. These tumor cells remain in the lung because they adhere to the lung lining and are not "cleared." A new way is needed to dislodge the remaining tumor cells from the lung. We have been studying an adhesion protein on the surface of lung cancer cells which permits adhesion of the tumor cells to a protein in the lung lining. We have recently discovered biologically active peptides which decrease the ability of tumor cells to adhere to laminin, a major component of the lining of the lung. This proposal will test the ability of these newly found peptides to interfere with tumor cell adhesion to laminin. The identity of the active peptides will be used to develop a "super" peptide as a lead agent for developing potential anti-adhesive agents. The discovery of the anti-adhesive agents specific for lung cancer cells will contribute to both clinical and basic science knowledge. Targeting of lung cancer cells can be used to: 1) dislodge the tumor cells, 2) direct delivery of therapeutic viruses or chemical agents and/or 3) improve detection of cancer for diagnostic purposes. Also, the discovery would impact other non-cancer disease states involving laminin defects including vascular disease, muscular dystrophy, blistering disease and wound healing. Finally, the discovery of agents which mimic the normal ligand of the integrin could be used to "map" the integrin for the ligand binding sites. This would be helpful in our basic science understanding of the ability of the integrins to serve as both adhesive and signaling receptors.

We have recently searched for anti-laminin adhesive peptides using a combinatorial peptide bead library approach. Our first results have shown the feasibility of this approach and have shown that previously identified peptides were found as well as novel peptide sequences. More recently, approximately two million peptide combinations were tested using this method and 33 lead peptides (termed the RZ series) were discovered which bind to the integrin a6 laminin receptor selectively. We are now ready to test our hypothesis that these newly discovered peptides will be biologically active in targeting lung cancer cells.

Our proposal is to 1) synthesize and purify the RZ peptides; 2) test the peptides for their ability to antagonize laminin adhesion of the lung cancer cells; 3) test the ability of the peptides to interact with the a6 integrin specifically; 4) hypothesize, synthesize and purify a consensus peptide based upon the active RZ peptides; and 5) submit a pharmacophore for electronic database searching of known chemical compounds and test any positive hits.

Davis, Ph.D., Thomas P.

University of Arizona
Award Amount FY 1999: $48,070

Nicotine Effects on Blood-Brain Barrier Integrity, Function and Permeability

Stroke is the third leading cause of death in Arizona, as well as in the United States, and is the leading cause of serious disability in the nation (Arizona Health Status and Vital Statistics, 1995). Cigarette smoking is associated with an increased risk of stroke. Clinical studies have indicated that application of nicotine patches alone can precipitate ischemic stroke in chronic smokers. The blood-brain barrier (BBB) is a system of small capillaries in the brain which regulates the levels of glucose, electrolytes, amino acids, iron, insulin and other metabolites that are allowed to cross from the blood into the cerebral fluid. Despite the importance of the BBB in maintaining the cerebral environment, remarkably little is known about the effects of nicotine on the blood-brain barrier. Since smoking is a risk factor for stroke, it is likely that nicotine (a major constituent of tobacco smoke) affects the permeability characteristics of the blood-brain barrier and thereby contributes to the occurrence and severity of stroke. Nicotine has already been found to increase the severity of stroke by increasing the build up of fluid in the brain and by depleting stores of brain tissue plasminogen activator (TPA) which is important in dissolving blood clots. These effects are almost certainly mediated through changes in the permeability of the BBB caused by nicotine. Our research will directly examine the effects of nicotine on the permeability and transport characteristics of the BBB.

Our hypothesis is that nicotine affects the structure and transport characteristics of the BBB. Our research goals are four-fold. One is to determine the effects of nicotine exposure on transport of important nutrients into the brain by examining whether nicotine affects the ability of several compounds to enter the brain. The second goal is to investigate whether nicotine and stroke have additive negative effects on the BBB. Third, we will examine the effects of nicotine on the structures of the BBB that constitute the physical barrier between the blood and brain. Our fourth goal is to determine nicotine effects on the expression of several protective proteins in the brain that are activated during stroke. Ultimately, we seek to define a mechanism whereby nicotine increases the risk and severity of stroke through effects at the BBB. We hope this research will facilitate the design and development of new strategies for the prevention and treatment of stroke.

DeLuca, Ph.D., Dominick

University of Arizona
Award Amount FY 1999: $122,269

Effects of Nicotine on Immune Cell Function

There is a common perception that the body's natural system of defense, the immune system, is compromised by exposure to tobacco smoke. However, surprisingly few scientific studies concern the effects of smoking or exposure to the biologically-active tobacco substance, nicotine, on the immune system, particularly during fetal development. These few studies suggest that smoking/nicotine induces imbalances in immune cells, such as subpopulations of thymic lymphocytes (T-cells). If so, this could either over-activate or over-suppress immune responses. Clearly, smoke or nicotine applied to the intact organism could act on brain or endocrine centers in complex ways to influence immune function. However, information is lacking about possible direct effects of smoke or nicotine on immune centers or on T-cells. Available data hints that mediators of nicotine's effects in the nervous system, called nicotinic acetylcholine receptors, are also made by immune cells. If so, then nicotine, whether provided *via* tobacco use or nicotine-like compounds used therapeutically to treat nervous system or other disorders, acting at these receptors could contribute to effects on T-cell function and balance. Nicotine effects on immune cells could contribute to susceptibility to tobacco-related diseases.

The purpose of this project is to critically determine whether nicotine exposure has effects on developing T-cells of human or mouse thymus and to identify the kinds of nicotine receptors that mediate those effects. This work will exploit a novel, *in vitro* fetal thymus organ culture (FTOC) system that mimics the growth and differentiation of both human and mouse T-cells. It also will exploit the newer and more powerful tools for studies of nicotine receptors. Preliminary data indicates that exposure to nicotine causes changes in the number of human T-cells produced by the FTOC system. It also suggests that nicotine receptors are also present on the surfaces of developing T-cells, providing a direct means for nicotine to affect T-cell development and function. Studies of nicotine effects on T-cells in the FTOC system are significant because they will critically define roles of nicotine that affect immune function, identify nicotinic receptors that mediate those effects, and suggest that neuronal control of immune function can be mediated through natural actions of chemical messengers acting at immune cell nicotine receptors. Information gained will clarify effects of tobacco use on the immune system and will suggest strategies for the design of nicotine-like drugs that could be used therapeutically to modulate function of the immune system, especially T-cell development.

Graves, Jr., Ph.D., Joseph L.

Arizona State University West
Award Amount FY 1999: $50,000

The Impact of Nicotine on the Genetics of Aging: Exposure and Selection for Resistance

Few studies exist in animals that look at the long-term multi-generational impacts of exposure to nicotine. This study utilizes fruit flies to examine possible genetic impacts of nicotine exposure in populations. It is possible that nicotine may influence subtle genetic systems that are not directly observed in specific disease systems. Drosophila fruit flies are well suited for these studies because of the existence of stocks that are genetically differentiated for longevity. Many of the physiological responses to nicotine exposure in animals are conserved between insects and mammals, as are fundamental mechanisms of aging.

The following will be accomplished in this study. Fly stocks will be assayed for acute and chronic impacts of nicotine exposure. We will particularly examine if nicotine differentially impacts genetic systems that control the rate of aging. Artificial selection will be used to breed flies that are resistant to nicotine exposure. Genetic changes will be surveyed to define genetic systems that may confer resistance to nicotine toxicity.

Gunatilaka, Ph.D., A.A. Leslie

University of Arizona
Award Amount FY 1999: $49,962

Mechanism-based Discovery of Novel Anti-tumor Agents from Plants

Cancer is a disease especially of the elderly and a major killer in the U.S. According to the Arizona Cancer Registry published by the Arizona Department of Health Services, in 1992, cancer was responsible for 7,400 deaths and 19,378 newly diagnosed cases. Cancer incidence and deaths in our state are on the rise, as many elderly continue to move here due to the desirable climate. A recent article published in *Scientific American* (Sept. 1996) suggests that more than 30 percent of all these cancer deaths can be attributed to tobacco smoke.

Cancer treatment currently relies on surgery, radiation and chemotherapy (treatment with anticancer drugs). Chemotherapy is the only effective way to treat a disseminated cancer. Unfortunately there are no good anticancer drugs available to effectively treat major solid tumors such as cancers of the lung, breast and colon, without any side effects. There is an urgent need for the development of new, effective and non-toxic anticancer drugs to treat these cancers.

The majority of anticancer drugs in use today are of natural origin. The search for anticancer agents from plants can be justified. With seed money from the University of Arizona Small Grants Program, we have installed two "in house" bioassays to screen plant extracts/pure compounds for their anticancer activity. We have discovered several plant extracts and pure compounds that exhibit encouraging results. The goals of the research proposed are to continue to work on these leads, and install additional selective bioassays to discover novel anticancer agents from plants of Arizona, Brazil, Bulgaria, China, Ethiopia and Sri Lanka to treat cancers which are suspected to be caused by tobacco use.

Plant extracts showing positive responses to one or more of these bioassays will be subjected to various separation techniques to isolate natural products responsible for their anticancer activity. The chemical structures of these promising leads will be elucidated by modern instrumental techniques and chemical methods as needed. Those active compounds with novel structures will be further developed for future animal testing and, if promising, for clinical trials. We are hopeful that having access to plants from different geographic locations of the world, combined with the use of selective and improved bioassays will lead to new drugs to effectively treat currently untreatable forms of cancer, some of which are tobacco-related.

Hersh, M.D., Evan M.

University of Arizona
Award Amount FY 1999: $150,000

Program Project to Develop Novel Gene Therapies for Tobacco-related Cancers

More Arizonans will die of tobacco-related lung cancer and head and neck cancer this year than any other malignancy. Despite chemotherapy, radiation therapy and surgery, the vast majority of patients diagnosed with these cancers die of their disease. For these people, new therapies are desperately needed. The goal of immunotherapy is to specifically increase patient immune responses against tumor cells. By directing therapy only at cancer cells, toxicities associated with other nonspecific therapies, such as chemotherapy and radiation, are eliminated. In the last few years, there has been a revolution in our understanding of the immune system, mechanisms by which cancer cells evade immune recognition, and gene therapy technology. Using gene therapy methods, we can directly target tumor cells to be recognized as foreign by our immune cells. We can also supplement patient immune function with the use of immuno-stimulatory proteins, known as cytokines. Using the resources of the Arizona Cancer Center, a center of excellence in both basic and clinical research, we intend to test and explore the mechanisms of a novel gene therapy approach for patients with these tobacco-related malignancies.

The goal of this program is to evaluate the role of dual modality gene therapy in lung and head and neck cancers. Prior studies conducted at the Arizona Cancer Center, as well as other institutions, have shown that gene therapy, or the transfer of genetic material into tumor cells, can produce clinical responses in tumors but that these responses are often transient or incomplete. Recent insights into the host (patient) immune response directed against tumor cells suggest multiple pathways of immune activation are required for optimal host anti-tumor responses. Cancer patients are often immuno-compromised at the time of their diagnosis. Thus, current gene therapy protocols using one immuno-stimulatory agent may generate insufficient immune responses to eradicate tumors. We hypothesize that a combined or dual modality approach, using systemic immunorestorative agents to improve patients' overall immune function coupled with gene therapy designed to target the specific tumor, will be superior to protocols currently in clinical trials.

Our specific objectives are:

1) To conduct a Phase I Clinical Trial of dual modality immunotherapy using low dose systemic IL-2 (an immunorestorative cytokine) and gene therapy, with intratumoral DNA injections of an allogeneic MHC molecule to target the response to the tumor, in patients with advanced head and neck cancer or metastatic lung cancer unresponsive to standard therapies;

2) To optimize dual modality therapy approaches using a mouse model;

3) To evaluate and define immunologic responses elicited by dual modality gene therapy in patients and animal models.

Lam, Ph.D., Kit S.

University of Arizona
Award Amount FY 1999: $50,000

Development of Peptide Therapy for Small Cell Lung Cancer

In 1997, an estimated 2,600 Arizonans will die of lung cancer and 2,900 new cases will be diagnosed. Although the incidence of this disease in men has recently declined, it continues to increase in women. Since 1987, lung cancer has been the major cause of death in women, surpassing the death rate for breast cancer, which for 40 years was the leading cause of cancer death in women. It is believed that as many as 90 percent of all lung cancers may be smoking-related. Secondhand smoke increases the cancer risk for non-smokers as well. There are two major types of lung cancer: small cell (SCLC) and non-small cell (NSCLC). Each affects a different type of cell in the lung and grows and spreads in a different way, and so are treated differently.

Approximately 40,000 people are diagnosed in the U.S. each year with SCLC. Although chemotherapy combinations, with or without radiation therapy, have substantially prolonged patient survival, almost all patients still die of the disease. Over the past 20 years, no combination of surgery, radiation or chemotherapy has produced substantial improvement in survival and new therapeutic therapies must be explored. Peptide therapy is currently one of the most promising approaches for cancer treatment. SCLC tumor cells have receptors on their surface that bind peptide hormones and directly stimulate cell growth. SCLC cells produce, secrete and have their growth modulated by a number of different peptide hormones, including gastrin releasing peptide (GRP), neurotensin, cholecystokinin and vasopressin. Tumor cell growth can be arrested by blocking the ability of the receptor to bind hormones. The challenge is to find compounds that bind strongly to SCLC hormone receptors and block cell division. This research grant application proposes to use a new drug discovery method invented in this laboratory (the "one-bead one-compound" combinatorial peptide library method) to rapidly identify peptides that bind very specifically to the surface of SCLC cells and block cell proliferation.

The overall goal of this research proposal is to apply a state-of-the-art combinatorial library method to discover cell surface binding peptides that will prove useful in the treatment and cure of human SCLC. In this proposal, we hypothesize that by screening several "one-bead one-compound" combinatorial libraries of tens of millions of compounds with a high throughput assay method, peptides that bind specifically to the SCLC cell surface can be identified. We further hypothesize that these peptides can be used to target and treat human SCLC. The main objective of this project is to test the above hypothesis by constructing peptide libraries and various analogs of antagonist G (a known peptide that binds to SCLC cells), screening the peptides to binding, followed by analysis of biological function of the peptides on human SCLC cell lines.

Leischow, Ph.D., Scott

University of Arizona
Award Amount FY 1999: $48,564

Diverted Youth: Testing a Tobacco Cessation Intervention for Adolescents

Extrapolating from California data and recent figures for Tucson, approximately 75,000 students in Arizona ages 11 to 14 may be tobacco users. While a number of programs in the state target tobacco prevention, there are far fewer that address tobacco cessation in adolescents and surveys of the published research reveal a remarkable lack of such programs nationwide. Moreover, existing programs suffer form a lack of evaluation; thus, very little is known regarding the effectiveness of different intervention strategies with adolescent tobacco users. The Arizona Smokers' Helpline (ASH), a free telephone counseling service based in Tucson but available to any Arizona resident, has recently initiated a "Diversion Program" targeting adolescent tobacco users. Adolescents found using tobacco on school property are referred to the 5-week program, in lieu of punishment (e.g., detention). Contact may also be initiated by parents, other adults and teens themselves. The present proposal outlines a research-oriented project designed to assess the relative effectiveness of the following: 1) counseling conducted by fellow peers who have successfully quit tobacco *vs.* counseling by adults, and 2) a counseling style that involves direct and explicit scrutiny of tobacco use *vs.* a more indirect counseling style that addresses broad topic areas without specific reference to tobacco use. All clients will complete the same set of activities at home; only the telephone counseling will differ. This project is to serve as a pilot study that will be utilized in developing a statewide tobacco cessation intervention for adolescents.

The goals of this project are to: 1) decrease tobacco use among Arizona adolescents, 2) evaluate the efficacy of intervention materials utilized in a preliminary cessation program, 3) evaluate the efficacy of new, theory-based cessation intervention materials, 4) evaluate the relative efficacy of adults and adolescent counselors and 5) evaluate theoretically derived-hypotheses addressing both the content and delivery of tobacco cessation interventions for Arizona adolescents. Generally stated, the hypotheses to be tested in this field experiment are as follows: 1) Adolescent counselors will be more effective than adult counselors. 2) A nondirective counseling style will be more effective than a directive counseling style. 3) Adolescent counselors utilizing a nondirective style will be most effective overall. 4) Adult counselors will be maximally effective when utilizing a directive style. 5) Attitudes regarding tobacco cessation and perceptions regarding social norms are significant predictors of program effectiveness. 6) Considering perceptions of control over tobacco cessation will enhance the predictability of program success.

McDonagh, Ph.D., Paul F.

University of Arizona
Award Amount FY 1999: $49,223

The Effects of Cigarette Smoke Exposure on the Leukocyte Contribution to Ischemia-Reperfusion Injury in the Heart

Cardiovascular disease is the number one killer in Arizona and the United States. Of all cardiovascular disorders, ischemic heart disease (coronary heart disease) causes the greatest mortality and morbidity. Ischemia refers to the condition in which the blood supply to an organ is significantly reduced. When ischemia is severe in the heart, a "heart attack" occurs. The American Heart Association has determined that the four key factors associated with heart attacks are: 1) smoking, 2) high blood pressure, 3) elevated lipids and 4) diabetes. Of these factors, cigarette smoking is the single greatest risk factor associated with sudden death from ischemic heart disease. Thus, there is a clear and significant association between cigarette smoke and ischemic heart disease.

Although the association between cigarette smoking and ischemic heart disease is known, it is not clear why and how cigarette smoke increases the rate and the severity of heart attacks. This research project aims to investigate a mechanism that we suspect links cigarette smoke exposure with the severity of heart attacks. Our hypothesis is that an important connection between cigarette smoke and the severity of heart attacks is through the circulating white blood cells (leukocytes). Specifically, we propose that smoking enhances the severity of ischemic heart disease *via* an exaggerated, leukocyte-mediated inflammatory response. That is, activated leukocytes accumulate in the ischemic heart, in increased numbers, releasing oxygen free radicals and protease, causing further tissue injury. Our hypothesis is based on the following: 1) We know that cigarette smoke exposure causes activation of the white blood cells (leukocytes). 2) Leukocyte activation complicates several cardiovascular disorders, including ischemia-reperfusion injury in the heart. 3) We have demonstrated that prior leukocyte activation makes ischemia-reperfusion injury worse. Thus, if cigarette smoke exposure activates leukocytes, then the leukocyte contribution to ischemia-reperfusion injury in the heart will likely be increased, increasing the severity of a heart attack. We will test this hypothesis in a series of well-controlled experiments. Understanding exactly how smoking influences the rate and severity of ischemic heart disease will aid in developing strategies for improved prevention and treatment of this significant killer.

For leukocytes to participate in a cardiac inflammatory response, these blood cells must first accumulate in the heart. The likelihood of leukocyte accumulation will increase when the number of cells increases, when the leukocyte deformability decreases and when leukocyte adhesion increases. Accordingly, in the first portion of this project, we will test the specific hypothesis that the magnitude and duration of exposure to second hand (sidestream) cigarette smoke (SSCS) alters leukocyte numbers, especially PMNs, and their state of activation in the blood. We will determine the relationship between exposure to sidestream smoke and leukocyte concentration as well as leukocyte deformability and adhesion.

Leukocyte activation is marked by an increase in the expression of adhesion proteins and an increase in the production of oxygen free radicals. The adhesion proteins contribute to retention in the microcirculation and the radicals cause tissue damage. In the second portion of the project we will investigate, at the cell and molecular level, leukocyte alterations caused by sidestream smoke exposure. We will specifically examine the effects of SSCS on the expression of the leukocyte adhesion proteins

(CD11b and L-selectin) and the production of oxygen-derived free radicals (Reactive Oxygen Species, ROS). We will also test two mechanisms by which the PMNs are activated, namely activation by a plasma factor and/or amplified leukocyte reactivity due to enhanced platelet-leukocyte interactions.

The initial step in leukocyte accumulation in the heart is by sequestration in the coronary microcirculation. In the third portion of this project, we will determine if cigarette smoke exposure leads to enhanced leukocyte deposition in the coronary microcirculation following ischemia. As mentioned, leukocyte sequestration is a key initial step in the inflammatory response, and we know that when deposition is enhanced, myocardial recovery from ischemia is significantly impaired. We will directly observe leukocyte deposition in the coronary microcirculation using intravital microscopy, a technique well established in our laboratory.

The lesson learned will help determine the pathophysiological mechanisms underlying the relationship between blood leukocyte activation and severe ischemic heart disease. This knowledge will also help further explain the interrelationship of cigarette smoke exposure and coronary (ischemic) heart disease. The results of these studies will aid in developing better strategies to prevent and treat ischemic heart disease, a significant killer in our society.

Nagle, M.D., Ph.D., Ray B.

University of Arizona
Award Amount FY 1999: $112,204

Improved Extracellular Matrix for Treatment of Tobacco-Related Occlusive Vascular Disease

Aging and smoking are major risk factors for the development of cardiovascular disease. Seniors aged 75 and older were the second fastest growing age group in Arizona from 1980 to 1990. In Pima County alone, their numbers increased during that period 62 percent. In 1990, 18 percent of the population was 60 years or older and it is estimated that by the year 2010 this percentage will reach 21.2 (*Arizona Daily Star* 6 Oct. 1996).

Recent data suggest that cigarette smoking leads to damaged components of blood and blood vessels. These altered blood components cause injury to vessels which lead to narrowing and eventual blockage of the vessel. This problem affects peripheral, coronary, and brain vessels. Modern surgical techniques employ either the patient's own veins or synthetic plastic grafts to bypass these lesions and restore critical circulation. The unsuitability of the patient's own veins for grafting is a severe limiting factor which has lead to the increased effort to develop synthetic grafts. The successful employment of synthetic grafts is largely dependent on how effectively the graft surfaces can be relined with the patient's own endothelial cells, which is essential to prevent clotting of blood and blocking of the vessel lumen.

This proposal is designed to improve endothelial regrowth on the surface of plastic vascular grafts by pretreating the grafts with the newly discovered extracellular matrix protein, laminin 5. Our first goal will be to determine whether the protein, laminin 5, increases endothelial cell adherence, spreading, and growth under tissue culture conditions. Our second goal will be to determine whether laminin 5 induces proper functional responses of the human endothelial cells. In the third goal we will determine whether a laminin 5 rich coating can increase endothelial regrowth in the artificial vessel itself. Our fourth goal will be to determine whether simply coating synthetic grafts with peptides which mimic laminin 5 could be used to support endothelial attachment in the artificial vessels. Our last goal will be to test the laminin 5 and peptide coated grafts in animal experiments to learn whether endothelial cells will reline their lumens and prevent clotting.

Parrish, Ph.D., Alan R.

University of Arizona
Award Amount FY 1999: $49,134

Role of Tobacco-Derived Cadmium in Prostate Disease

Prostate cancer is the second leading cause of cancer death in men, responsible for approximately 40,400 deaths per year in the United States. The probability of developing invasive prostate cancer increases dramatically with age; therefore, this cancer is a tremendous concern to the state of Arizona which supports an aging population. As of 1995, mortality due to prostate cancer in Arizona was in the top 40 percent of the United States. Prostate cancer incidence and mortality have been linked with cigarette smoking and cadmium, a carcinogenic metal that is a component of tobacco products. Thus, the prostate cancer linked with smoking may be related to cadmium, a hypothesis to be examined in this proposal. As there is currently no acceptable animal model of prostate cancer, we propose to perform experiments in human prostate tissue. We have recently begun to develop a tissue slice model for human prostate, a system which retains the complex cellular nature of the organ and can be used to examine the distinct pathologies associated with different areas of the prostate. These studies will serve to: 1) develop and validate a new system to understand prostate biology and pathology and 2) elucidate the basis of tobacco-derived cadmium in prostate cancer.

Three specific aims have been designed to test the hypothesis that *the cadmium associated with tobacco products is responsible for the prostate cancer linked to cigarette smoking.* As there is currently no accepted animal model for prostate cancer, we will first establish an *in vitro* model of human prostate which maintains the complex architecture and heterogeneity of the organ. Having established the precision-cut slice model, we will examine the toxic effects of cadmium in peripheral and transitional zone slices, two distinct regions in the prostate each associated with different, yet clinically important, pathologies. Finally, we will examine the impact of cadmium on two interconnected signaling pathways that are associated with the development of prostate cancer. In these studies, cadmium will be studied at concentrations associated with exposure due to cigarette smoking. It is anticipated that completion of the proposed studies will serve to: 1) establish a novel *in vitro* system to investigate prostate biology which will significantly advance our understanding of the organ and 2) provide insight into the molecular mechanism by which cadmium contributes to prostate cancer.

Pettit, Ph.D., Robin K.

Arizona State University
Award Amount FY 1999: $150,000

Biological Research for Advancing New Anti-infective Drugs to Clinical Trials

Each year, nearly 60,000 Arizona residents die of tobacco-related cancer or associated infectious disease. Smoking-attributable medical and economic costs to the state of Arizona approximate one billion dollars yearly. Nationally, tobacco use is responsible for approximately 200,000 cancer deaths a year, with medical and economic losses well over 100 billion dollars.

Cancer patients often die of infectious disease because their immune systems are so severely depressed. As such, there is an urgent need to develop new anticancer and antimicrobial drugs. The Arizona State University Cancer Research Institute (ASU-CRI) discovered and developed six compounds that are currently in human cancer clinical trials. Scientists at the CRI continue to discover new compounds active against tobacco-related diseases, but these cannot enter human trials until they are extensively evaluated in animal models. ADCRC Tobacco Tax Research Funding is requested to expand the CRI's drug discovery program to include antimicrobial compounds, and to test these new compounds in animal models. The proposed ACDRC contract will allow promising drugs to proceed much more rapidly from the laboratory to thousands of Arizona patients with tobacco-related cancers and associated infectious disease.

Infectious disease is a leading cause of death in cancer patients. Thus, the goal is to expand our drug discovery program to include antibiotics. ADCRC contract funding will allow the discovery and development of new antibiotics at the CRI, and preclinical screening at a major animal research facility. These data will facilitate rapid selection of compounds for infectious disease clinical trials. The long-term goal of the proposed research is to provide new antitumor anti-infectives to thousands of Arizona residents with tobacco-related cancer or associated infectious disease.

Philipps, M.D., Anthony

University of Arizona
Award Amount FY 1999: $49,748

The Effects of Nicotine Patches on Maternal and Fetal Health

The Arizona Department of Health Services estimates that approximately 9,000 women gave birth in Arizona in 1995. Of these, 990 voluntarily reported using tobacco products during pregnancy. As many as 2 percent of all surgeries performed on women each year occur during pregnancy and more than 25 percent of pregnant women require cesarean delivery. Almost 40 percent of cesarean deliveries are performed under general anesthesia. This means that in 1995 alone, approximately 180 women in Arizona had emergency surgery while they were pregnant, 2,250 delivered by cesarean section and 900 required general anesthesia. Due to the deleterious effects of smoking on unborn children, it is strongly recommended that women quit smoking during pregnancy. With the current popularity of nicotine patches, it is likely that some women will arrive in the operating room wearing a nicotine patch. Currently, the effects of nicotine patches on the unborn child or its interactions with anesthetics is unknown. Studies need to be undertaken *to evaluate the effects of nicotine patches on the mother and baby and their potential interaction with the anesthetic drugs* commonly administered to pregnant women during emergency surgery, labor and delivery.

The goal of this study is to determine what effects the nicotine patch has upon the mother and baby and how those effects relate to the administration of anesthetic drugs. The physiologic effects of nicotine on the mother and fetus have been documented by studies utilizing animals. Measurement as simple as blood pressure cannot be performed in the human fetus because this requires instrumentation. The pregnant sheep (ewe) model is the best animal model available today for physiologic investigations in pregnant animals. This study will be accomplished in three phases. First, we will show that when nicotine patches are applied to ewes they will produce the same nicotine blood levels as they do in humans. Second, we expect that slow, continuous administration of nicotine from patches will have similar effects on the mother and baby as nicotine from smoking, and that those effects will last longer with a continuous source of nicotine. Third, we want to show that the constant presence of nicotine will alter the effects of anesthetic drugs. We think that mothers using nicotine patches will need more anesthetic drugs to maintain a surgical level of anesthesia compared to women not using patches because nicotine is a stimulant. If mothers using nicotine patches do require more anesthetic drugs, they will encounter more cardiovascular compromise under general anesthesia. In addition, the fetus will be stressed more than what normally occurs under general anesthesia. Increased fetal stress can ultimately lead to miscarriage.

Rider, M.D., Evelyn D.

University of Arizona
Award Amount FY 1999: $49,998

Environmental Tobacco Smoke Exposure in Developing Lungs: Effects on Surfactant Metabolism

This application proposes to define the effects of environmental tobacco smoke exposure on surfactant metabolism in developing lungs. Pulmonary surfactant, a complex mixture of proteins and phospholipid, possesses unique surface tension-lowering properties, modulates the immune and inflammatory responses in the lung, and is absolutely essential for normal lung function. Cigarette smoking is known to alter both pulmonary surfactant lipid composition and function and may have inhibitory effects on surfactant secretion at the alveolar surface. Functional surfactant is further reduced in the airways by cigarette smoke-induced increase in alveolar macrophage ingestion of surfactant. The effect of environmental tobacco smoke exposure on surfactant metabolism remains unknown. This is an important area of research as it is estimated that over 50 percent of children, five years of age and under, are exposed, postnatally, to environmental tobacco smoke.

Epidemiological evidence indicates that postnatal exposure to environmental tobacco smoke increases the risk of lower respiratory tract illness, increases the incidence and severity of asthma, and leads to alteration in lung growth and development in young infants and children. Surfactant provides a monomolecular covering for the alveolar and bronchiolar surfaces of the lung. Disruption of this protective monolayer surface may lead to development of disease by opening the way to toxic injury of the epithelial surface on the lung. Since environmental tobacco smoke exposure of young infants and children leads to serious lung diseases that may have abnormalities in surfactant as associated findings, the following experiments were designed to investigate the effects of postnatal exposure of the developing lung to environmental tobacco smoke on pulmonary surfactant homeostasis and metabolism.

This application proposes to define the effects of postnatal environmental tobacco smoke exposure on surfactant metabolism *in vivo* in the developing lung. The overall objective is to determine mechanisms involved in regulation of surfactant metabolism in the injured developing lung and enhance understanding of the role of surfactant in the serious lung diseases associated with environmental tobacco smoke exposure. The central hypothesis is that exposure to environmental tobacco smoke causes structural and cellular changes in the developing lung that lead to altered pulmonary surfactant metabolism.

SPECIFIC AIMS:

1) Define cell-specific effects of environmental tobacco smoke (ETS) exposure on surfactant metabolism in the developing postnatal lung.
    a. Investigate developmental changes in surfactant phosphatidylcholine metabolism by type II cells and alveolar macrophage isolated from 3-day, 2-week, and 4-week old rabbits.
    b. Determine the effects of postnatal environmental tobacco smoke exposure on developmental changes in surfactant metabolism by type II cells and alveolar macrophage isolated from 3-day, 2-week, and 4-week old rabbits.
2) Determine if prolonged postnatal exposure to ETS results in alveolar epithelial injury and altered type II cell proliferative activity.
3) Investigate the effects of environmental tobacco smoke exposure on lung function and surfactant metabolic pathways *in vivo*.

Shapiro, Ph.D., Daniel E.

University of Arizona
Award Amount FY 1999: $49,998

Physician-Patient Communication with Heavy Smokers: Comparing Motivational Interviewing with the Prescriptive Approach

Of the 550,000 Arizonans who smoke, roughly 75 percent will visit a health professional during 1998. In surveys, the majority of smokers report that if they were asked by health professional to quit, they would make an attempt to quit. Unfortunately, most health professionals are confused about optimal methods to use with smokers and frequently do not counsel smokers to quit. In particular, they frequently avoid counseling ambivalent smokers and smokers of Hispanic origin. Given this obvious opportunity, how should our health professionals counsel heavy smokers so that the chances for a quit attempt are optimized? There are two approaches:

1) Most physicians, nurses, and nurse practitioners currently use the "prescriptive" approach in which the professional takes an authoritative role and seeks to convince the smoker to quit immediately: The health professional demands that the smoker quit, logically argues with any of the smokers protests, and explains the hazards inherent in not quitting.
2) A new and innovative model is known as "motivational interviewing." Motivational interviewing takes the opposite approach. The health professional assumes the role of supportive consultant and encourages the patient to generate their own negative reactions to smoking. Instead of arguing, demanding or explaining, the professional gently directs the smoker to consider how smoking affects their health, their lives and their well being.

These two contrasting communication approaches have never been compared in a head-to-head trial for smokers. When they were compared for diabetics, drug users, alcoholics and hypertensives, the supportive style of motivational interviewing was shown superior to the authoritative, prescriptive approach in adopting healthier lifestyles. We know that 375,000 Arizona smokers visit a health professional every year. This study can help assure that communication during this visit is optimally effective.

Our long term goal is to insure that smokers who meet with Arizona-trained health professionals receive the most effective cessation counseling available. Our immediate objective is to test the hypothesis that motivational interviewing is superior to the commonly-used prescriptive approach. Because Hispanics and ambivalent smokers are least likely to receive smoking cessation counseling from health professionals, our study focuses on these groups. Our secondary objective is to test which approach works best for men, women, Hispanics, Caucasians, older and younger Arizonans. From these results, we will be able to provide clear, practical, research-based guidelines for health professionals who counsel the Arizona smoker.

Sherrill, Ph.D., Duane

University of Arizona
Award Amount FY 1999: $49,942

Assessment of Patterns of Mendelian Inheritance of Addiction to Cigarettes

It is well established that nicotine is an addictive drug and, as such, it is critical to understand the role that familial inheritance plays in this addiction. Evidence from animal studies has suggested that there may be important genetically determined differences in people's susceptibility to nicotine. In addition, several twin and family studies have shown a consistent degree of heritability of the smoking habit. By examining the patterns of cigarette addiction among randomly sampled families, we plan to determine if these patterns are consistent with those expected assuming a Mendelian mode of inheritance, with a single major gene with two alleles. This information is a first step in identifying a specific gene or genes responsible for increased susceptibility to nicotine addiction among smokers and has the potential to increase our understanding of the addiction mechanism, develop new preventive strategies and identify target high risk populations for early intervention.

The primary objective of the proposed study is to assess patterns of Mendelian inheritance associated with addiction to cigarettes among pedigrees in which smoking assessments were reported by parents and offspring in repeat surveys (1972-1996). Segregation analysis, a statistical method for detection of Mendelian ratios in the transmission of a trait from one generation to the next, will be performed on discrete smoking variables. These models specify a regressive relationship in which the phenotype of an individual is conditional on the phenotype (trait value) of his or her antecedents and any covariates in the model. Subjects will be classified, based on self-reported smoking status, into current and never-smokers, quitters, or restarters. To determine if inheritance of susceptibility to addiction is related to the level of dependency, secondary analyses will be performed using light, moderate and heavy smoker caterogries. The goal of the proposed study is to identify patterns of familial inheritance of increased susceptibility to nicotine addiction.

Witten, Ph.D., Mark L.

University of Arizona
Award Amount FY 1999: $132,752

Effects of Vitamin E Dietary Supplementation on Sidestream Cigarette Smoke-induced Lung Injury

The adverse effects to tobacco smoke exposure on human lung function and disease have been investigated extensively; the lung has responses to tobacco smoke that range from chronic inflammation to cancer. The effects of chronic exposure to sidestream cigarette smoke is less understood.

Eighty-five percent of the people of Arizona live in the Tucson and Phoenix areas. It is estimated that urban residents spend 90 percent of their time indoors and, thus, are exposed to sidestream cigarette smoke. Our pilot study has demonstrated that only 10 days of vitamin E dietary supplementation can partially attenuate the adverse effects of sidestream cigarette smoke exposure in a mouse model.

It is the overall goal of this proposed study to determine the effectiveness of a 150-fold increase in vitamin E dietary supplementation, to attenuate sidestream cigarette smoke-induced injury to the lungs and immune system, in three different age groups; neonate (immature immune system), adults, and elderly (immunosenescence).

Hypothesis:

A diet with vitamin E supplementation, 150-fold over normal levels, will attenuate the sidestream cigarette smoke-induced injury to the lungs and immune system even in neonatal (immature immune system) and elderly (immunosenescence) mouse model.

Specific Aim:

Determine the effectiveness of a 150-fold increase in Vitamin E to attenuate the sidestream cigarette smoke-induced injury to the lungs and immune system.

Winzerling, Ph.D., Joy Johnson

University of Arizona
Award Amount FY 1999: $50,000

The Effects of Chemicals Found in Cigarette Smoke on Iron Metabolism of Lung Cancer Cells

Each year 2500 persons die of lung cancers in Arizona (American Cancer Society, 1996). Free radicals are thought responsible for the development of many of these cancers. Nitric oxide (NO·), found in cigarette smoke, and superoxide, produced by smoking, are free radicals. In lung tissues, these free radicals can damage the cells and promote cancer. When NO·, superoxide and related compounds, like hydrogen peroxide, are mixed with iron, more free radicals are produced. Iron can be introduced into the lung from the tar in cigarettes by smoking. Normal cells protect themselves from iron by storing the iron in a protein called ferritin. How much ferritin is available for storage of iron is determined by another protein called the iron regulatory protein (IRP1). IRP1 controls how much ferritin is made by binding to a very specific control site, the iron regulatory element (IRE), on the ferritin subunit messenger RNA (mRNA). NO· and superoxide have been shown to enhance the binding of the IRP1 to the IRE in normal cells. Thus, these chemicals can decrease the amount of ferritin and available iron storage inside the cell. Ferritin and iron are increased in the lung fluids of smokers with tumors. Where this ferritin comes from is not known. It could be secreted from the cancer cells or leak from damaged tissues. Since chemicals in tobacco smoke are known to alter the available amount of ferritin, and to cause increased free radical formation in the presence of iron, we want to study their effects on iron metabolism in lung cancer cells.

We propose to study the effects of free radicals on lung cancer cells by exposing the cells directly to free radical-producing compounds or cigarette smoke. Our immediate goals are: 1) to evaluate the effect of NO·, superoxide and hydrogen peroxide on the synthesis of ferritin and IRP1, and on IRP1 binding activity for the IRE of ferritin mRNA in the small cell lung cancer cell line NCI-H510 and 2) to determine if cigarette smoke can produce these same effects. Based upon current knowledge, we hypothesize that these chemicals or cigarette smoke will alter the synthesis of ferritin, and possibly IRP1, and will enhance the binding of IRP1 for the IRE of ferritin mRNA and NCI-H510 cells. Our specific aims are to evaluate the effects of 1) iron, 2) NO·, superoxide and hydrogen peroxide, or 3) cigarette smoke, on the synthesis of ferritin and IRP1, on ferritin and IRP1 mRNA levels and on IRP1 binding activity for the IRE in NCI-H510 cells.

The objectives of this project are to provide a preliminary picture of changes in iron metabolism in lung cancer cells that results from cell exposure to free radicals, iron or cigarette smoke, and to obtain preliminary data for a proposal to the NIH. The long-term goals of this research are to study the effect of cigarette smoke on iron metabolism of lung cancer cells, to determine changes in iron metabolism that could contribute to the development of tobacco-related diseases and to examine various agents that could reduce or prevent these effects.

Wu-Wang, Ph.D., (Grace) C.Y.

University of Arizona
Award Amount FY 1999: $50,000

Effect of Tobacco on the Cellular Functions of Oral Epithelium: Roles of Prostaglandin E2 and Epidermal Growth Factor

Numerous studies have shown strong associations between smokeless tobacco (snuff or chewing tobacco) use and various oral diseases, including a high incidence of oral cancer in man. There has been a three-fold increase in the use of smokeless tobacco in the United States over the past 20 years. The consumption of smokeless tobacco is still on the rise in the United States, particularly among young individuals. This trend has serious health implications. Unfortunately, the mechanism of tobacco-induced oral cancer remains largely unknown. Salivary secretions are essential for the maintenance of the health of the oral cavity. Prostaglandin E2 (PGE2) and epidermal growth factor (EGF) are two important biologically-active compounds found in saliva. In our previous studies, we found that salivary levels of PGs and EGF were diminished in cigarette smokers. Since these two compounds are known to have a cytoprotective effect on the mucosa of the alimentary tract, the reduction of salivary PGE2, and EGF may be associated with tobacco-induced oral mucosal diseases. Investigation of the effect of tobacco on various cellular activities will help us to understand the mechanism by which tobacco causes a malignant effect on oral mucosa.

The goal of this proposed study is to elucidate the physiological and pathological roles of PGE2 and EGF in protection of the oral mucosa. Understanding the functions of PGE2 and EGF in the oral cavity will help us to establish strategies for prevention and treatment of oral diseases (including cancer) induced by tobacco. The hamster is an excellent animal model to study the progression of carcinogenesis (especially for oral cancer) and will be used for the proposed project. The hamster's buccal pouches will be treated by swabbing both sides of the pouch with mineral oil (control group), or components of tobacco in mineral oil by cotton swab. The salivary glands and buccal pouch tissues will be used for the following objectives: 1) to study the effects of tobacco components on PGE2, and EGF syntheses in the major salivary glands. Biochemical and molecular biological analysis will be conducted to study the enzyme proteins or mRNAs responsible for the syntheses of PGE2 and EGF. 2) to investigate the effect of tobacco on PGE2- and EGF-mediated cellular responses in oral mucosa. Hamster buccal pouch epithelium (HBPE) will be incubated with various concentrations of PGE2 or EGF in culture medium with other reagents to study cell proliferation and intracellular signaling systems. It is hypothesized that the tobacco-related decrease of PGE2 or EGF level is due to impairment of the PGE2- and EGF-synthesizing mechanism in salivary glands. The decrease of PGE2 or EGF may weaken the normal cellular functions in the protection of oral mucosa.

Yamamura, Ph.D., Henry I.

University of Arizona
Award Amount FY 1999: $49,500

Adenylyl Cyclase Superactivation after Chronic Opioid Receptor Stimulation

Smoking has been implicated in several forms of cancer, including lung cancer. Lung cancer has been responsible for 44 deaths per 100,000 population in the period 1986-1990 in Arizona. Despite the advances in treatment, lung cancer has only a 13 percent survival rate five years after the diagnosis. Much of the care provided lung cancer patients is, by necessity, supportive, designed to reduce the pain associated with disease. Opiate drugs are the primary choice in the management of severe pain and also, a main concern as drugs of abuse. Classical opiates like morphine, heroin and levorphanol act through a type of opioid receptor (mu receptor) that cause a number of side effects, like constipation, breathing problems and physical dependence. Breathing problems (respiratory depression) is the major cause of death in opiate overdose and can be especially dangerous in the case of lung cancer where lung function is already compromised. For these reasons, a new class of opioid drugs is under clinical trial that act through a different type of opioid receptor (delta) and show fewer side effects than classical opiates. Drugs that act through the delta opioid receptor do not cause respiratory depression. However, this new drug class also displays tolerance, a loss of responsiveness to the drug after long-term treatment. This proposal is designed to study the biochemical mechanisms underlying drug tolerance at the human delta opioid receptor. A better understanding of this process should aid in the development of pain-relieving agents that cause less side effects and tolerance in the treatment of lung cancer pain.

Chronic exposure to opioids leads to tolerance, the loss of responsiveness to the drug, and dependence, a condition when the presence of the drug is necessary to maintain normal function. Cells of the nervous system communicate using special membrane proteins called receptors. Binding of a drug to the receptor leads to the change of activity of enzymes (such as adenylyl cyclase) and ion-channels. Chronic activation of the opioid receptors in the brain has been shown to lead to an increase in forskolin-stimulated cAMP accumulation upon withdrawal of the inhibitory agonist. This mechanism is referred to as adenylyl cyclase (AC) superactivation or cAMP overshoot. Adenylyl cyclase superactivation after chronic agonist exposure has been proposed to play an important role in opiate tolerance, dependence and withdrawal. This proposal is designed to study the mechanism of AC superactivation after chronic delta opioid agonist treatment in a model Chinese Hamster Ovary (CHO) cell line, genetically modified to express the human delta opioid receptors. The central hypothesis in this proposal is that chronic opioid agonist treatment activates cellular enzymes (protein kinase) that chemically modify (phosphorylate) the adenylyl cyclase present in the CHO cells, leading to increased activity of the enzyme. We will use specific protein kinase inhibitors to determine the role of different kinase in the process of AC superactivation. We will determine what types of AC isoenzyme are present in the CHO cells, where AC superactivation occurs after chronic opioid agonist treatment and in the B82 cells, where AC superactivation does not occur. We will directly measure the change in the phosphorylation of the different AC isoenzyme after chronic opioid treatment. Finally, we will compare the sequence of the AC isoenzyme present in CHO and B82 cell lines, identify possible phosphorylation sites and prove their role in AC superactivation by destroying the phosphorylation site using site-directed mutagenesis.

Principal Investigators, Continuing Contracts

Principal Investigators, New Awards