Arizona Disease Control Research Commission

1996-97 Annual Report

Arizona Disease Control Research Commission

ANNUAL REPORT
1996-97

Fife Symington, Governor

Jack Dillenberg D.D.S., M.P.H., 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
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January 1998

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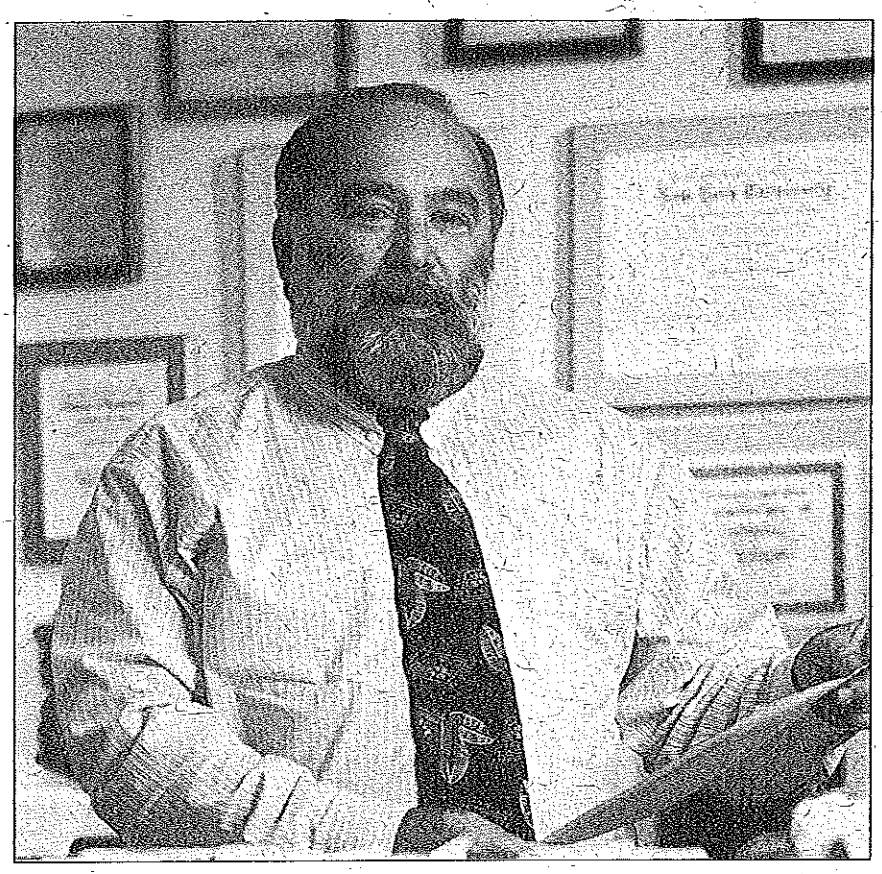


Message from the Chairman

As Chairman of the Arizona Disease Control Research Commission (ADCRC), I am pleased to forward this agency's report on its contributions to improving the health of Arizonans through funding essential scientific research. Established by the Arizona Legislature in 1984 to support research on the cause, epidemiology, diagnosis, treatment and prevention of diseases affecting Arizonans, the ADCRC is empowered to direct public funds to disease research on a wide range of medical and public health concerns. Funding comes through the legislative appropriations process and the Tobacco Tax Initiative, passed by the voters in November, 1994. The ADCRC receives five percent of the revenues collected from the tax to fund tobacco related medical research. The first recipients of these funds were selected in June of 1995. The "Healthy Arizona Initiative" passed in 1996 provided two million dollars annually for non-tobacco related essential scientific research. The initiative remains unfunded and the Commission will continue to seek avenues to reestablish the unrestricted medical research program.

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. The terms of three members expire each year; Commissioners may be reappointed. The Director of the Department of Health Services serves as Chairman of the Commission and *ex-officio* member. The Chairman and Commissioners who served during 1996-97 are presented below.

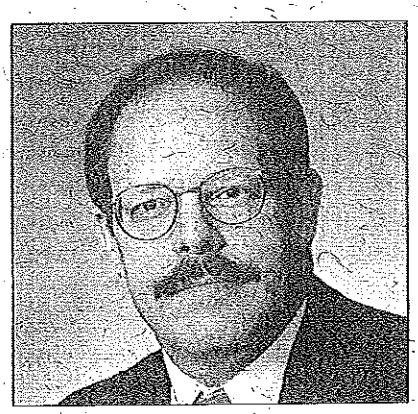
Jack Dillenberg, D.D.S., M.P.H.
Director
Arizona Department of Health Services

Chairman Dillenberg was appointed Director of the Arizona Department of Health Services by Governor Fife Symington in late August 1993. Dr. Dillenberg began with the ADHS in 1986 when he became Chief of the Office of Dental Health. In 1991, he became Assistant Director for Community and Family Health Services, where he served until appointed ADHS Acting Director on March 12, 1993. Chairman Dillenberg is a graduate of Tulane University and earned his D.D.S. degree at New York University and his Masters in Public Health from the Harvard School of Public Health, where he was honored as recipient of the 1993 Alumni Award of Merit in recognition of his major contributions to public health.

Dr. Dillenberg was replaced as Director of the Department of Health Services by Acting Director James Griffith in June of 1997.

General Public

Jose Cardenas, J.D.
Partner, Lewis and Roca Commercial Litigation Group
Phoenix

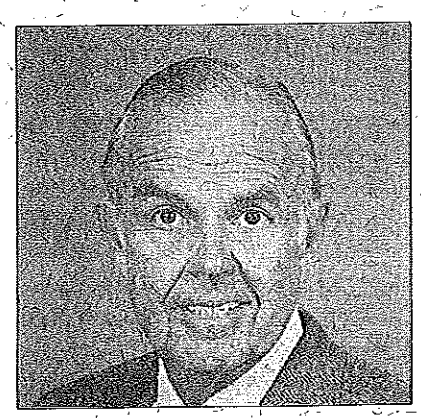


Commissioner Cardenas received his B.A. from the University of Nevada, Las Vegas in 1974 and his J.D. from Stanford University in 1977. He joined the firm of Lewis and Roca in 1978 where he practices primarily in the areas of commercial and civil litigation, intellectual property, and international law. 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 her 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 of 2000.

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



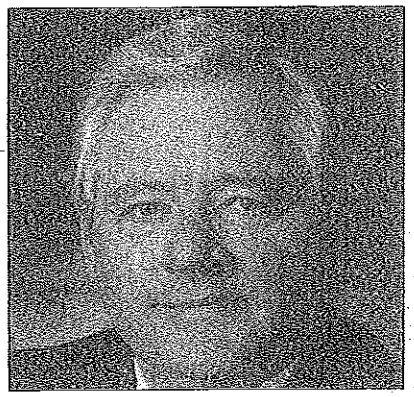
Commissioner Lewis oversees commercial real estate interests and participates in environmentally sensitive businesses. He currently serves on the governing boards of the Arizona Historical Foundation, Arizona State University Foundation, Phoenix Children's Hospital, Polycystic Kidney Research Foundation and 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 Legislature. He received his B.S. in Economics in 1958 from the University of Arizona. Commissioner Lewis was appointed by Governor Symington to the Commission in 1995 and his term will expire in May, 1998.

Medical Community

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

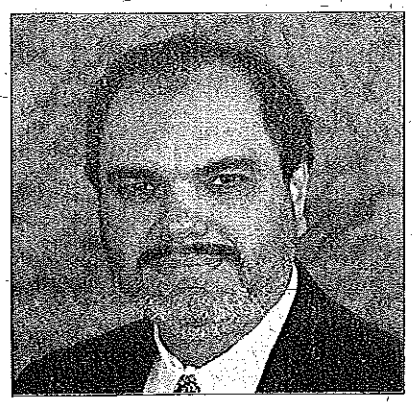
Commissioner Moore received her Doctorate and Master's degrees in Public Health from the Johns Hopkins University School of Hygiene and Public Health and a Master's degree in Nursing from the Catholic University of America. Commissioner Moore is an Associate Professor and Chair of the Division of Community 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



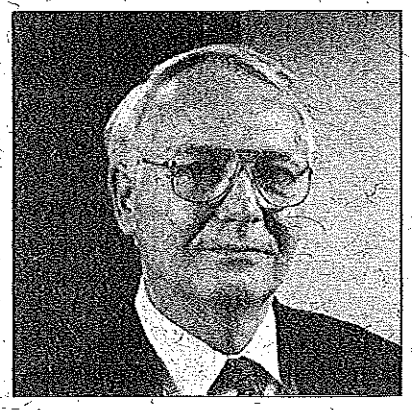
Commissioner Pereira received his B.S. in Chemistry from Georgia Institute of Technology in 1979. He graduated *Magna Cum Laude* from Emory University School of Medicine in 1983. He completed his internal medicine residency in 1986 at the Emory University Affiliated Hospitals and joined the staff of the Mariposa Community Health Center that year. He returned to Emory University where he was an Assistant Professor of Medicine and Director of the Intensive Care Unit at Grady Memorial Hospital from 1990 to 1992. He has been a Fellow of the American College of Physicians since 1993. In 1994, he initiated and continues to oversee, as program planner, annual Southern Arizona Conferences for primary care physicians. He was appointed to the Commission by Governor Symington in 1995 to complete the term of Commissioner Carlos Gonzales. His term expired in May, 1996 and he was reappointed to a second term which expires in 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.

Henry Reeves, Ph.D.
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. 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.

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.

Summary of 1996-97 Commission Activities

The Commission had 81 contracts with medical and health researchers in Arizona as of July, 1996. Contract summaries are contained in Sections A-C. The section headings list the source of funding (unrestricted medical research supported by appropriated funds and tobacco related research supported by tobacco tax funds) and the year the contracts are in, first second or third. Seven contracts received funding from both appropriated and tobacco tax revenues. These are marked as supplemental award amounts. Some of these projects will have two abstracts if two distinct research avenues were pursued.

Abstracts for each project outlining the progress made have been included here as have scientific publications and published abstracts. Lay summaries for new awards have been added to Section E to provide an overview of the new research. These changes should provide a more complete description of the work of the Commission and the impact this program has on both the scientific community and the state of Arizona.

Approximately 1050 Requests for Proposals (RFPs) for 1996-97 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 111 proposals in November 1996. Section D lists the research proposals received.

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 310 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 33 award-winning research projects from among the 111 applications submitted. The New Awards are listed in Section D. During 1997-98, the ADCRC will be managing 95 contracts.

SECTION A

CONTINUING CONTRACTS

UNRESTRICTED MEDICAL RESEARCH

YEAR THREE

Christopher P. Appleton, M.D.

Mayo Clinic, Scottsdale
Award Amount FY 1997: $26,383

Experimental Determinants of Transmitral and Pulmonary Venous Flow During Atrial Contraction

The purpose of this experimental study in dogs was to define the factors which alter venous flow from the lungs to the heart's main pumping chamber when heart pressures change because of heart rate, reparation, or disease states. By simultaneously recording pressure in the lung, veins, and heart chambers together with recording blood flow velocity from the lung veins, we were able to, for the first time, establish the fundamental mechanisms which result in these blood flows. These results explain the different components of blood flow coming from the lungs during the heart cycle and how they are altered under various circumstances. They provide insight into how these same blood flow variables might be used in heart patients when obtained non-invasively, using ultrasound methods. We are more hopeful than ever that these ultrasound methods will replace the need for direct invasive measurement of heart pressures and thus replace patient morbidity and mortality in the future.

Robert Berg, M.D.

University of Arizona
Award Amount FY 1997: $26,400
Supplemental Award FY 1997: $3600

Evaluation of the Need for Ventilation During Cardiopulmonary Resuscitation in a Swine Cardiac Arrest Model

Cigarette smoking is a major contributor to atherosclerotic heart disease and its major endpoints, myocardial infarction and sudden cardiac arrest. This study was designed to determine whether assisted ventilation during simulated single rescuer bystander cardiopulmonary resuscitation improves outcome. After steel cylinders were inserted in the coronary arteries of animals in order to simulate coronary artery disease, we induced cardiac arrest and provided cardiopulmonary resuscitation with either chest compressions alone, chest compression plus assisted ventilation, or no "bystander" cardiopulmonary resuscitation. In this very realistic model of "bystander" CPR for sudden cardiac arrest we demonstrated that assisted ventilation did not improve initial resuscitation, 24-hour survival, or neurologically intact outcome.

The implications of this research are clear: If assisted ventilation is not necessary to improve outcome in "bystander" CPR, then a simpler, easier to perform CPR technique, chest compressions alone, may be equally effective and more frequently performed in light of its greater acceptability to the public.

Publications:

Berg RA, Kern KB, Hilwig RW, Berg MD, Sanders AB, Otto CW, Ewy GA. Assisted ventilation does not improve outcome in a porcine model of single rescuer bystander CPR. Circulation 95: 1635-1641; 1997.

Janis M. Burt, Ph.D.

University of Arizona
Award Amount FY 1997: $26,400

Vascular Smooth Muscle Dysfunction Early in Atherogenesis

Smooth muscle cell (SMC) proliferation is an integral step in atherogenesis. Consequently, identification of factors which cause SMC proliferation and development of strategies to prevent such proliferation are logical goals for studies whose ultimate aim is prevention of atherosclerosis. Diseased vessels exhibit high levels of cholesterol and other lipids; and a major risk factor for atherosclerosis is elevated blood levels of low density lipoproteins (LDL). Diseased vessels also exhibit abnormally high levels of growth factors. There are numerous data which suggest that changes in the proliferative status of cells involves alteration of the cells' abilities to communicate with each other. We hypothesized that LDL and growth factors compromise communication between SMCs, predisposing the vessel to atherosclerotic disease. Since gap junctions are the structures responsible for communication between SMCs, we hypothesized that LDL and growth factors compromised gap junction function. Thus far, our data support this hypothesis. The data indicate that 1) LDL and growth factors stimulate proliferation on SMCs, 2) when proliferating, the ability of SMCs to communicate with each other is reduced and 3) oxidized LDL and growth factors compromise gap junction function. We have also demonstrated that the change in gap junction function associated with proliferation reflects a change in the permeability of the gap junctions channels. This change in permeability may reflect a change in the composition of the junction and a simultaneous change in either the phosphorylation of the channels or their heteromeric composition. In summary, our data suggest that interventions designed to prevent LDL induced loss of gap junction function would also prevent SMC proliferation. If this proves to be the case, then development of strategies to enhance gap junction function or expression would be the next logical goal for therapeutic intervention in this disease.

Eldon J. Braun, Ph.D.

University of Arizona

Award Amount FY 1997: $26,400

A Role for Serum Albumin in the Excretion of Uric Acid

Avian urine contains significant amounts of protein and it has been suggested that this urinary protein may be involved in the excretion of uric acid. Uric acid is excreted by birds (and other uricotelic animals) in the form of small spherical structures that have been shown to consist primarily of uric acid and protein. In an attempt to identify the urinary and sphere protein(s), western blot analyses were carried out. Primary antibodies were generated both commercially and by ourselves and used in the identifications process. Commercially prepared antibodies to chicken serum albumin specifically bound to protein from the urine and to protein from the spheres, showing a large part of the urinary protein to be serum albumin and identifying the sphere protein as serum albumin. By isolating and purifying the sphere protein and injecting it into sheep, we were able to generate antibodies to the sphere protein. The sphere protein antibodies specifically bound to urinary protein, the sphere protein, and to a chicken albumin standard. Together, these data show avian urine to contain multiple plasma proteins, including serum albumin, and that the protein found in the uric acid containing spheres is serum albumin.

Laura McCloskey, Ph.D.

University of Arizona

Award Amount FY 1997: $26,444

Violence and Substance Use in Hispanic and Anglo Youth

The goal of our study was to identify risk markers for substance use among Hispanics and Anglo adolescents from violent and non-violent family backgrounds. We found few ethnic differences in the use of alcohol or drugs within our sample, although Hispanics were slightly more likely to report the use of marijuana, and Anglos were much more likely to sell drugs than Hispanics (42% *vs.* 17%). Children form violent homes from both ethnic groups displayed a higher rate both of drug use and delinquency, especially if the violence was ongoing. The strongest predictor across ethnic groups for early and sustained substance abuse was family income. Other findings reveal relatively few ethnic differences when children were matched for family income, although Hispanic adolescents are more likely to display internalized psychological problems than Anglos.

John J. Marchalonis, Ph.D.

University of Arizona
Award Amount FY 1997: $111,304
Supplemental Award Amount FY 1997: $14,622

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 1-1.5% of Americans with female to male ratio 3:1. The incidence of the disease is increased to approximately 5% in certain tribes of Native Americans and the prevalence in the Tucson area is approximately 5% due to the influx of individuals suffering from the disease and the high percentage of Native Americans in the area. We have found that individuals suffering from RA tend to have increased levels of autoantibodies directed against the recognition molecules on their own thymus-derived lymphocytes. These autoantibodies are predominantly of the immune macroglobulin (IgM) class and react with the combining site region of the T-cell receptor. The autoantibodies against self Tcrs probably play a role in immune regulation and the elevation of these antibodies in RA patients may indicate 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 their presence offers new approaches for diagnosis and potential therapy for this prevalent and crippling autoimmune disease. Adverse lung complications occur in approximately 20% of rheumatoid arthritis patients. Smoking causes 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:

Dehghanpisheh K, Bona CA, Marchalonis JJ. Peptide epitope binding specificity and V_k and V_H gene usage in a monoclonal IgM natural autoantibody to T-cell receptor CDR1 from a viable moth-eaten mouse. Immunological Investigations 25: 241-252; 1996.

Dehghanpisheh K, Marchalonis JJ. Retrovirally induced mouse anti-TCR monoclonals can synergize the *in vitro* proliferative T-cell response to bacterial superantigens. Scand J Immunol 45: 645-654; 1997.

Lake DF, Helgerson D, Landsperger, WJ, Marchalonis JJ. Physical and epitope analysis of a recombinant human TCR Vα-Vβ construct support the similarity to immunoglobulin. J Prot Chem 16: 309-319; 1997.

Landsperger WJ, Schluter SF, Garza A, Yocum DE, Marchalonis JJ. Fine specificity analysis of autoantibodies to T-cell Receptor CDR1 segments in rheumatoid arthritis. Annals NY Acad Sci 815: 459-461; 1997.

Marchalonis JJ, Kaymaz H, Schluter SF, Lake DF, Landsperger WJ, Sucui-Foca N. Autoantibodies to Tcr β-chains in human heart transplantation: epitope and spectrotype analyses and kinetics of response. Expt & Clin Immunogenet 13: 181-191; 1997.

Marchalonis JJ, Ampel NM, Schluter SF, Garza A, Lake DF, Galgiani JN, Landsperger WJ. Analysis of autoantibodies to T-cell receptors among HIV-infected individuals: epitope analysis and time course. Clin Immunol & Immunopathol 82: 174-189; 1997.

Richard G. Posner, Ph.D.

Northern Arizona University
Award Amount FY 1997: $26,210

Development of Strategies to Inhibit Allergic Responses

Aggregation of cell surface receptors is an important mechanism for initiating responses in cells of the immune system. It is well established that allergic responses are initiated when antibodies (IgE) that are bound to the surface of certain immune cells are cross-linked by the allergen (antigen) to which they are specific. Individuals who are allergic to a specific antigen produce IgE that specifically binds that antigen. When cell surface bound IgE is cross-linked by that antigen, a biochemical cascade is initiated that results in the release of histamine. We have developed an assay that directly measures how a multivalent antigen interacts with cell surface IgE and synthesized some model compounds that can block these IgE-antigen interactions. Because these molecules have the potential to serve as potent inhibitors of specific IgE mediated allergic responses, they have the potential to provide a model system for the design of new therapeutics in the treatment of allergies.

Emmanuel Akporiaye, Ph.D.

University of Arizona
Award Amount FY 1997: $30,000

Analysis of Tumor-Rejections Responses Using a Retrievable Matrix of Tumor Implantation

Using a gelatin-sponge model of tumor implantation, we have determined the expression of cell surface integrins (LFA-1 and VLA-4a) and adhesion molecules (l-selection) on sponge derived tumor-rejecting lymphocytes (TRLs) and activated tumor-specific draining lymph node cells from immunized mice. We have shown that in both T-cell populations, the expression of LFA-1 and VLA-4a is up-regulated while the expression of L-selection is down-regulated. *In vivo* trafficking studies of the tumor-specific lymphocytes demonstrate that they do not preferentially home to sites of antigen deposition. These studies demonstrate the need for the development of strategies that redirect the traffic of adoptively transferred lymphocytes to tumor sites for successful cancer therapy.

Publications:

Tsang TC, Harris DT, Akporiaye ET, Chu RS, Brailey J, Liu F, Vasanwala FH, Schluter SF. A mammalian expression vector with two multiple cloning sites for expression of two foreign genes. Biotechniques 22: 68; January, 1997.

Ana L. Moore, Ph.D.

Arizona State University
Award Amount FY 1997: $26,165

Carotenofluorophores: Imaging Agents for Diagnosis of Neoplastic Disease

The goal of this investigation was to develop drugs that can be used as markers for the early detection of malignancy. We have chosen chemicals that fluoresce as potential markers because fluorescence is one of the most sensitive measurements available in a clinical environment. One of the problems associated with drugs that fluoresce is sensitization and damage to healthy skin and other nonmalignant tissue. To prevent skin sensitization, derivatives of B-carotene have been prepared and attached to the fluorescence markers (fluorophores). During this contract period of three years, a series of new carotenofluorophores have been synthesized and their photophysical properties determined. In these molecules the fluorophore is hematoporphyrin which is a widely studied, tumor-localizing substance. Also, two new fluorophores have been prepared. One is a porphyrin derivative and the other a phthalocyanine derivative. These new fluorophores include structural modifications which in principle should facilitate their elimination from the liver.

The new carotenofluorophores are presently being tested *in vivo* to characterize their tumor localization and fluorescence properties in living tissues.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY 1997: $108,370

Discovery and Development of New Anticancer Drugs

The Arizona Disease Control Research Commission support for the Arizona State University Cancer Research Institute this past year has led to exceptionally good 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 human clinical trials of bryostatin 1 to the phase II level at over 20 different collaborative institutions. Furthermore, nine phase II trials of bryostatin 1 are now being initiated in Canada and another several in England. The initial phase I human clinical trials of dolastatin 10 through the U.S. National Cancer Institute have been completed. For example, at the Mayo Clinic in Rochester, Minnesota the testing of dolastatin 10 is now being expanded to over 20 phase II human cancer clinical trials. Both of these new anticancer drugs required ADCRC assistance for their development into human clinical trials and that requirement continues to be essential. Another very exciting development this past year based on ADCRC support has been the further confirmation that our combretastatin A-4 prodrug is apparently the most powerful tumor antiangiogenesis drug known to date. That has motivated the British Cancer Research Campaign to place the combretastatin A-4 prodrug into accelerated preclinical development with the first clinical trial scheduled for early next year. Other advances include the discovery of phenstatin, X-ray crystal structure of xestospongin D from a Singapore marine sponge, the isolation and structure of hemibastadins 1-3 from a Papua, New Guinea marine sponge, isolation and crystal structure determination of dibromophakellistatin from an Indian Ocean sponge, and isolation and structure of spongilipid from a Republic of Singapore marine sponge. In summary, the ADCRC support has led to outstanding research progress over the past year.

Publications:

Carey JO, Posekany KJ, deVente JE, Pettit GR, Ways DK. Phorbol ester-stimulated phosphorylation of PU1: association with leukemic cell growth inhibition. Blood 87: 4316-4324; 1996.

Chaplin DJ, Pettit GR, Parkins CS, Hill SA. Antivascular approaches to solid tumour therapy: evaluation of tubulin binding agents. Br J Can 74: S86-S88; 1996.

Dorr RT, Dvorakova K, Snead K, Alberts DS, Salmon SE, and Pettit GR. Antitumor activity of combretastatin a-4 phospate, a natural product tubulin inhibitor. Invest New Drugs 14: 131-137; 1996.

Lee HW, Smith L, Pettit GR, Vinitsky A, Smith JB. Ubiquitination of protein kinase c-α and degradation by the proteasome. J Biol Chem 271: 20973-20976; 1996.

Pettit GR, Orr B, Herald DL, Doubek DL, Tackett L, Schmidt JM, Boyd MR, Pettit RK, Hooper JNA. Isolation and x-ray crystal structure of racemic Xestospongin D from the Singapore marine sponge, *Niphates sp.* BioMed. Chem. Lett 6, 1313-1318; 1996.

Pettit GR, Hoard MS, Doubek DL, Schmidt JM, Pettit RK, Tackett LP, Chapuis JC. Antineoplastic agents 338. The cancer cell growth inhibitory constituents of *Terminalia arjuna* (Combretaceae).

Ethnopharmacology 53: 57-63; 1996.

Pettit GR, Butler MS, Williams MD, Filliatrault MJ, and Pettit RK. Antineoplastic agents 331. Isolation and structure of hemibastastatins 1-3 from the Papua, New Guinea marine sponge, *Ianthella basta*. J Nat Prod 59: 927-934: 1996.

Pettit GR, Holman JW, Boland GM. Synthesis of the cyclic heptapeptides axinastatin 2 and axinastatin 3. J Chem Soc, Perkin Trans. 1: 2411-2416; 1996.

Dark GG, Hill SA, Prise VE, Tozer GM, Pettit GR, Chaplin DJ. Combretastatin A-4: an agent that displays potent and selective toxicity towards tumour vasculature. Cancer Research 57: 1829-1834; 1997.

Pettit GR, McNulty J, Doubek DL, Chapuis JC, Schmidt, JM, Tacket LP, Boyd MR. Antineoplastic agents 362. Isolation and x-ray crystal structure of dibromophakellistatin from the Indian Ocean sponge, *Phakellia mauritiana*. J Nat Prod 60: 180-183; 1997.

George R. Pettit, Ph.D.

Arizona State University

Supplemental Award Amount FY 1997: $343,458

Discovery and Development of New Anticancer Drugs

With the absolutely essential financial assistance provided by the Arizona Disease Control Research Commission, the scientific and medical progress this past year has been extraordinarily good. The Tobacco Supplemental Assistance was directed at the further development of dolastatins 10 and 15 as well as the structurally related auristatin PE as promising new anticancer drugs for improving human cancer treatment. Furthermore, a total synthesis of dolastatin 11 now in clinical development at the U.S. National Cancer Institute was completed and has greatly increased the probability that dolastatin 11 will go to clinical trials. In addition, the new dolastatins 16, 17, and 18 we discovered in the Papua, New Guinea shell-less mollusk, *Dolabella aureicularia*, are being subjected to very determined efforts to complete practical total syntheses. Dolastatin 16 especially offers promise of becoming a useful anticancer drug. Meanwhile, the clinical promise of spongistatin 1 has been advanced by continuing the isolation from a Republic of Maldives marine sponge to meet preclinical development requirements. The result has been another outstanding year of progress for our ADCRC research sharply focused on the development of new anticancer drugs for improving human cancer treatment.

Publications:

Bai R, Schwartz RE, Kepler JA, Pettit GR, Hamel E. Characterization of the interaction of cryptophycin 1 with tubulin: binding in the Vinca domain, competitive inhibition of dolastatin 10 binding, and an unusual aggregation reaction. Cancer Research, 56: 4398-4406; 1996.

Maki A, Mohammad R, Raza S, Saleh M, Govindaraju KD, Pettit GR, Al-Katib A. Effect of dolastatin 10 on human non-Hodgkin's lymphoma cell lines. Anti-Cancer Drugs 7: 344-350; 1996.

Bates R, Brusoe KG, Burns JJ, Caldera S, Cui W, Gangwar S, Gramme MR, McClure KJ, Rouen GP, Schadow H, Stessman CC, Taylor SR, Vu VH, Yarick GV, Zhang J, Pettit GR, Bontems R. Dolastatin 26. Synthesis and stereochemistry of dolastatin 11. J Am Chem Soc 119: 2111-2113; 1997.

Pettit GR, Xu J-P, Williams MD, Hogan F, Schmidt, JM, Cerny RL. Antineoplastic agents 370. Isolation and structure of dolastatin 18. BioMed Chem Lett 7: 827-832; 1997.

Garth Powis, D.Phil.

University of Arizona

Award Amount FY 1997: $618,207

Arizona Cancer Center Multidisciplinary Research Program

The last year of the three-year ADCRC grant has been very productive and some important basic and clinical research observations have been made. A new cancer-causing gene, thioredoxin, that is found expressed at high levels in a number of human cancers, including one-half of lung cancers, has been identified. The work also shows that thioredoxin is an attractive target for anticancer drug development. Studies were also conducted to introduce the thioredoxin gene into the lung tissue of mice as a transgene to evaluate its effects on the development of lung cancer. Other basic studies have focused on specific gene mutations in normal and tumor colon tissue of azoxymethane treated rats and have demonstrated the ability of certain chemopreventive agents to prevent these genetic changes. Gene therapy studies were directed towards developing suitable vectors for delivering genes to tumor cells and a new, more efficient vector has been developed. Clinical studies have been conducted in advanced breast cancer on the effects of drugs that sensitize the tumor to high dose chemotherapy and the effects of a drug that protects against the toxic side effects of chemotherapy.

Publications:

Baker A, Briehl MM, Dorr R, Liebler D, Powis G. Decreased antioxidant defense and increased oxidant stress during dexamethasone-induced apoptosis: *bcl-2* selectively prevents the loss of catalase activity. Cell Death & Differ 3: 207-213; 1996.

Berggren M, Gallegos A, Gasdaska JR, Gasdaska PY, Warneke J, Powis G. Thioredoxin and thioredoxin reductase gene expression in human tumors and cell lines, and the effects of serum stimulation and hypoxia. Anticancer Res 16: 3459-3466; 1996.

Garth Powis, D.Phil. University of Arizona

Supplemental Award Amount FY 1997: $81,679

Arizona Cancer Center Multidisciplinary Research Program

Studies in the final year of the ADCRC grant related to tobacco use have focused on the effects of potential anticancer drugs and chemopreventive agents in tobacco-related tumor models. Preliminary studies have been conducted on vectors for delivering gene therapy to lung cancer. Several potential anticancer drugs have been tested for activity in the severe combined immunodeficient (scid) mouse model with tobacco related tumors. Potential chemopreventive drugs have been tested for their ability of induce apoptosis in colon cancer. Non-steroidal antiinflammatory drugs (NSAIDS) was shown to induce apoptosis by a ras-mediated mechanism. Preliminary studies have provided the intriguing observation that the chemopreventive agent difluoromethylornithine may block NSAID-induced apoptosis without reversing the antiproliferative effects of NSAIDS. Selenium, a chemopreventive agent that has been shown to increase the levels of a newly discovered selenium-containing protein, thioredoxin reductase, has been measured in human colon tumors. Studies of gene therapy of lung cancer have compared different vectors to increase the delivery of the gene products to lung cancer cells. These studies have been extended to a gene animal model.

Publications:

Einspahr JG, Alberts DS, Gapstur SM, Bostick RM, Emerson SS, Gerner EW. Surrogate endpoint biomakers as measures of colon cancer risk and their use in cancer chemoprevention trials. Cancer Epidemiology, Biomakers & Prevention 6: 37-48; 1997.

Erdman SH, Wu HD, Hixson LJ, Ahnen DJ, Gerner EW. Assessment of mutations in Ki-ras and p53 in colon cancers from azoxymethane- and dimethylhydrazine-treated rats. Molecular Carcinogenesis 19: 137-144; 1997.

Adrienne C. Scheck, Ph.D.

St. Joseph's Hospital
Award Amount FY 1997: $21,209
Supplemental Award Amount FY 1997: $2,884

Identification of Genes Associated with BCNU Resistance in Human Malignant Gliomas

Brain tumors are an almost uniformly fatal disease, affecting over 15,500 Americans annually. This figure is climbing, particularly in Arizona, as the incidence of primary brain tumors in people over 65 is increasing. Current therapies are not completely effective, and improved treatment requires the design of new therapies based on an understanding of therapy resistance. Our work is designed to understand the basis for resistance to a commonly used chemotherapeutic agent. We are defining previously unidentified mechanisms of resistance by studying differences in gene expression between drug resistant and drug sensitive brain tumor cells. To date, we have obtained evidence of a number of previously undescribed genes in our drug resistant cells. We have also demonstrated the over-expression of a gene involved in the activation of a growth-controlling protein. Our current and future studies will involve the identification and/or characterization of the putative novel genes, and the elucidation of the role played by growth control in drug resistance.

Ted A. Weinert, Ph.D.

University of Arizona
Award Amount FY 1997: $26,396

Checkpoint Genes and Genomic Instabilities

We study genes involved in cell cycle controls called checkpoints that block eukaryotic cell division after DNA damage. All normal cells stop cell division when their chromosomes are damaged, and damaged cells then repair their chromosomes before cells resume dividing. Cancer cells are notably defective for checkpoints; they continue to divide even when damaged. As a consequence of cell division with damaged chromosomes, the damaged cells end up with abnormal chromosomes and a phenomenon termed "genomic instability." This leads to rearrangements of chromosomes, often times activating key cancer genes that lead directly to cancer.

Checkpoint genes are relevant to cancer because when defective they cause genomic instability. The exact forms and mechanisms of instability are poorly understood. Our continuing goal discussed in this summary is to unravel the mechanisms of genomic instability, using the highly experimentally tractable yeast cell as a model. A basic understanding of mechanisms of genomic instability may lead to useful insights into how cancer evolves and how to better diagnosis its onset.

We are continuing to characterize genomic instability in yeast cells and find an association between checkpoint mutations and chromosome loss and mitotic recombination. We have identified and characterized an instance of genome instability (we call "unstable 7") useful in understanding how genomic instability can arise in checkpoint mutants. This form of genomic instability is probably related to a breakage-fusion-bridge cycle, one mechanism by which cancer cells may undergo genome rearrangements. Ongoing studies seek to fully describe this instability.

Jean M. Wilson, Ph.D.

University of Arizona
Award Amount FY 1997: $26,146

Membrane Dynamics in Colon Carcinoma Cells

There are two problems in the area of the chemotherapy of cancer: the presence of multidrug resistance and the inefficient killing by immunotoxins. We are studying how molecules move through the cells to discern how to prevent cancer cells from avoiding their toxic effect. To accomplish these goals we have studied a novel protein that is present in the intracellular membranes of intestinal cells. We have determined that this protein is synthesized as a large molecule that is processed and lives for several hours before degradation. These results may help to evaluate how quickly an immunotoxin conjugate will be delivered to the lysosomal compartment after binding to the cell surface. We also found that this protein cycles between the surface of the cell and inside the cell. Therefore, chemotherapeutic drugs present in the compartment could be extruded from the cell, eliminating its ability to kill the cancer cell.

Mary I. Johnson, M.D.

University of Arizona
Award Amount FY 1997: $20,286

Anticonvulsant (Phenobarbital) Effects on the Developing Nervous System: An *In Vitro* Model

Phenobarbital may adversely affect a number of steps in central nervous system development, including early morphogenesis of the nervous system, nerve cell proliferation and migration, as well as the initiation of nerve processes and the establishment of contracts on target cells. That therapeutic or even subtherapeutic levels of phenobarbital may have unacceptable deleterious effects on neuronal differentiation is debated. Phenobarbital, however, is the most commonly used anticonvulsant in newborn and young infants during a time when there is still considerable brain development occurring. In these studies, we use a tissue culture model of neuronal development to test for the adverse effects of phenobarbital and eventually to study the mechanisms by which such effects might occur. Nerve cells can be treated with increasing concentrations of phenobarbital during a critical time period of normal process development induced by either coculture with normal companion (glial) cells or by a purified growth factor.

Publications:

Johnson MI, Rueger D, Loegering MB. Therapeutic levels of phenobarbital inhibit development and maintenance of dendrites induced by osteogenic protein-1 (OP-1). Ann. Neurol. 40(2):286; 1996.

Claudia Kappen, Ph.D.

Mayo Clinic, Scottsdale
Award Amount FY 1997: $26,386

Analysis of Transgenic Mice Expressing the Precursor Protein of Alzheimer's Disease B-Amyloid

The goal of this research was to generate transgenic mice as a model for Alzheimer's Disease (AD). In particular, we sought to recreate the early stages of pathogenesis which cannot be adequately investigated in humans. Initially, AD is believed to be caused by highly elevated levels of B-amyloid precursor protein in the brain. We characterized and identified elements that lead to high level expression of the gene for this protein, specifically in neurons of genetically manipulated mice that we created. Current research focuses on cell differentiation in the brain of these transgenic mice and on the analysis of neuron cell death, a hallmark of AD. Since AD afflicts predominantly elderly, results from this project will be important to a major segment of the population in Arizona.

John W. Bloom, M.D.

University of Arizona
Award Amount FY 1997: $26,108

Development of a Recombinant Glucocorticoid Receptor with Constitutive Activity-Potential Therapy for Asthma

Asthma is the most important cause of chronic illness in children and affects over 5% of adults. This illness is a particularly serious problem in Arizona. The prevalence rate of asthma in Arizona is twice the national average, and the death rate is 1.5 times the national rate. At present, glucocorticoids (e.g., cortisone, prednisone, "steroids") are the most effective medications for asthma. The goal of our research is to understand the mechanisms of glucocorticoids action in the lung. We have characterized the mechanisms through which glucocorticoids produce anti-asthma effects in lung cells, termed airway epithelial cells, that line the walls of the bronchial tubes. We have found that glucocorticoids function, at least partially, by decreasing the expression of genes that are involved in producing the asthmatic condition. This knowledge should contribute to the development of safer and more effective therapy for asthma.

Publications:

LeVan TD, Behr FD, Adkin KK, Miesfeld RL, Bloom JW. Glucocorticoid receptor signaling in a bronchial epithelial cell line. Am J Physiol 16: L838-L843; 1997.

John W. Bloom, M.D.

University of Arizona
Supplemental Award Amount FY 1997: $3,498

Development of a Recombinant Glucocorticoid Receptor with Constitutive Activity-Potential Therapy for Asthma

The obstructive lung disease, chronic obstructive pulmonary disease (COPD) and asthma, are major causes of disability and death nationally. Although the cause of asthma is unknown, almost all cases of COPD are due to cigarette smoking, Because of the in-migration of patients with these diseases, the prevalence and mortality rates for obstructive lung diseases in Arizona are greater than the national averages. Glucocorticoids (e.g, cortisone, prednisone, "steroids") are effective medications for obstructive lung disease. The goal of our research is to understand the mechanisms of glucocorticoid action in the lung. We have characterized the mechanisms through which glucocorticoids produce anti-inflammatory effects in lung cells, termed airway epithelial cells, that line the walls of the bronchial tubes. We have found that glucocorticoids function, at least partially, by decreasing the expression of genes that are involved in producing the inflammatory condition. This knowledge should contribute to the development of safer and more effective therapy for obstructive lung disease.

Evelyn D. Rider, M.D. — University of Arizona

Award Amount FY 1997: $23,620

Pulmonary Surfactant Degradation in Newborn Rabbit Lung Lysosomes *In Vivo*

In the current funding period, biochemical characterization of lung lysosomes from 3-day, and 6-week-old rabbits was completed. Significant differences in peak lysosome enzyme-specific activities were demonstrated. Peak lysosomal enzyme activity in the younger rabbits was 50% lower than in the 6- week-old rabbits and occurred at lighter densities compared to the 6-week-old group. This may be significant as data in the literature indicate that more immature lysosomes isolated at lighter densities than mature lysosomes. Morphologic characterization of lysosomal preparation from 3-week-old rabbit lung was prepared and correlated well with the biochemical data. The processing of trace amounts of labeled surfactant lipid (DEPC and DPPC) into lung lysosomes of 3-week-old rabbits was also studied this year. We found that giving the animals the radioactively labeled surfactant did not alter the isolation characteristics of the lung lysosomes. We also found that the amount of lipid material measured in the lung lysosome fractions from the 3-week-old animals was 10-fold greater than the amounts measured previously in lung lysosome fractions from 6-week-old rabbits. This observation is consistent with 3-week-old rabbits being capable of surfactant degradation rates similar to 6-week-old rabbits, despite 50% less lysosome enzyme activities, and supports the hypothesis that lung lysosomes are important in surfactant lipid breakdown in the lung. Understanding how surfactant balance is maintained in the lung is important as this material is crucial to normal lung function. Disturbances in surfactant can result in significant lung dysfunction and potential alterations in processes of inflammation and immune function in the lung, particularly in conditions of chronic disease such as asthma or bronchopulmonary dysplasia.

Evelyn D. Rider, M.D. — University of Arizona

Supplemental Award Amount FY 1997: $3,280

Pulmonary Surfactant Degradation in Newborn Rabbit Lung Lysosomes *In Vivo*

Significant progress was made during this final funding year. Biochemical characterization of lung lysosomes from 3-day, 3-week and 6-week-old rabbits was completed. Peak lysosome enzyme-specific activities in the younger rabbits was 50% lower than in the 6-week-old rabbits and occurred at lighter densities compared to the 6-week-old group. This is potentially significant as data in the literature indicate that more immature lysosomes isolate at lighter densities than more mature lysosomes. Morphologic characterization of lysosomal preparation from 3-week-old rabbit lungs were prepared and correlated well with the biochemical data. The processing of trace amounts of labeled surfactant lipid (DEPC and DPPC) into lung lysosomes of 3-week-old rabbits was also studied. We found that giving the animals the radioactively labeled surfactant did not alter the isolation characteristics of the lung lysosomes. The amount of lipid material measured in the lung lysosome fractions from the 3-week-old animals were 10-fold greater than the amounts measured previously in lung lysosome fractions from 6-week-old rabbits. The observation is consistent with 3-week-old rabbits being capable of surfactant degradation rates similar to 6-week-old rabbits, despite 50% less lysosome enzyme activity and supports the hypothesis that lung lysosomes are important in surfactant lipid breakdown in the lung. Data obtained in these studies will help in our understanding of how surfactant metabolism changes with development.

SECTION B

CONTRACTS

TOBACCO RELATED RESEARCH

YEAR ONE

David S. Alberts, Ph.D.

University of Arizona
Award Amount FY 1997: $145,258

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

In year one of the project we have completed the design, pilot testing and reproduction of the smoking assessment questionnaire. The questionnaire was designed as a scannable form to be used on the optical scanner purchased for this project. All programs required to use the scanner, including scanning and verifying procedures have been completed. To date, 550 questionnaires have been completed; of these, 350 have been scanned into the database. Approximately 70% of all baseline and recurrent polyp tissue materials have been retrieved and reviewed to confirm histopathologic diagnosis. Of the 535 baseline tissue samples sent to the laboratory, 290 K-ras analyses have been completed. Of the 405 recurrent polyp tissue materials sent for analysis, 196 have had the K-ras, P53 and DCC analyses completed. Because of the low rates of P53 and DCC seen in these polyps, a special focus has been placed on completing the K-ras mutation analyses. Overall, we have met our time-line goals for this complex project in terms of smoking assessment, retrieval of polyp tissue materials and the analysis of genetic mutations. Our efforts will continue through the remaining years of this grant to assure that this project is completed in a timely manner and we can properly test these important hypotheses.

Paul Consroe, Ph.D.

University of Arizona
Award Amount FY 1997: $49,522

Antiemetic Drug Development for Cancer Treatment

We have found that "knocking-down" (destroying) mouse brain cannabinoid (CB1) receptors by injecting an "antisense oligo" (a special gene) prevents analgesic and other effects of cannabinoid drugs. This proved that there must be receptors for cannabinoid drugs to work. We found that some cannabinoid drugs act on CB1 receptors: 1) THC can stimulate, but also block the mouse brain CB1 receptor; 2) AM630 can block CB1 receptors in mouse and guinea pig brains, but can stimulate CB1 receptors in guinea pig intestines; 3) SR141716A can block mouse and human CB1 receptors, but also can inhibit the binding of the CB1 receptor to its "messenger protein" (GTPγS) in the brain; and 4) CP55,940 is the most effective drug for binding CB1 brain receptors to the messenger protein. Mouse, guinea pig and human CB1 receptors are virtually identical and our findings of opposing effects are important for further research.

Publications:

Edsall SA, Knapp RJ, Vanderah TW, Roeske WR, Consroe P, Yamamura HI. Antisense oligodeoxynucleotide treatment to the brain cannabinoid receptor inhibits antinociception. Neuro Report 7: 593-596; 1996.

Burkey TH, Quock RM, Consroe P, Roeske WR, Yamamura HI. Delta-9-THC is a partial agonist of cannabinoid receptors in mouse brain. Eur J Pharmacol 323: 3-4; 1997

Dominick DeLuca, Ph.D.

University of Arizona

Award Amount FY 1997: $50,000

Organ Culture Approaches for Transplantation of Human Stem Cells

During the first year of this project, we have determined the best means to purify the progenitors of T-cells that must be replaced in transplants of patients undergoing intensive chemotherapy for smoking-induced lung cancer. Currently, bone marrow transplantation is the only effective therapy for these patients, but the low frequency of successful "takes" for these procedures and the limited amount of donor material are major difficulties in treatment. We have also found that treatment of the tissues with "chemokines" that regulate the migration of T progenitor cells can affect the development of T-cells (thymus derived cells that are responsible for cellular immunity) in our system. Manipulation of progenitor cell migration offers a means of rapidly increasing the development of T-cells that must be achieved if transplant recipients are to have their immune systems restored after high dose chemotherapy. 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-cells in organ culture can increase the production of T-cells at low doses and decrease their production at low 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 1997: $29,212

The Synthesis of C-glycoside Sulfones as Potential Cancer Therapeutics

The goals of our research program are to prepare compounds that will inhibit the incorporation of certain carbohydrate moieties onto the surface of lung cancer cells. These carbohydrates play an important role in tumor metastasis. If one can block their incorporation onto the cell surface, then the spread of lung cancer may be stopped or slowed. The inhibitors are designed to look very much like the natural carbohydrates except that they have carbon and sulfur atoms where the natural carbohydrates have oxygen and phosphorous atoms, respectively. We have classified the inhibitors as C-glycoside sulfones. During the past year, we have prepared a simplified form of the target molecule using new chemical reactions developed in our laboratory. These reactions allow the incorporation of both carbon and sulfur in a highly efficient manner. This research is continuing toward the preparation of fully functionalized inhibitors.

Anna R. Giuliano, Ph.D.

University of Arizona
Award Amount FY 1997: $125,562

Effects of Smoking on Persistent HPV Infection Among Reproductive Age Women

In the last fiscal year, our first year of operations, we designed and finalized all study procedures. This includes the development of an operations manual complete with eligibility forms, consent forms, questionnaires, interview and clinical procedures protocols, participant invitations, and letters of notification. We have successfully recruited and interviewed 431 participants into the baseline phase of this study, and have recruited and interviewed 98 participants in the follow-up phase of this study. Overall, 35 % of screened women are current smokers and 30 % are positive for intermediate and high risk type human papillomavirus (HPV) infection. Both rates are higher than anticipated at the start of the study. As we interview more participants in the coming fiscal year, we will be able to start estimating the rate of HPV persistence among smokers and non-smokers.

Arthur F. Gmitro, Ph.D.

University of Arizona
Amount FY 1997: $50,000

A Fiber-Optic Confocal Microscope for *In Vivo* Imaging

This research project is aimed at the development and demonstration of a new type of instrument for *in vivo* microscopic examination of diseased tissues. The instrument combines the basic elements of a laser-scanning confocal microscope with a fiber-optic image bundle to allow the unique depth discrimination capability of confocal microscopy at remote locations inside the human body. The primary application of the instrument is as a tool for use in the diagnosis of lung cancer and other pre-cancerous conditions of the bronchial airways. Significant progress has been made on the construction of the instrument and on the evaluation of chemical agents to stain living tissues for better visualization of disease. The major challenge of instrument development is making a miniature catheter with the necessary imaging optics and focusing mechanism. A design for such a catheter has been completed and prototypes are being evaluated.

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

University of Arizona
Award Amount FY 1997: $46,609

Role of c-fos in the Regulation of Neuropathic Pain

Our research focuses on a mechanism which may regulate neuropathic pain (pain caused by injury to nerves or disease of nerves). We are testing the hypotheses that 1) neuropathic pain increases production of the regulatory protein Fos by turning on the c-fos gene and 2) production of Fos protein turns on other genes which make substances which decrease pain intensity. To date, we have concentrated on the effect of blocking Fos production on the intensity of neuropathic pain. Blocking Fos production gives an indication of the function of Fos in pain regulation. In preliminary studies, we have shown that our method for blocking Fos production is effective and specific. We have examined the effect of blocking Fos production in animals without neuropathic injury. Finally, we have shown that blocking Fos production has little effect on pain intensity in animals with neuropathic pain. We are currently building upon these findings.

Ana M. Pajor, Ph.D.

University of Arizona
Award Amount FY 1997: $33,359

Cloning and Expression of Renal Na/Nucleoside Cotransporter

The focus of our research is the way in which nucleoside-based cancer drugs enter 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 1997: $50,000

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

We have recruited 14 smokers (35 to 65 years old) for the first cycle of the intervention study. They were selected because they had low intakes of fruits and vegetables (2-3 servings/day) as estimated for the Food- Frequency Questionnaire. After a 1-month period of baseline data collection, they were divided into control (n=7) and intervention groups (n=7). The subjects in the intervention group were provided with 35 servings of fruits, vegetables and juices weekly. Most were able to increase and maintain their intake of fruits and vegetables from the first month. The plasma concentration of lutein, cryptoxanthin, *a*-carotene and *B*-carotene (especially the two carotenes) in the intervention group were increased when compared to the control and baseline level. This finding suggests that most smokers are able to increase plasma concentrations of carotenoid, potential cancer preventive agents, by increasing fruit and vegetable intake. More subjects will be recruited to confirm this finding in years 2 and 3.

William A. Remers, Ph.D.

University of Arizona
Award Amount FY 1997: $84,147

Design of Non-cross Resistant Agents for Lung Cancer

The goal of this project is to discover new compounds that are effective against resistant 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. Active compounds will be tested further against a human lung tumor in mice with deficient immune systems and then a data base will be created for use in the design of future compounds with improved activity. To date, eight of the fourteen target compounds have been synthesized on a scale adequate for complete testing. Testing results have been obtained for six compounds in the Lewis lung tumor assay and two of these compounds have definite effectiveness. The results have been entered into the data base, together with the physical and chemical properties of the compounds.

The results obtained thus far demonstrate that the study is feasible and that it should be possible to accomplish all of the goals in synthesis and testing. Furthermore, the two active compounds in mice are encouraging for future testing, especially because they have already shown activity in cell cultures against the human lung cancer cells to be used in the immunodeficient mice. The latter type of assay tends to show good correlations with activity against cancers in human patients.

Donato Romagnolo, Ph.D.

University of Arizona
Award Amount FY 1997: $49,500

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

The state of Arizona ranks 16th in the United States in deaths (5,700 in 1990) attributable to smoking. Estimated years of potential life lost approach 67,000 years and the state medical care expenditure related to smoking is about $52,000/death. Tobacco smoking may be a risk factor in the etiology of breast and ovarian cancer. Cigarette smoking and other tobacco derivatives may expose women of reproductive age to carcinogenic substances and increase the risk of developing malignancies. We investigated whether tobacco derivatives influence the expression of BRCA-1, a tumor suppressor gene. Our investigations using breast and ovarian cancer cells provide preliminary evidence that acute exposure to benzo[a]pyrene, a polyaromatic carbon present in tobacco smoke, may alter the normal expression of BRCA-1. The translational significance of these findings is that tobacco smoking may be a risk factor that predisposes women to the onset of breast and ovarian cancer.

Seth D. Rose, Ph.D.

Arizona State University
Award Amount FY 1997: $47,882

Chemotherapy by Contravention of Oncogenesis in Smoking-induced Lung Cancer

Approximately one-third of lung cancers result from the action of a faulty protein. To obstruct this protein's detrimental effect, we have devised and synthesized new chemical compounds and evaluated them for anticancer activity. Nine compounds were tested at the National Cancer Institute against lung cancer cells grown in culture. Of these, two showed sufficient activity to encourage the development of close analogues that might have higher activity. Three were more weakly active, which suggested some chemical features to incorporate into new analogues, and four were almost inactive, thereby identifying noncritical molecular features. An additional eight new compounds were prepared for evaluation in a biochemical test of obstruction of the protein as a guide to whether tests against cancer cells grown in culture are warranted. 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 1997: $32,757

Transgenic Models for Leukemia and Myelodysplasia (Preleukemia)

The goal of this project is to determine the role of a gene, called EVI-1, in tobacco-induced hematologic malignancies, acute myelogenous leukemia and myelodysplastic syndromes. We will construct artificial gene-expressed transgenic mice. An artificial gene was created and successfully injected into mouse eggs. Although mice gave birth to pups in which the gene was detected, none expressed the artificial EVI-1. Detecting the EVI-1 protein expressed by the artificial gene and distinguishing it from the native mouse gene was difficult. A new artificial gene has been created which is coupled to a "reporter" molecule. This reporter (green fluorescence protein) causes the protein expressed from the artificial gene to glow green and allows us to detect EVI-1 protein in cells. This construct is being tested in human and mouse cells. Using this artificial gene, it will be possible to detect EVI-1 in cells by examining the fluorescing protein. Although delays encountered were significant, current study designs are more powerful than those originally proposed. If present approaches are not successful, we will create new genes with more specific stimulating elements and obtain transgenic mice from other investigators.

Ann L. Baldwin, Ph.D.

University of Arizona

Award Amount FY 1997: $48,861

What Cellular Mechanisms are Responsible for Histamine-Induced Alterations 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, histamine an inflammatory agent, is applied to a network of small blood vessels in the rat mesentery, a thin sheet of tissue that holds the intestine together. The degree of leakage of albumin, the condition of the cytoskeleton or "scaffolding" of the endothelial cells, and the presence of junctional proteins holding the cells together (cadherin 5, or C5) is determined. This year, we have demonstrated that an inflammatory agent cause focal leaks in the small veins, and these leaks are accompanied by local disruption of the cytoskeleton and/or C5. We have also developed techniques to determine the role that white blood cells play in forming the leaks. Such leakage is thought to contribute to atherogenesis, or hardening of the arteries. In Arizona, 25% of the population smokes, and in 1991, cardiovascular disease was responsible for 38% of total deaths.

Publications:

Abstracts:

Al-Naemi H, Thurston G, Baldwin AL. Changes in venular permeability due to L-NMMA and histamine treatments. Microcirculation 4(1): No. 179, 150; 1997.

Wong R, Heimark RL, Baldwin AL. Characterization of cytokine-induced microvascular leaks in the rat mesenteric window. Microcirculation 4(1): No. 177, 150; 1997

Mary C. Davis, Ph.D.

Arizona State University
Award Amount FY 1997: $43,264

The Effects of Smoking and Oral Contraceptive Use on Physiological Stress Responses in Young Women

This study explored the possibility that the combination of smoking with oral contraceptive (OC) use may increase women's risk of heart disease, at least partly, by magnifying their physiological responses to smoking and stress. Toward this end, physiological measures were obtained from 52 female smokers and nonsmokers, some of whom used OCs, while they rested and then while they performed several challenging tasks. Just prior to the tasks, half of the smokers smoked a single cigarette. Comparisons among groups showed that smokers who also used OCs had larger increases in blood pressure during both smoking a cigarette and stress than did smokers who did not use OCs. Women who smoked a cigarette just prior to stress also showed larger increases in cholesterol during stress than did women who did not smoke. All women showed increases, during stress, in fibrinogen, an important blood clotting factor that research suggests may increase the risk of heart disease. Roughly 25% of women in Arizona smoke, and many of them are likely to use OCs and to smoke more when under stress. The current findings suggest that the natural physiological changes that occur in response to stress are exaggerated in women smokers who also use OCs, particularly if they are smoking and experiencing stress simultaneously. This may be one means whereby they experience an increased risk of heart disease.

Cheryl A. Dyer, Ph.D.

Northern Arizona University
Award Amount FY 1997: $46,863

The Effect of Nicotine on Ovarian Steroid Hormone Production

Our research is to understand how nicotine, due to smoking, alters the type of steroids made by the ovary. One particular ovarian steroid, allopregnanolone, is active in the brain, acting like Valium™ to calm and relieve anxiety. Our data indicates that nicotine stimulates ovarian production of allopregnanolone. We think that the increase in endogenous Valium™ may contribute to the addictiveness of smoking, making it more difficult for women who smoke to quit. Our next goal is to determine if the effect of nicotine on allopregnanolone occurs in the whole animal and analyze the effect of nicotine withdrawal on behaviors, such as anxiety and poor coping responses to stress. Women quit with greater difficulty or are treated with less successful methods than men. With increased understanding of the pharmacology of smoking that is unique to women's physiology, we can provide more appropriate programs and/or treatments to help more women be successful at quitting.

Irwin L. Flink, Ph.D.

University of Arizona
Award Amount FY 1997: $39,507

Cardiac Cell-cycle Progression and Terminal Differentiation

The retinoblastoma protein (pRb) family members, p107, pRb, and p130 are regulators of cellular proliferation, differentiation, and cell cycle exit and entry, respectively. The active, under-phosphorylated form of these proteins, targets the E2F family of transcriptional factors, which play a critical role in the control of genes associated with DNA synthesis (S-phase). The interaction of pRb family members with E2F, and the cyclin-dependent kinases (cdks) and kinase inhibitors (cdk_is) responsible for modulating the phosphorylation of pRb, were examined during the fetal (day 17) to neonatal (day 2) developmental transition. Electromobility shift analyses demonstrated that E2F was complexed with p107 in proliferating fetal cardiomyocytes, whereas in neonatal cells E2F was associated with pRb and p130. The interaction of E2F with underphosphorylated pRb correlated with the decreased protein levels of cyclins D_2, D_3, and E and cdks 2 and 4. The transcripts for cyclins A and E, and cdk4 also were diminished. Cdk assays showed reduced activities of cdks 2, 4 and 6 cdc2 in neonatal heart cells. RNA blot analysis and immunodepletion studies indicated that p21 was strongly induced in day 2 neonate and progressively upregulated into the adult stage. Although mRNA levels of the cdk_i, p27 were unchanged, its inhibitory activity also was increased. Thus, cardiomyocytes retain the capacity to proliferate until the neonatal period when cdk_i activity is induced. Unlike skeletal muscle, cdk_i activity is uncoupled from myogenesis during cardiomyocyte development. An understanding of the regulation of cell-cycle proteins will be useful in the replacement of damaged cardiomyocytes by surrounding muscle cells.

Joseph Heiserman, Ph.D.

St Joseph's Hospital
Award Amount FY 1997: $46,115

Clinical Utility of High Performance Gradient

Our research examines the ability of a new medical test, Magnetic Resonance Angiography (MRA), to detect narrowing of the carotid arteries in the neck. Narrowing of these arteries is made worse by, among other things, cigarette smoking, increasing the risk of devastating stroke. New developments and improvements in MRA make it possible to accurately depict these arteries without risk to the patient. The standard method for measuring narrowing, x-ray angiography, is itself associated with a risk of stroke. After obtaining studies of patients who volunteer for MRA and who have already undergone x-ray angiography, we will be able to compare the two methods in order to decide whether MRA can replace the standard method. During the first year, we enrolled 44 out of our goal of 100 patients for this comparison. During the second year, we plan to enroll additional patients and perform the comparison studies.

Publications:

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Heiserman JE, Keller PJ. Magnetic resonance angiography. In Press, Scientific American.

Brant-Zawadzki M, Heiserman JE. The roles of MR angiography, CT angiography, and ultrasound in vascular imaging of the head and neck. In Press, AJNR

Genetic Markers for Susceptibility to Tobacco Related Diseases

Duane Sherrill, Ph.D.

University of Arizona

Award Amount FY 1997: $46,412

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% of office visits to physicians and 13% of hospitalizations. Cigarette smoking is important in the development of COPD, yet only 15-20% of smokers develop clinical disease. In the initial phase of 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 (FEV_1) as markers for susceptibility to COPD, since patients with COPD generally have reduced levels of FEV_1. Using FEV_1 data from 2613 subjects, representing 746 Tucson families, we performed genetics analyses to determine if low levels of FEV_1 follow patterns consistent with Mendelian inheritance. The results indicate that the FEV_1 data do not conform to a model which assumes a Mendelian gene. Instead the analyses suggest that the level of FEV_1 is most likely determined by environmental factors and/or related to multiple genes (termed polygenic).

Ronald J Lukas, Ph.D.

St, Joseph's Hospital
Award Amount FY 1997: $149,313

Molecular Basis for Nicotine Dependence

Addiction to nicotine 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, n AchR.

It is well known that acute nicotine exposure stimulates function of nAChR. Some of the work in this collaborative project shows that chronic nicotine exposure induces increases in numbers of all nAChR subtypes, but with distinct nicotine dose dependencies and at different rates across nAChR subtypes. Both chemical and electrophysiological measures also show that chronic nicotine exposure induces a distinctive phase of long-lasting loss of function for all nAChR subtypes, but again at different rates and with distinct nicotine dose dependencies. This latter finding has potentially high significance. It counters the common view that tobacco users act as they do to intermittently stimulate nAChR. Instead, chronic nicotine exposure would disable chemical signaling mediated by nAChR. We suggest that habitual users of tobacco products have adapted to nervous systems with low nAChR signaling, and that withdrawal from nicotine might provoke an unpleasant, hyper-stimulation of neuronal activity.

Publications:

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Lukas RJ, Ke L, Bencherif M, Eisehour CM. Regulation by nicotine ot its own receptors. Drug Devel Res 38: 136-148; 1996.

Lukas RJ, Eisenhour CM. Interactions between tachykinins and diverse, human nicotinic acetylcholine receptor subtypes. Neurochem Res 21: 1245-1257; 1996.

Lukas RJ. "Neuronal" nicotinic acetylcholine receptors. Neuroscience Intelligence Unit, In Press; 1997.

Andrea J. Yool, Ph.D.

University of Arizona
Award Amount FY 1997: $49,952

Influence of Nicotine on Glucose-sensitive Neurons of the Hypothalamus

The hypothalamus is a region of the brain that serves important roles in governing behavior and maintaining body functions. We are investigating the idea that long-term exposure to nicotine alters the ability of a subset of neurons within the hypothalamus to sense sugar (glucose) levels, and thus, compromises the ability of the brain to control feeding behavior, leading to obesity and related problems. The first aim of our research program has been to identify and characterize the neurons responsible for sensing the glucose levels, and to understand the mechanisms that enable this sensing process. A glucose-sensitive subset of neurons has been suggested to exist based on the known functions of the hypothalamus, but have not previously been fully identified or characterized.

Our work during the last year has established several important advances in our research project. We have established the techniques for growing hypothalamic neurons (obtained form postnatal rats) under culture conditions. This allows us to observe the development of neurons, apply defined stimuli in controlled conditions, and evaluate the responses using calcium imaging and electrophysiological recording techniques. We have identified a subpopulation of neurons that respond to increases in external glucose with increases in intracellular calcium and changes in membrane firing patterns. These responses are physiologically significant and the effects are in accord with what we would predict should cause appropriate changes in the level of release of systemic signals form the hypothalamus. Interestingly, there is more than one pattern of response observed in the cultured neurons, suggesting that different neurons may contribute to different networks of control. That, in theory, could allow for a more complex regulation of feeding behavior. Once the system is defined, we will investigate the consequences of exposure to nicotine on the responses of the glucose-sensitive neurons.

Hugh S. Miller, M.D.

University of Arizona
Award Amount FY 1997: $90,944

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

The independent impact of smoking and adolescent pregnancy continues to plague residents of Arizona. We have spent the last year studying how the intersection of these two behaviors contributes to several significant health hazards. Our retrospective evaluation of approximately 2500 hundred adolescents delivering babies over the past six years has led to several important observations that extend beyond the mere prevalence of smoking in the population. We are currently in the process of analyzing our data, but our preliminary conclusions suggest that second hand smoke exposure may play a more important role in the teen population. In addition, adequate nutrition, anemia and appropriate weight gain are critical elements to a healthy teen pregnancy. During this past year, we have developed and initiated the first intervention phase of this project. In order to better assess the value of our smoking cessation program for teen mothers, we are also enrolling adult women in this prospective randomized trial so we can determine what the relative benefit is for 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.

Mathuram Santosham, M.D.

John Hopkins University
Award Amount FY 1997: $49,530

Youth and Childbearing Women in The Gila River-Maricopa Indian Community: Assessment of Tobacco Use and Exposure in Children

Data collection has been completed for this study. All research objectives have been addressed. An interim report has been presented to the Gila River-Maricopa Indian Community. Currently, no results have been submitted for publication. A final report containing complete results will be submitted to the Commission and the Community within 45 days. Survey results indicate that smoking is prevalent among teens and young women in this Community. Data looking at the effects on the health of young children are currently being analyzed. Focus groups suggested that although a problem, smoking is not the highest priority problem in the Community; a smoking prevention program could be useful if young children were targeted. Results from this study will be instrumental in formulating effective prevention efforts to decrease tobacco and other drug use in the community, and may be useful to similar communities in Arizona in their efforts to formulate similar prevention programs.

Dean E. Carter, Ph.D.

University of Arizona
Award Amount FY 1997: $138,340

Synergism Between Smoking and Arsenic Exposure in Lung Injury

The overall goal of this research is to compare the relative risk of lung injury following inhalation of tobacco smoke and arsenic. This is important to Arizonans because arsenic is a major emission from copper smelters and coal-fired power plants and this may be an additional risk to smokers. During the first year, we have collected data from unexposed animals that have been exposed to smoke only or to smoke and arsenic. Our data indicate that the combined exposure to arsenic and smoke causes more lung toxicity than smoke exposure alone. We have found that combined smoke and arsenic exposure causes decreases in the ability of the lung to protect itself from injury with concomitant increases in indicators of DNA damage. Using this data, we hope to develop indicators that will identify Arizonans at risk for lung injury from these combined exposures.

Thomas P. Davis, Ph.D.

University of Arizona
Award Amount FY 1997: $45,884

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

Small cell lung cancer (SCLC) is a serious health problem in Arizona. No satisfactory treatment for this disease exists, emphasizing the need for basic research into the causes of the disease. A hallmark of SCLC is the expression of autocrine (self-stimulating) growth factors (AGFs). Several gene products regulate the level and activity of AGFs. Among these is a gene known as *prohormone convertase 1 (PC1)*, which cleaves growth factors from an inactive to an active form. We discovered that PC1 is expressed in SCLCs, while it is not expressed in normal lung cells. We have also discovered that PC1 is expressed in SCLCs that are inhibited by treatment with an anti-cancer drug, but not in cells which are unaffected by treatment with the drug. These results suggest that PC1 plays a major role in the development of SCLC and may be useful target for the treatment of SCLC.

Richard L. Friedman, Ph.D.

University of Arizona
Award Amount FY 1997: $50,000

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

Tuberculosis is probably the most important infectious disease of humans. The World Health Organization estimates that one third of the world's population is infected with the tubercle bacillus, that ten million new cases of tuberculosis occur annually, and that over three million people die of tuberculosis every year. For citizens of the state of Arizona, tuberculosis is a serious and rapidly increasing health problem.

The goal of this research is to identify and characterize the genes possessed by the tubercle bacillus *(Mycobacterium tuberculosis)* that enable it to enter and survive in phagocytic cells (macrophages). Using this system, we have begun to screen an avirulent strain of mycobacterium *(Mycobacterium smegmatis)* containing genetic material form virulent *M. tuberculosis*. A new tissue culture assay was developed which utilizes a human macrophage-like cell line (U-937). These cells more readily internalize the microbe but kill *M. smegmatis* at a slower rate than the previously used cell line (J774A.1). Thus, this system should be better suited for the detection of recombinant clones which survive in the assay. In this manner, it is hoped that bacterial genes involved in the production of human disease will be identified and characterized.

Information about the genetic basis of the disease producing process in tuberculosis will help devise more effective drug and vaccine therapies for the prevention and cure of the 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 1997: $38,052

The Effect of Micronutrient Antioxidants on Exhaled Ethane in Cigarette Smokers

Only 15-20% of cigarette smokers develop significant lung disease. Antioxidant ingestion may determine disease susceptibility. Ethane is released in breath during free radical injury. Our previous work indicates antioxidants (vitamin E, C and β-carotene) reduce ethane. The more ethane is reduced, the better preserved is lung function. *Questions:* Can vitamin E alone reduce ethane in smokers? Does this ethane reduction predict smokers at risk for lung disease? We gave vitamin E to 30 smokers for three weeks. *Results:* Vitamin E reduced ethane, but not significantly. However, more reduction in ethane was associated with poorer lung function supporting previous work. These results were not statistically significant. *Conclusion:* Vitamin E alone fails to explain completely changes found in our initial studies but the changes seen were in the same direction. We will study vitamin C/β-carotene alone to determine their influence on exhaled ethane as a potential marker of susceptible smokers.

John A McDonald, Ph.D., M.D.

Mayo Clinic, Scottsdale
Award Amount FY 1997: $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 artificial 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.

John A. McDonald, Ph.D., M.D. (Wu)*

Mayo Clinic, Scottsdale
Award Amount FY 1997: $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.

* Dr. McDonald took over as Principal Investigator for Dr. Wu on this project.

Anne L. Wright, Ph.D.

University of Arizona
Award Amount FY 1997: $119,152

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. In Arizona, over 20,000 infants have LRIs annually, hundreds of whom are hospitalized.

This project is designed to investigate the role of passive smoke exposure, infant feeding practices, and infant immunologic responses in relation to the occurrence of LRIs in a cohort of infants. Specifically, we are investigating 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 almost 80 infants and collected blood and LRI specimens for the majority of subjects. Continued enrollment and characterization of these subjects will permit identification of preventable risk factors for this common type of illness, which could have a major impact on the health of infants and on health care costs in Arizona, as well as elsewhere.

Charlene A. McQueen, Ph.D.

University of Arizona
Award Amount FY 1997: $42,936

Genetic Variation in N-acetyltransferase and the Developmental 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 developmental toxicity of 4-aminobiphenol (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). Genetic variation in NAT results in different abilities to detoxify 4AB. Adults are classified as having high or low abilities. In Arizona, about 50% of the population has low ability. There is little information on NAT activity in the developing fetus. Studies funded by this project have shown, in a mouse model, that NATs are present at mid-gestation. Thus, the fetus has enzymes that can alter the toxicity of 4AB. Future studies will determine the relationship between NAT activities in the mother and fetus and the developmental toxicity of 4AB.

Catherine Racowsky, Ph.D.

University of Arizona
Award Amount FY 1997: $48,571

Investigation of the Mechanisms Underlying the Deleterious Effect of Cigarette Smoking on Human Fertility

During the first year of this research project, we established all the clinical and laboratory protocols necessary to perform the studies as described in our application. However, we encountered unanticipated problems with respect to subject recruitment. As a result, we were only able to recruit three subjects for our study, but only one of these became a participant. Nevertheless, our findings from this one individual, who was a cigarette smoker, did show a reduction in egg quality as determined by the proportion of eggs with the normal number of chromosomes. Based upon this single subject, the data obtained are consistent with the hypotheses proposed in our original application.

SECTION C

CONTRACTS

TOBACCO RELATED RESEARCH

YEAR TWO

Ronald L. Heimark, Ph.D.

University of Arizona

Award Amount FY 1997: $30,220

Smoking and Pericytes: Their Roles in Angiogenesis

This proposal addresses a common cellular mechanism that affects the increased health care cost of smokers in the state of Arizona. Given the central role of cell-cell communication of endothelial cells and pericytes in angiogenesis, we believe that understanding the molecular basis of cell-cell communication may lead to therapeutic agents capable of preventing lung tumor growth and enhancing wound healing in smokers. Angiogenesis is the formation of new blood vessels from existing small blood vessels. The increased incidence of lung tumors in smokers cannot occur without increased angiogenesis to provide a blood supply for the tumor to grow. Lung tumors in the absence of angiogenesis can grow to the size of a cubic millimeter, and it is the induction of angiogenesis that allows for increased growth and, in addition, provides new routes for metastasis. Moreover, in wound healing, angiogenesis is required for repair of skin lesions. The process of wound healing is impaired in smokers, partially as a result of altering angiogenesis. The studies we are performing are to understand the communication between the two cell types, the endothelial cell and the pericyte, in small blood vessels. The endothelial cell lines the lumen of all blood vessels and the pericyte surrounds the endothelium with cellular processes.

Our results demonstrate, for the first time, new markers for the identification of pericytes. Pericytes show similarities to smooth muscle cells in their location and function, but they retain their differentiated markers during pathogenesis in contrast to smooth muscle. The angiogenesis model system we have used in these studies, cultures intact blood vessel fragments, which maintain the association of pericytes and endothelial cells in a three-dimensional collagen gel. Other studies have shown evidence for soluble factors in the cell-cell communication and we have identified proteins associated with physical contact. We have found that both pericytes and endothelial cells are proliferating during angiogenesis and suspect that different factors are responsible for the proliferation. We are in the process of the quantitation of proliferation using neutralizing antibodies to determine the effect on the two cell types. Moreover, we have identified cytoskeletal proteins that are involved in the pericyte processes that contact the microvascular endothelium. Our current aims are to quantitate the temporal pattern of proliferation in response to different angiogenic factors that are released during tumor angiogenesis and wound healing. We have also found a probe that will block angiogenesis and we will determine the effect on pericytes and endothelial cells.

Bertram L. Jacobs, Ph.D.

Arizona State University

Award Amount FY 1997: $30,000

Regulation of Programmed 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 in preventing VV from inducing cancer cells to commit suicide. Our results suggest that it functions by inhibiting the well-characterized interferon system. These results reinforce the role of this system in fighting cancer.

Publications:

Jacobs BL, Landland JO. When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA. Virology 219:339-349; 1996.

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Langland JO, Jacobs BL. Viral inhibitors of interferon action: inhibitors of the PKR and 2'5' oligoadenylate synthetase/RNase L pathways. in *Gamma Interferon in Antiviral Defense* (G. Karupiah, ed.) R.G. Landes, Texas; 1997.

Claire M. Payne, Ph.D.

University of Arizona
Award Amount FY 1997: $30,000

Evaluation of Novel Biomarker 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 59 patients during colonoscopy, a procedure used to identify and remove polyps, the precursor lesions to colon cancer. It was determined that patients at high risk for colon cancer (HRCC) could be distinguished from those at low risk for colon cancer (LRCC) using this bioassay. The % apoptosis for the HRCC group was 36+ 3.2 (S.E.M.) and that for the LRCC group was 52+ 2.1 (S.E.M.). Biopsies were also taken from two additional sites within the colon to determine site-to-site variability. The HRCC group showed a "patchiness" in apoptosis induction, whereas the LRCC group showed good correlation between the three biopsy sites.

Publications:

Zheng ZY, Bernstein H, Bernstein C, Payne, CM, Martinez JD, Gerner EW. Bile activation of the *gadd*153 promoter and of p53-independent apoptosis: relevance to colon cancer. Cell Death and Differentiation 3: 407-414; 1996.

Henry I. Yamamura, Ph.D.

University of Arizona
Award Amount FY 1997: $29,700

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

In Arizona, opiates, such as morphine, have been used to manage the pain of tobacco-induced cancers. 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 third extracellular loop of the human delta opioid receptor determines the selectivity of drugs acting at this receptor.

Denise A. Drumm, Ph.D.

St Joseph's Hospital

Award Amount FY 1997: $22,350

Glycemia Subarachnold Hemorrhage, Neurobehavioral Outcome in Smokers, Passive Smokers and Non-Smokers

The goal of this investigation is to quantify factors contributing to secondary ischemic injury and neurobehavioral outcome following aneurysmal subarachnoid hemorrhage. Glycemia and tobacco exposure are the primary factors being examined. We hypothesize that these factors are related to poor neurobehavioral outcome even for patients described as having attained good neurological recovery. During this recruitment period, 43 research participants met the rigorous selection criterion for this study, representing 86% (43/50) of the anticipated goal. Initial blood samples, obtained within 48 hours of the initial hemorrhage, were acquired for 100% of the sample (glycosylated hemoglobin). Urine samples for urinary nicotine metabolites were also obtained for 100% of the patient sample well within the specified window. Although neurobehavioral assessments continue at specified follow-up periods, several research participants have completed 12-month or 8-10 hours of cumulative cognitive assessments. Interim analyses have not been completed to avoid examiner bias in the construction of companies for group matrices.

Maria Luz Fernandez, Ph.D.

University of Arizona

Award *Amount FY 1997*: $29,700

Cardiovascular Disease Risk Reduction by Dietary Fiber

Dietary soluble fiber has been shown to lower plasma cholesterol concentrations and elevated plasma cholesterol is associated with increased risk for cardiovascular disease. It is known that soluble fiber lowers both plasma VLDL and LDL cholesterol concentrations and that both lipoproteins can be potentially atherogenic. These studies were undertaken to determine the mechanisms by which three sources of soluble fiber, pectin, guar gum and psyllium lower plasma cholesterol levels and to assess whether gender has an effect on the hypocholesterolemic response. Guinea pigs were used as the animal model for these studies since these animals have plasma lipoprotein profile and responses to dietary fiber similar to humans. Guinea pigs were fed a hypercholesterolemic diet. From the results, it can be concluded that the three sources of fiber were equally potent in lowering plasma cholesterol although their actions are slightly different which results in different mechanisms. Gender had a major effect on plasma lipid levels. Female guinea pigs, responded similarly to males to dietary fiber but their plasma cholesterol values were higher than males in control and soluble fiber-treated animals. These results indicate that females are more responsive than males to hypercholesterolemic diets. In addition, female guinea pigs had lower concentrations of hepatic cholesterol than males and some of the mechanisms of secretion and catabolism of lipoproteins were different. From these studies, we conclude that gender had a more potent effect in determining plasma lipid levels than dietary soluble fiber. The distinctive effects of the different sources of soluble fiber are not so significant when compared with the gender effects. This situation has to be considered when making dietary recommendations to men and women who are at risk for cardiovascular disease.

Publications:

Fernandez ML, Vegara-Jimenez M, Conde K, Behr T, Abdel-Fattah G. Regulation of apo B containing lipoproteins by dietary soluble fiber in the guinea pig. Am J Clin Nutr 65: 814-822; 1997.

Shen H, He L, Fernandez ML. Gender differences on the effects of pectin, guar gum and psyllium on plasma VLDL metabolism in guinea pigs. FASEB J 11: 886; 1997.

Abstracts:

Fernandez ML, Verfara-Jimenez M, Conde K, Behr T, Abdel-Fattah G. Regulation of VLDL-LDL apo B metabolism by pectin (PE), guar gum (GG) and psyllium (PSY) in guinea pigs. *FASEB J* 10:A180; 1996.

Elizabeth A. Krupinski, Ph.D.

University of Arizona
Award Amount FY 1997: $29,785

Interactive Three-Dimensional Display for Evaluation of Coronary Artery Disease

Coronary artery disease is the leading cause of death in the United States. Tobacco consumption has been shown to increase even further the chance of coronary artery disease in long-time users, augmenting the risk for elderly people, an important part of the population of the state of Arizona. This project is focused towards improving the diagnostics of early detection of coronary artery disease to provide more appropriate, prompter and more cost-effective patient management.

To do so, we have developed an interactive 3D rendering display of 3D radioisotope myocardial perfusion images, a usually difficult type of image to display, comprehend and interpret. This interactive display is based on standard desktop personal computer technology. This software is now in the final software-testing phases. Earlier, we showed, using conventional evaluation techniques, that there is no significant difference between film and monitor reading for nuclear medicine myocardial images displayed as a set of slices.

Arnold R. Martin, Ph.D.

University of Arizona
Award Amount FY 1997: $28,525

New Antitubercular Agents: Products and Isosteres of Isoniazid and Pyrazinamide

Eleven (11) additional analogs of the standard antitubercular drugs isoniazid (INH) and pyrazinamide (PZA) were synthesized and evaluated against a standard strain of *Mycobacterium tuberculosis 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, fifteen (15) boronic acid derivatives, including two (2) 2,3,1-benzodiazaborines and eleven (11) 2,4,1-benzodiazaborines were also synthesized and evaluated. The selection of boronic acid derivatives as new targets (not part of the original proposal) was based on literature reports of significant general antibacterial activity of such compounds, particularly the 2,3,1-benzodiazaborines.

None of the additional isoniazid or pyrazinamide analogs synthesized during the second year of the project exhibited significant antitubercular activity *in vitro*. However, one of the 2,3,1-benzodiazaborine compounds (reported earlier in the literature) exhibited *in vitro* activity, and nearly all the new 2,4,1-benzodiazaborines exhibited antitubercular activity *in vitro*. Although the antitubercular potencies of the diazaborines thus far are still significantly less (60-350X) than that of isoniazid, the compounds are much more (4-70X) potent that pyrazinamide, encouraging us to attempt to structurally optimize the series. Compounds that have exhibited promising activity *in vitro* will be tested *in vivo* in infected mice and their toxicities in mice will be determined. If the results of the *in vivo* tests are promising then patenting and commercial development of selected compounds will be considered.

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*. University of Arizona, 1997. Dissertation Director: Arnold R. Martin, Ph.D.

Eugene Morkin, M.D.

University of Arizona
Award Amount FY 1997: $94,054

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

The major objective of this study is to investigate the actions of diiodothyropropionic acid (DITPA), a thyroid hormone analogue, in the treatment of heart failure. Despite advances in the prevention and treatment of heart disease, heart failure remains a major public health problem. In fact, it is the only form of heart disease that seems to be increasing. American Heart Association data suggest that there are 3 to 5 new cases per 100 persons per year with an overall incidence of 10 per 1,000. Development of better drugs for the management of this condition is essential. Experimental studies indicate that DITPA improves the performance of the heart after infarction, but the mechanism for this action is largely unknown. In this multidisciplinary program, investigators in *Section 1* are studying the time that the drug spends in the body after intravenous or oral administration. Such information is important to determine correct dosage and dosing intervals. In addition, they plan to distinguish the effect of DITPA on the peripheral circulation from the direct actions of DITPA on the heart by studies to be carried out in isolated cardiac muscle preparations. Investigators in *Section 2* are assisting in the analysis of levels of the drug and its breakdown products in the blood and tissues. Also, they are studying the actions of DITPA on intracellular components often depressed in heart failure, and they hope these studies will determine whether these changes can be reversed by treatment with the drug.

Mark A. Nelson, Ph.D.

University of Arizona
Award Amount FY 1997: $29,361

Functional and Clinical Evaluation of the p-16 Protein in Melanoma and Human Lung Cancers

This project involves evaluating the frequency of p-16 gene alteration in human lung cancer and melanoma specimens and correlation of these data to clinical variables. We have developed PCR-based methods for analysis of p-16 gene alterations and applied these techniques to archival lung cancer and melanoma specimens. We have also evaluated staining of the p-16 protein product as a surrogate for determining p-16 gene inactivation in lung cancer cases.

In another series of experiments, the effects of biochemical and molecular basis for the anticancer effects of dietary selenium against lung and colon cancer are being studied. We have observed that selenium, in the form of selenomethionine, can induce alterations in intracellular polyamine levels and cause cell cycle alterations (i.e., G1 arrest) in HT-29 colon cancer cells and A549 lung cancer cells. The exact mechanisms responsible for decreased polyamine level and the cell cycle arrest induced by selenomethionine are under investigation. The involvement of p-16 in selenomethionine-induced cell cycle changes is being evaluated.

Marvin J. Slepian, M.D.

University of Arizona
Award Amount FY 1997: $29,547

The Role of Beta-3 Integrin Expression in Arterial Smooth Muscle Cell Migration Following Injury

The migration of smooth muscle cells (SMCs), the constituent cells of the arterial wall, into the lumen of the artery leading to arterial narrowing, is a vital mechanism in the development of atherosclerosis. It has recently been recognized that cell-extracellular matrix interactions, mediated through specialized cell surface adhesion receptors, known as "integrins," are vital in cell migration. We previously demonstrated that blockade of SMC B3 integrin-matrix interactions resulted in reduced SMC migration. The present proposal is designed to identify mechanisms responsible for the predominant role of B3 integrins in SMC migration. Over the past year, we demonstrated that migrating rat aortic SMCs express a 2-3-fold increase in detectable levels of B1 and B3 integrins on their surfaces, compared to stationary SMCs. Further, the level of B3 integrin expression in migrating SMCs was greater than B1, with B3:B1 ratio in migrating SMCs of 1.46 ± 0.25 *versus* 0.76 ± 0.12 for static SMCs ($p<0.01$). We have also demonstrated that exposure of SMCs to defined underlying extracellular matrix substrata *in vitro* does not alter integrin expression. These findings support our working hypothesis that B3 integrins are preferentially expressed in migrating cells, acting as the "sticky feet" needed for forward locomotion. Defining the role of integrins in SMC migration will open the door for Arizona citizens to new therapies which limit SMC migration and therefore, interfere with the development and progression of atherosclerosis.

Alison Stopeck, M.D.

University of Arizona
Award Amount FY 1997: $28,294

Genetically-Modified Endothelial Cells in Vascular Biology

Damaged blood vessels caused by atherosclerosis and peripheral vascular disease are the major cause of mortality in Arizona. New vessels are also needed for access in patients on dialysis. While damaged vessels can be repaired and new vessels (grafts) surgically placed, their function is limited by early clogging and narrowing of the vessel lumen from abnormal smooth muscle cell growth. Smooth muscle cells are the major component of vessels and their growth is controlled by the vessel-lining endothelial cells. We have successfully transferred a gene, gamma interferon, into endothelial cells. This enables endothelial cells to produce a protein that inhibits smooth muscle cell growth in culture. We have also determined the mechanism by which gamma interferon inhibits smooth muscle cell growth in culture. Hopefully, the future use of genetically-modified endothelial cells in vessel grafts or repaired native vessels will improve graft longevity and vessel function in patients.

Yi Ran Wang, Ph.D.

University of Arizona

Award Amount FY 1997: $29,729

Apolipoprotein A-I Gene Promoter: Functional Change in a Common Point Mutation

This project was designed to understand the mechanisms that regulate the production of apolipoprotein (apo) A-I, the major component of high density lipoproteins. A common variant within the apo A-I gene promoter region, found in 10-20% of the population, is created by the single substitution of nucleotide, the building block of DNA. The activity of apo A-I promoter, was determined with reporter gene constructs containing the liver-specific apo A-I promoter region, with or without the mutation, fused to that of a reporter gene whose product can be easily measured. A 30% increase in promoter activity was observed with the reporter gene construct, with the mutation than without, transfected into cells of human liver cell line. This increased promoter activity indicates that the mutated apo A-I promoter is capable of inducing an enhanced apo A-I gene expression and may explain why this mutation is associated with elevated plasma apo A-I.

Linda Larkey, Ph.D.

University of Arizona

Award Amount FY 1997: $29,754

Curbing the Trend of Tobacco Related Cancer Deaths: Identifying Factors Influencing Late Presentation of Symptomatic Hispanics

Hispanics in Arizona often postpone visits to the doctor when symptoms appear leading to late detection of treatable smoking-related and other cancers. Focus groups were used to identify ten issues affecting Hispanics' decision to visit the doctor when facing symptoms. Then, these issues were translated into a questionnaire and administered to 133 Hispanic participants who pre-qualified as having had recent symptoms. Path analytic methods revealed a pattern of effects on visits to the doctor. Attitudes about going to the doctor are troublesome and can be remedied by the availability of Latino doctors of both genders who communicate trustworthiness *(confianza)* and warmth *(personalismo)*. Severity of symptoms and faith in God both lead to an expressed desire for a doctor one can trust and the search for a doctor to trust is positively associated with going to the doctor. With the strength of these contributing factors assessed, prevention campaigns may be designed to provide more culturally appealing messages and services for Hispanics in Arizona.

James Bloedel, Ph.D.

St. Joseph's Hospital
Award Amount FY 1997: $30,000

Genetic Engineering of Receptors for Nicotine

Nicotine acetylcholine receptors (nAChR) play important roles in chemical signaling throughout the brain and body. nAChR also are targets of nicotine, thought to be the addictive substance in 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. In the past year, we have mastered two ways to create human cells that now make the simplest possible form of human nAChR, a7-nAChR, which is ideally suited for studies to elucidate basic principles of nAChR structure and function. In other breakthroughs, we now also can control amounts of human a7-nAChR made by genetically-engineered cells, and we can create mutant forms of those nAChR. Whereas nicotine triggers only a brief burst of signaling by normal a7-nAChR, such signaling lasts much longer when nicotine interacts with one type of mutant a7-nAChR and is triggered at much lower nicotine concentrations. Further, some cell types that make mutant a7-nAChR have reduced survival. These findings suggest the possibility that minor genetic differences may affect properties of nAChR. 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.

Publications:

Bencherif M, Eisenhour CM, Prince RJ, Lippiello PM, Lukas RJ. The "calcium antagonist" TBM-8 [3,4,5-trimethoxy benzoic acid 8-(diethylamino) octyl ester] is a potent, non-competitive, functional antagonist at diverse nicotinic acetylcholine receptor subtypes. J Pharm Exper Thera 275: 1418-1426; 1995.

Ke L, Lukas RJ. Effects of steroid exposure on ligand binding and functional activities of diverse nicotinic acetylcholine receptor subtypes. J Neurochem 67: 1100-1112; 1996.

Lukas RJ, Eisenhour CM. Interactions between tachykinins and diverse, human nicotinic acetylcholine receptor subtypes. Neurochem Res 21: 1245-1257; 1996.

Lukas RJ, Ke L, Bencherif M, Eisenhour CM. Regulation by nicotine of its own receptor. Drug Devel Res 38: 136-148; 1996.

Quik M, Choremis J, Komourian J, Lukas RJ, Puchac E. Similarity between rat brain nicotinic α-bungarotoxin receptors and stably expressed α-bungarotoxin binding sites. J Neurochem 67: 145-154; 1996.

Lukas RJ. "Neuronal" nicotinic acetylcholine receptors. Neuroscience Intelligence Unit, In Press, 1997.

Edward D. French, Ph. D.

University of Arizona
Award Amount FY 1997: $28,446

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

Nicotine containing products, such as cigarettes, have been recognized recently as possessing addictive qualities. Moreover, the use of these substance has been considered a gateway to the abuse of other substances. Since considerable basic research has identified the neurotransmitter dopamine as playing a pivotal role in the reinforcing effects of most drugs of abuse, it seems reasonable to hypothesize that the addictive effects of nicotine may be produced through nicotine-induced activation of dopamine neurotransmission in the central nervous system. Moreover, the repeated activation of this pathway may produce long-lasting neuronal adaptations which could further act to augment or diminish the rewarding effects of other drugs, such as marijuana. Using electrophysiological and behavioral methods, we have found that nicotine is a potent stimulator of dopamine neuronal activity within the ventral tegmental area of the anesthetized rat. Nicotine also elevates gross motor behavior leading to hyperactivity. Moreover, the psychoactive ingredient in marijuana, delta-9-tetrahydrocannabinol stimulates the same dopamine neurons. Once daily injections of nicotine to rats will cause an augmented behavioral response to nicotine or sensitization. During the past funding period, we sought to examine the response of midbrain dopamine neurons in the brain slice preparation to the effects of nicotine, and to ascertain whether the electrophysiological concomitant of the behavioral sensitization is expressed at the single neuronal level. Moreover, in those animals in which nicotine treatments evoked sensitization to nicotine, we hypothesized that cross-sensitization would develop to the electrophysiological effects of the psychoactive cannabinoid, delta-9-tetrahydrocannabinol.

Ronald M. Lynch, Ph.D.

University of Arizona
Award Amount FY 1997: $29,968

Regulation of Insulin Secretion from Individual Beta Cells

Diabetes afflicts 9 million Americans and is often associated with obesity. Normally, insulin secretion increases when blood glucose increases. In diabetes, insulin secreting beta cells lose sensitivity to glucose causing 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 year, probes were developed to measure insulin secretion from single cells on a microscope, and beta cells that do not sense glucose normally were produced. In the coming year, the factors required for translating cell excitation into insulin secretion will be studied. This will shed light on alterations in these processes which occur during development of Type II Diabetes and long term nicotine exposure and withdrawal.

Y. Howard Lien, Ph.D.

University of Arizona

Award Amount FY 1997: $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. We found that CA II deficiency is associated with a lower 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 preliminary results of intravenous gene therapy using the human CA II gene showed that there was a significant improvement in CO_2 retention. Further work is needed to substantiate our preliminary findings and to delineate the contribution of red blood cell and pulmonary CA II to pulmonary elimination of CO_2. The results of this study will enhance our understanding of the mechanisms of CO_2 exchange in the lung and facilitate the development of gene therapy targeted to the respiratory diseases.

Nafees Ahmad, Ph.D.

University of Arizona

Award Amount FY 1997: $30,000

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

Infants born to HIV-1 infected mothers are at risk of acquiring HIV-1 infection and subsequently developing AIDS. 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 highest rate of smoking 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 viral factors associated with HIV-1 mother-to-infant transmission, we have analyzed *vif* sequence from five infected mother-infant pairs following perinatal transmission. The coding potential of the *vif* open reading frame directly derived from uncultured peripheral blood mononuclear cell DNA was maintained in most of the sequences in 78, 912 base pair sequenced. We found that 123 of the 137 clones analyzed conserved an intact *vif* open reading frame with an 89.8% frequency of intact *vif* open reading frames. There was a low degree of heterogeneity of *vif* genes within mothers, within infants, and between epidemiologically linked mother-infant pairs. However, the distance of *vif* sequences between epidemiologically unlinked individuals was more compared to epidemiologically linked mother-infant pairs. Furthermore, the epidemiologically linked mother-infant pairs' *vif* sequences displayed similar patterns that were not seen in epidemiologically unlinked individuals. The functional domains essential for *vif* protein function were highly conserved in most of the sequences. In conclusion, the conservation of an intact *vif* open reading frame and its limited variability following mother-to-infant transmission of HIV-1 are consistent with a role for *vif* in perinatal transmission. This finding may be helpful in developing strategies for prevention and treatment of HIV-1 infection.

Felipe G. Castro, M.S.W., Ph.D.

Arizona State University
Amount Awarded FY 1997: $29.679

The Evaluation of a Peer Health Worker Model: Is It Effective in Preventing Cigarette Smoking and Promoting Women's and Children's Preventive Health Care?

In a case-controlled experimental design, this project examines the hypothesis that women and their infants participating in the Arizona Health Start Program will enjoy better health outcomes as compared with non-participants. Health Start is a community outreach program that employs community lay women (peer Health Workers; *Promotoras*) as health educators. The current study examines health outcomes from self-report and birth certificates. For the infants, health outcomes examined include normal birth weight and good APGAR score. For the mothers, health behaviors examined include: cigarette smoking, alcohol consumption, and access to prenatal care. Also examined is the role of the Peer Health Workers in promoting good self-care. The influence of sociocultural factors, such as acculturation status and traditional beliefs and practices, are also examined as contextual factors affecting health among these low-income Hispanic and other minority women. Results of this study can yield policy-relevant information on this Peer Health Model.

John N. Galgiani, M.D.

University of Arizona

Award Amount FY 1997: $30,000

Structure of Protein Antigen from Cryptococcus Neoformans

Complications of smoking tobacco include both scarring and cancer of the lungs. These consequences make smokers more prone to infection with unusual fungi such as *Cryptococcus neoformans* which is resisted by normal human immunity and therefore, does not infect the lungs. We are interested in determining the specific antigens that stimulate immune protection against *C. neoformans*, and that interest has led to the current project. Preliminary work identified a fungal protein which immune animals respond to and which could be the stimulus of immunity. During the period of funding, we have discovered the DNA sequence for the gene which encodes this protein. Further, we have developed the procedures to use this gene to produce ample quantities of this protein in a common bacterium. Thus, as a result of this ADCRC-funded research, it is now possible to test whether the protein stimulates immune protection.

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

Arizona State University

Award Amount FY 1997: $29,730

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

Selection for delayed reproduction has been carried out for two years in the five chosen species, *D. melanogaster, D. pseudoobsura, D. hydei, D. arizoane, and D. virilis.* Selection for delayed reproduction has been shown to result in both postponed aging and resistance to toxic compounds. In year two, stocks from *D. pseudoobscura* were shown to have been differentiated for life span. Year three examined the physiological performance of these same stocks. Weak evidence was found for the differentiation of these stocks in desiccation and starvation resistance, while no statistically different result 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.

Dianne Lorton, Ph.D.

Sun Health Research Institute

Amount FY 1997: $29,969

Neuroimmune Involvement in the Progression of Experimental Arthritis

Since two of the most concentrated geriatric communities in the world are in Arizona, and the incidence of arthritis increases with age, rheumatoid arthritis (RA) represents a significant health problem for Arizona residents. Data support smoking may adversely affect RA by changing sympathetic outflow. Our studies examine whether increased severity of arthritis following denervation of sympathetic nerve fibers supplying immune organs is due to altered ability of immune cells to process the foreign antigen that induces arthritis. Denervation during the time of antigen processing and at the effector phase of the disease both resulted in an increased severity of arthritis. Denervation altered the expression of several markers of immune cell activation, altered the distribution of immune cells, 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 phases of the disease.

Publications:

Lorton D, Bellinger DL, Felten SY, Felten DL. Selective sympathectomy of lymphoid organs exacerbates the expression of adjuvant-induced arthritis. J. Neuroimmunology 64: 103-113; 1996.

Lorton D, Lubahn C, Felten SY, Bellinger D. Norepinephrine content in primary and secondary lymphoid organs is altered in rats with adjuvant-induced arthritis. Mechanisms Aging and Development, 94: 145-163; 1997.

Abstracts:

Lubahn C, Schaller J, Bellinger DL, Lorton D. Noradrenergic innervation of lymphatic organs and joints in experimental arthritis. Soc Neurosci Abstr. 22; 1995.

Bellinger DL, Lorton D, Lubahn C, Felten SY. Norepinephrine content in primary and secondary lymphoid organs is altered in rats with adjuvant-induced arthritis. First International Conference on Aging; 1996.

SECTION D

PROPOSALS RECEIVED

TOBACCO RELATED RESEARCH

FY 1997

Adams	University of Arizona	Regulation and Roles of the Actin Cytoskeleton	$45579 46334 47112
Adams	University of Arizona	Vitamin E Protects from Free Radical Damage	$50000 50000 50000
Aiken	University of Arizona	Phosphomonoester Metabolism in Lung Cancer Cells	$49995 49995 49982
Akporiaye	University of Arizona	Active Specific Immunotherapy of Breast Cancer by Inhibition of an Immunosuppressive Cytokine	$50000 50000 50000
Aldous	University of Arizona	Development of a Program for Teaching Medical Students and Residents to More Accurately Diagnose Acute Otitis Media and Middle-Ear Effusion in Children	$44463 49909 41384
Allen	Arizona State University	Structure Based Strategy for the Design of Cancer Drugs	$50000 50000 50000
Appleton	Mayo Clinic, Scottsdale	Experimental Determinants of Transmitral and Pulmonary Venous Flow: Further Relations to Left Ventricular Filling Pressure	$50000 50000 50000
Bellamy	University of Arizona	Inhibition of Angiogenesis as a Novel Target for Lung Cancer Therapy	$49669 49942 49741
Bieber	Arizona State University	Composition of a Unique Receptor for Nicotine	$144563 146231 148480
Blankenship	Arizona State University	Purification and Characterization of a Novel Form of Superoxide Dismutase	$50000 50000 50000
Bloom	University of Arizona	The Airway Epithelium as a Target for the Anti-Inflammatory Action of Inhaled Steroid Therapy	$49908 49984 49990

Bloom	Arizona State University	Molecular Mechanisms for the Formation of Mutations Due to Tobacco related DNA Damage	$48950 48707 49532
Borchers	University of Arizona	Mechanism of Matrix Degrading Protease Expression in Oral Squamous Cell Carcinoma	$49994 49994 49994
Boswell	University of Arizona	Modulation of Angiogenesis by Nicotine: Cellular and Molecular Mechanisms	$49714 50000 50000
Brinton	Carl T. Hayden VA Medical Center	Smoking Cessation: (1) Its Impact on HDL and Other Atherosclerotic Risk Factors; (2) Its Effects on the Artery Wall; and (3) Its Psychometric Prediction and Outcomes	$164957 155309 146685
Brown	University of Arizona	Effects of *In Utero* Passive Smoke Exposure on Early Human T-Helper Cell Differentiation	$49309 47718 44617
Brucks	University of Arizona	The Cumulative Impact of Tobacco Advertising on Young Children's Socialization to Pro-tobacco Attitudes and Behaviors	$50000 50000 50000
Burgoon	University of Arizona	When Gains Go Up in Smoke: Explaining Adolescents' Negative Reactions to Smoking Prevention Campaigns	$89961 59711
Burt	University of Arizona	Tumor Suppressor Function of Cx43-role of Phosphorylation	$50000 50000 50000
Cesarotti	Arizona State University	Interventions to Decrease Second-Hand Smoke Exposure in School Children with Asthma	$60068 63821
Chang	Arizona State University	Structure and Function of DNA-Dependent Protein Kinase in DNA Repair	$49989 49989 50000
Chong	University of Arizona	Changing the Odds: Research for Prevention	$49839

Chow	University of Arizona	Pharmacokinetic Modeling of the Anabolites of Nucleoside Analogues	$49999 49999 49999
Coons	St. Joseph's Hospital/ BNI	The Microangiopathy of Smoking in Nerve: An Additional Cause of Peripheral Nerve Disease	$40092 35938
Cress	University of Arizona	Cytokeratin-Dependent Multiple Drug Resistance in Small Cell Cancer	$49959 49959 49959
Crisp	Arizona State University	The Development of an Intestinal Pacemaker	$50000 50000 50000
Davis	Arizona State University	The Effects of Smoking on Cognitive Functioning in Young and Middle-Aged Men and Women	$43317 42077
DeLuca	University of Arizona	HIV Infection of the Developing Thymus *In Vitro*: Effect of Smoking	$144793 143839 149593
Dembroski	University of Arizona	Biobehavioral Risk Profile of Smokers	$85396 84993 84020
Dieckmann	University of Arizona	Peroxisome Assembly: The Importance of Organelle Integrity for Proper Lipid Metabolism	$50000 50000 50000
Disney	University of Arizona	Smoking Cessation in Rural Communities Targeting Pregnant Women	$114083 171301 166828
Donnerstein	University of Arizona	Clinical and *In-Vitro* Evaluation of Factors Affecting Closing Dynamics of Bileaflet Mechanical Heart Valves	$35396
Drumm	Sun Health Research Institute	Interaction Effects of Smoking Exposure and Glycemia on Neurobehavioral Outcome Following Surgical Intervention for Ruptured and Unruptured Cerebral Aneurysms	$55221 54341 40041

Enright	University of Arizona	Spirometry for the Detection of High Risk Smokers in Southern Arizona Primary Care M.D. Offices	$49953 49776 49838
Fleming-Moran	University of Arizona	The Arizona Border Cardiovascular Risk Study	$149927 149950 109320
Floyd-Smith	Arizona State University	Protein Kinase C as a Regulator of Proliferation in Lung Cancer Cell Lines	$50000 50000 50000
Foster	Valley Neurological Headache Clinic	The Effects of Nicotine Addiction on Intravenous Dihydroergotamine Treatment for Headache	$45240
Fry	Airpark Medical Center	Low Cost Spacer for Enhanced MDI Medication Delivery	$46652
Galons	University of Arizona	Discrimination of Lung Tumor Phenotypes by Multimodal MR	$46476 46471 48077
Gillies	University of Arizona	Endosomes and Drug Resistance in Lung Cancer	$49993 49844 49978
Grimes	University of Arizona	HLA and p53 an *In Vitro* Study of Peptide Immunotherapy in Human Lung Cancer	$50000 50000 50000
Guerriero, Jr.	University of Arizona	Stress Proteins in Cardiac Tissue	$49013 49955 49060
Hammond	Arizona State University West	Cigarette Smoking and Visual Health: Implications for Age-Related Macular Degeneration and Cataract	$49933 49940 49163
Harris	University of Arizona	Reversal of Tumorigenic and Pulmonary Effects of Cigarette Smoke by Administration of Substance P	$98054 98054 98054

Hatch	University of Arizona	HPV, Chlamydia and Cervical Dysplasia Prevalence Among Smokers *vs.* Non-Smokers Along the United States-Mexico Border	$149826 149916
Heimark	University of Arizona	Mechanisms of Vascular Dysfunction in Atherogenesis: Cell-Cell Interactions	$150000 150000 150000
Hempleman	Northern Arizona University	Chronic Nicotine Exposure: SIDS; and the Maturation of Hypoxic Chemosensitivity	$48500 49325 49325
Hoyer	University of Arizona	Mechanisms of Ovarian Follicular Cell Death Initiated by Polycyclic Aromatic Hydrocarbons	$49990 49907 49999
Huo	University of Arizona	Molecular Mechanism of Hormone-Regulated Calcium Transport in Kidney	$50000 50000 50000
Jin	University of Arizona	Synergistic Effects of Tobacco Smoking: Air and Biological Contaminants on Human Health	$48510 50158 43469
Johnson	University of Arizona	Cardiac Teratogenicity: The Combined Effects of Exposure to Trichlorethylene and Cigarette Smoke in the Pregnant Sprague-Dawley Rat Model	$47954 49530 49940
Kappen	Mayo Clinic, Scottsdale	Tobacco's Effect on the Fetus: Molecular Analysis of Abnormal Skeletal Development	$50000 50000 50000
Kay	University of Arizona	Tobacco and Free Radical Damage to Brain Anion Transporters During Aging & Neurological Disease: Tandem MS-MS Spectrometry and Cellular Studies	$150000 150000 150000
Kelley	Northern Arizona University	Structural Studies of the Binding of Cadmium by Glutathione to Metallothioneins: New Directions for the Design of Therapeutic Agents for the Detoxification of Cadmium	$49998 49997 49997

Klewer	University of Arizona	The Effects of Fetal Smoke Exposure Upon Congenital Cardiovascular Malformations: A Clinical and Molecular Epidemiological Analysis of Environmental and Genetic Influences	$148499 148501 148444
Kline	Grand Canyon Learning Center	Infant/Toddler Tobacco Smoke Exposure Developmental Study	$50000 50000 50000
Kling	University of Arizona	The Effect of Smoking on Oxidants, Erythropoietin and Growth Factors in Human Milk	$50000 50000 50000
Krupinski	University of Arizona	Improving Cancer Detection Performance on Film Images Via Perceptual Feedback	$33736 13524
Kuberski	Boswell Memorial Hospital	The Influence of Cigarette Smoking on the Acquisition of Coccidioidomycosis in a Geriatric Population	$49719 50000 50000
Kurth	St. Joseph's Hospital	Nicotine Acetylcholine Receptors and Monoamine Oxidase B as Potential Genetic Markers for Nicotine Dependence	$149362 148490 143100
Lai	University of Arizona	Opioid Modulation of Tobacco Addiction	$45255 45348 47160
Lai, L	University of Arizona	Enhancement of Non-Viral Gene Transfer in the Lung	$50000 50000 50000
Lake	University of Arizona	Generation of Recombinant Antibodies from Lung Tumor Infiltrating B Lymphocytes (TIL-B)	$49941 49993 49964
Lake	University of Arizona	Genetic Immunization Against Mutant P53 for Lung Cancer	$49941 49883 49997
Lam	University of Arizona	Elucidation of Group A Streptococci Adhesion Binding Motif Using a Combinatorial Library Method	$50000 50000 50000

Lebowitz	University of Arizona	Evaluation of the Effects of Smoking on the Development of Chronic Pulmonary & Cardiovascular Diseases in Arizona	$108314 112647 114535
Leischow	University of Arizona	The Effectiveness of a Toll Free Smoking Cessation Telephone Helpline Alone and in Combination With Over-the-Counter Nicotine Patch as an Intervention to Smoking Cessation	$149180 149076 149531
Lien	University of Arizona	Brain-Specific Gene Therapy for Acute Ischemic Stroke Using Antisense Oligonucleotides Against Endothelin-1	$150000 150000 150000
Lippiello	Maricopa Medical Foundation	Direct Effects of Smoking Components on Osteosynthesis	$47309
Lue	Sun Health Research Institute	Nicotine Effects on Amyloid B Peptide Cytotoxicity in Alzheimer's Disease	$50000 50000 50000
Manseau	University of Arizona	Analysis of DrtGEF, a Regulator of Rho-type GTPase Signaling	$47416 48491 49600
Marchalonis	University of Arizona	Analysis of Autoantibodies to T-Cell Receptors in Rheumatoid Arthritis	$150000 150000 150000
Martinez	University of Arizona	Linkage of Asthma and Asthma-Related Traits to Chromosomes 5q and 11q	$496251 499988 420500
Matsunaga	ImaRx Pharmaceutical Corp.	Targeted Microbubbles for Diagnosis and Treatment of Vascular Thrombosis	$50000 50000 50000
McDonagh	University of Arizona	The Effects of Cigarette Smoke Exposure on the Leukocyte Contribution to Ischemia-Reperfusion Injury in the Heart	$49268 48238 49431
Mills	University of Arizona	The Influence of Smoking and Associated Atherosclerotic Risk Factors on Restenosis After Carotid Endarterectomy and Lower Extremity Revascularization	$49939 49393 49393

Montfort	University of Arizona	Structural Studies of Human Thioredoxin: Target for Anticancer Drug Design	$50000 50000 50000
Moore	Arizona State University	Carotenofluorophores in Imaging and Therapy of Neoplastic Disease	$50000 50000 50000
Nagle	University of Arizona	Improved Extracellular Matrix for Treatment of Tobacco Related Occlusive Vascular Disease	$140339 144354 146559
Noecker	University of Arizona	Effects of Smoking on Intraocular Pressure and Glaucoma: Tobacco Associated Diseases	$45073 47521 47246
Nogami	University of Arizona	The Effects of Nicotine Patches on Maternal and Fetal Health	$49847 49924 48897
Orbach	University of Arizona	Isolation of Infection Cycle Genes of the Valley Fever Fungus Coccidioides Immitis	$50000 50000 50000
Pettit	Arizona State University	Preclinical Development of New Anticancer Drugs Necessary to Improving Treatment of Tobacco Related Human Cancer	$500000 500000 500000
Porreca	University of Arizona	Pathological Role of Dynorphin in Cancer-Related Pain	$49000 49500 50000
Powis	University of Arizona	Arizona Cancer Center Interdisciplinary Basic Science Program Project	$150000 150000 150000
Reaves	University of Arizona	Regulation of Human Tumor Supressor p53 Gene Expression by Zinc Status	$50000 50000 50000
Rider	University of Arizona	Effects of Environmental Tobacco Smoke Exposure on Alveolar Surfactant Catabolism by Macrophages	$49995 50000 50000

Roeske	University of Arizona	Regulation of the Human Delta Opioid Receptor	$49500 49500 49500
Salmon	University of Arizona	Developmental Treatment of Smoking-related Cancers	$499904 499904 499904
Samson	University of Arizona	Nicotine-Induced Delay After Depolarizations in Hypertrophied Hearts	$23585
Schumacher	University of Arizona	Human b Cell Development *In Vitro*: Implications for Human Stem Cell Transplantation	$49984 49984 49984
Selmin	University of Arizona	Characterization of a Molecular Marker for Tobacco Derivatives Exposure: 25-Dx, a Novel Cytokine Receptor	$47795 49228 49850
Shapiro	University of Arizona	Pregnant Smokers: A High-Impact Cessation Program	$48814 42172 41528
Skibo	Arizona State University	Antitumor Agents Targeting Topoisomerase II	$37402 46570 37402
St. John	University of Arizona	Nicotine Receptors in the Spinal Cord	$120836 125646
Stachowiak	St. Joseph's Hospital	Long-Term Effects of Nicotine: New Molecular Mechanisms	$148236 147916 148828
Stelmach	Arizona State University	Smoking and Motor Control	$143037 142939 147372
Thomas	Arizona State University	The Relationship of Tobacco Use and Youth Sport Participation in Arizona: Preventing Addiction	$49999 49983 40660
Tseng	University of Arizona	Development of New Vectors and Gene Therapy Techniques for Gene Therapy	$49772 49772 49772

Ulreich	University of Arizona	Arizona Liver Transplantation Research: Optimizing Organ Replacement in Tobacco Related Liver Disease	$123897 118619 116277
Vaillancourt	University of Arizona	Intracellular Signal Transduction Pathways Activated by Nicotine	$48510 48954 49183
Wang	University of Arizona	Modulation of Transcription by a Frequent Point Mutation in the Human Apolipoprotein A-I Gene Promoter	$50000 50000 50000
Watson	University of Arizona	Hormone Replacement to Reduce Cardiovascular Disease Risk Factors in Old Smokers	$106605 126583 130230
Weinert	University of Arizona	Checkpoint Gene Function in Yeast: Order of Gene Function and the Many Roles of MEC1	$48772 49856 49872
Whitten	University of Arizona	The Role of the Arylhydrocarbon Hydroxylase Enzyme in a Chronic Sidestream Cigarette Smoke Model	$45276 47913 49499
Whitten	University of Arizona	Effects of Cessation and Vitamin E Supplementation on Lung Function After Long Term Exposure to Sidestream Cigarette Smoke	$134453 130257 135433
Wijeweera	University of Arizona	Hepatotoxicity of Smokeless Tobacco Extract	$49766 49900 49888
Williams	Arizona State University	Development of a Protein-Based Test for Early Diagnosis of Tobacco Related Cancers	$50000 50000 50000
Winzerling	University of Arizona	The Effects of Chemicals Found in Cigarette Smoke on Iron Metabolism of Lung Cancer	$50000 50000 50000
Wright	University of Arizona	Effect of Nicotine on Renal Organic Cation Transport	$49419 47629 49424

SECTION E

NEW CONTRACT AWARDS

TOBACCO RELATED RESEARCH

BEGINNING IN FY 1997

Akporiaye, Ph.D., Emmanuel

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

Active-Specific Immunotherapy of Breast Cancer by Inhibition of An Immunosuppressive Cytokine

Breast cancer is the leading cause of death in women between the ages of 35 and 55. It is estimated by The American Cancer Society that there will be 21,200 new cases of cancer in 1996 in Arizona. 1200 of these cases will be breast cancer of which 50% of afflicted individuals will die from the disease. Recent studies have demonstrated an association between smoking and fatal breast cancer, as well as increased disease recurrence in survivors implicating smoking as a high risk factor. Another impediment to survival of breast cancer patients is the limited efficacy of adjuvant therapy due to drug or radiation-induced toxicity, development of resistant cancer cells and generalized immunosuppression. This limitation has fueled efforts to develop therapeutic approaches that stimulate the body's immune system to specifically recognize and destroy cancer cells while sparing normal cells. A major obstacle to the success of this approach is the reduced immune responsiveness of the cancer patient with progressing disease. One substance that has been prominently implicated in this immunosuppression is transforming growth factor-beta (TGF-β). At the high concentrations produced by breast cancer cells, TGF-β is a potent inactivator of the immune response. Recent studies is breast cancer patients have shown a direct correlation between tumor-derived TGF-β production and disease spread (metastases), recurrence and mortality. Collectively, these findings have led to the widely held notion that TGF-β production by tumor cells is an effective strategy of sabotaging the immune system to promote tumor growth and spread. Given this deleterious role of tumor-derived TGF-β, its elimination should constitute an effective strategy for restoring antitumor immune functions capable of eradicating primary and metastatic disease.

The goal of this study is to genetically inhibit TGF-β production by mammary tumor cells and to evaluate the efficacy of gene-modified tumor cell vaccines in treating established primary tumors and metastatic disease. TGF-β production will be interrupted by inserting an antisense copy of the TGF-β gene into TGF-β-producing mammary tumor cells. Once the antisense gene is converted to messenger RNA (mRNA), it will bind to the complementary endogenous TGF-β mRNA and prevent its conversion to protein. By so doing, the immunosuppressive effect of tumor-derived TGF-β is mitigated or eliminated. The hypotheses to be tested are: 1) inhibition of TGF-β production by mammary tumor cells eliminates tumor-mediated immunosuppression and restores effective antitumor responses and 2) introduction of genes that encode immune-enhancing substances such as IFN-γ and B7.1 improves the effectiveness of antisense TGF-β-expressing vaccines in eradicating established primary tumor and residual metastatic disease.

The specific aims of the study are to determine the following: 1a) the efficacy of antisense TGF-β tumor cell vaccines in eliminating primary tumors and residual metastatic disease, 1b) the ability of IFN-γ and B7.1 genes transfer to improve the efficacy of antisense TGF-β tumor cell vaccines and 2) the mechanism by which antisense TGF-β expression inhibits tumor formation. This study will demonstrate the effectiveness of antisense TGF-β tumor vaccines in treating established tumors and eradicating metastatic disease and the ability of IFN-γ and B7.1 to potentiate antitumor activity. The fulfillment of these objectives will provide a novel and effective approach to treat metastatic breast cancer. In the long term, this approach of activating the immune system by genetically inhibiting an immunosuppressive cytokine is expected to find widespread use in treating other forms of cancer.

Appleton, M.D., Christopher P.

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

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

Heart disease is the number one cause of death in the state of Arizona. Congestive heart failure (feeling of breathlessness) is the major symptom in these patients which detracts from their quality of life. This shortness of breath occurs when excessive blood pressure in the lungs is needed to fill an abnormally stiff heart before each ejection. Since shortness of breath can result from a problem with either the heart or the lungs, implicating heart dysfunction has previously required advancing plastic tubes into the heart from peripheral blood vessels (cardiac catheterization) and directly measuring the pressure. The problem is that this procedure is uncomfortable, and has a risk of bleeding, infection, stroke or heart attack. Therefore, less "invasive" procedures which give the same information are highly desirable.

We believe that Doppler echocardiography (a technique using sound waves to measure the speed and direction of blood in the heart) may be a safe, widely available method to estimate blood pressure in the heart and lungs. In recently completed patient studies, we have shown that blood flow from the lung veins to the heart is altered in patients with elevated filling pressures. However, the reason why these changes occur is undetermined, and the reliability of these new methods in normal and abnormal conditions is unknown.

The purpose of this experimental study in dogs is to define the factors which alter venous flow in the lungs when heart pressures become elevated. We hypothesize that as the main pumping chamber becomes stiff and pressures increase, the way the atrial chamber fills becomes abnormal, and the atrial "booster" pump that helps fill the main chamber pumps an abnormally large amount of blood backwards into the lungs instead of forward. Testing this hypothesis requires analyzing blood flow from the lung veins using Doppler methods together with measuring the pressures form both the lung veins and the heart. Since measuring lung vein pressures is not possible in humans except at surgery, we propose studying lung vein pressure-flow relationships at different pressures in normal dogs, and in dogs with thickened heart walls or heart failure. The results of this work should improve the non-invasive assessment of heart and lung pressures using Doppler techniques. The hope is that these ultrasound methods will replace the need for direct measurement of heart pressures (cardiac catheterization) and thus reduce patient morbidity and mortality.

Brucks, Ph.D., Merrie

University of Arizona

Award Amount FY 1998: $50,000

The Cumulative Impact of Tobacco Advertising on Young Children: Socialization of Pro-Tobacco Attitudes and Behaviors

Over half of 3 to 6-year-old children in the U.S. recognize the logo for Camel cigarettes and associate it with smoking. By age six, "Smooth Character Joe Camel" is as recognizable to children as the Disney Channel's logo "Ronald McDonald." Thus, we know from previous research that children notice and remember at least some advertising for tobacco products. What we don't know is what effect this familiarity with tobacco advertising has on young children's attitudes towards tobacco use or their likelihood of trying cigarettes later during adolescence. If pro-tobacco advertising influences children to have positive attitudes towards smoking, then the state of Arizona should be concerned. Outdoor advertising is regulated at the state and city level, not the federal level. The research described in this proposal will help state lawmakers decide whether and how to regulate the placement and content of billboards and other forms of outdoor advertising that are likely to be seen by young children and influence their tobacco related attitudes and behaviors.

We propose three related studies, to be completed over a three-year-period, to address the following critical questions: *Which children understand what from tobacco advertising, and when do they understand it? Furthermore, how do these understandings contribute to the onset of tobacco use in later adolescence?* The first study explores 1st, 3rd, and 5th graders' perceptions of advertising for tobacco products and their beliefs about social and psychological benefits of smoking (such as, being "cool"). We propose a new method for getting children to communicate their perceptions and beliefs. This method will provide more valid information than previously used research methods. In drawing conclusions about children's perceptions of tobacco use, we take into account the children's age, sex, ethnicity, and whether anyone in their household smokes. The second study examines changes in perceptions and behaviors as fifth graders sampled in Year 1 make the transition into middle school. In the third study, we return to the entire Year 1 sample to learn how the children's perceptions have changed. Most importantly, we analyze the relationship between perceptions in Year 1 and any tobacco usage behavior that has occurred by Year 3. From this information, we will be able to conclude which kinds of images in advertising lead to positive perceptions of smoking and later smoking initiation. From these conclusions, we will be able to offer guidance to public policy-makers interested in tobacco use prevention among youth populations in Arizona.

Burgoon, Ph.D., Michael

University of Arizona

Amount Awarded FY 1998: $89,961

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

Children between the ages of 9-15 are a high risk group for tobacco use. Results from a recent California study indicate this population appears to be the only group taking up smoking in significant numbers – with 1/3 to 1/2 of adolescents susceptible to smoking. There are more that 390,000 children in Arizona between the ages of 9-16. Based on the California data, approximately 75, 000 students in Arizona from ages 11-14 may have used tobacco and more than 140,000 Arizona adolescents are susceptible to smoking. Moreover, research indicating that awareness and liking of cigarette advertisements is higher among adolescents than adults underscores the need to devote more effort to understanding reactions to tobacco related messages. Adding to this problem is the fact that the early gains of successful anti-tobacco interventions disappear as adolescents age – with 20-30% of adolescents initiating smoking behaviors despite earlier held anti-smoking beliefs, attitudes and intentions.

Broadly stated, the project aims to examine the relative effectiveness of pro- and anti-smoking messages targeted at youth; examine message factors affecting both initiation and promotion of smoking; test reactions to various message strategies; and to test a theoretical model explaining the effectiveness of current anti-smoking campaigns in primary grades and their relative ineffectiveness in adolescence.

Drawing on the theoretical model, we propose to test a number of hypotheses that address the impact of a variety of factors on outcomes that include participants intended behaviors, evaluation of message sources, and seeking disconfirming information. Informally stated, the hypotheses are as follows: 1) negative evaluation of message sources, intentions to perform prohibited behaviors, and the seeking of disconfirming information will peak during middle school years; 2) messages focusing on freedom of choice will elicit less of the aforementioned negative outcomes when compared to messages restricting choice; 3) existing anti-smoking messages will result in more of the above-mentioned negative behaviors than new messages emphasizing freedom of choice, while existing pro-smoking messages will result in less of the above-mentioned negative outcomes when compared to new messages restricting choice; 4) both existing anti- and pro-smoking messages will result in more of the above-mentioned negative outcomes than new messages.

Cesarotti, Ph.D., R.N., Evelyn

Arizona State University
Award Amount FY 1998: $60,068

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

Since 1979, deaths from asthma have increased, and the states with the highest death rates are Hawaii, Arizona and New Mexico (Sly, 1989). Maricopa county's mortality rate due to asthma has been cited as one of the highest in the nation (Weiss & Wagner, 1990). In a recent 1995 statewide needs assessment conducted by the Arizona Department of Health, Office of Children with Special Needs, 26.4% of 5,155 families interviewed reported that the most frequent health care concern that lasts more than a year with their children was asthma.

The Environmental Protection Agency estimates that between 200,000 and 1,000,000 asthmatic children have their condition made worse by exposure to second hand smoke. Passive smoking may also cause thousands of nonasthmatic children to develop the condition (EPA, 1993). Asthma accounts for more than 20 million lost school days per year, and can also have the potential of adversely affecting school performance (Kay et. al., 1995; Swanson &Thompson, 1995; and Richards, 1986). Among families with incomes of less than $20,000, school children with asthma had twice the odds of grade failure compared to well children (Fowler et. al., 1992).

Research has shown that several health care interventions have had positive results in modifying second hand smoke exposure in asthmatic children. First, parents are more likely to change their smoking behavior when it is related to their child's health (Moe, et. al., 1992). Second, education regarding the effects of second hand smoke on children with asthma can be enhanced when the information is provided by health providers to parents while treating the child (Substance Abuse, 1994; Young, 1998). And thirdly, children with asthma who receive health education classes have fewer absences and better school performance than those who do not receive the classes (Celano & Geller, 1993).

The goal of the research is to develop, implement and evaluate a program of interventions intended to reduce second hand smoke exposure in children with asthma through school based health clinics and parent-child asthma education programs.

The specific aims of the research proposal are: 1) Describe the difference in smoking behaviors between parents of asthmatic children who participate in structured counseling sessions related to recognition of the relationship of second had smoke exposure to symptoms of asthma in school-based health clinics and those who receive traditional school health care. 2) Describe the difference in parent outcomes (early recognition of symptoms of asthma, increased knowledge about asthma management and change in parent smoking behaviors) between parents of asthmatic children who participate with their children in an asthma education program in school-based health clinics and those parents and children who receive traditional school health care. 3) Describe the difference in child outcomes (second hand smoke exposure, knowledge related to asthma, self-care management, absentee rate, and school performance) between asthmatic children who participate in an asthma education program in school-based health clinics and those who receive traditional school health care.

The hypotheses to be tested are: 1) Parents who receive counseling regarding the relationship of second

hand smoke to asthma symptoms will report more change in smoking behaviors than parents who do not receive counseling. 2) Parents who participate in the asthma education program will have greater knowledge regarding asthma symptoms and the relationship to second hand smoke and will modify their smoking behaviors. 3) Children who participate in the asthma education program will have decreased school absences and increased school performance.

Chong, Ph.D., Jenny

University of Arizona

Award Amount FY 1998: $49,839

Changing the Odds: Research for Prevention

Over the past few years, smokers have had to comply with smoking prohibitions in buildings and smoking only in designated areas. In most states, including Arizona, few workplaces in recent years have allowed smoking to occur indiscriminately within their walls. (Hodgets, R.S. 1990) Casinos are one of the few such building facilities. With the advent of casinos on reservations, smokers and nonsmokers alike gather within a relatively small space, and as a consequence, increase the number of passive smokers (inhaling second hand smoke). Anecdotal reports have suggested that casino employees who did not smoke prior to becoming employees have begun the habit. Other reports include individuals being unsuccessful in attempts to quit because of "the environment." To further increase the number of smokers among Native Americans and other employees through association with casinos would be against the spirit of the venture into casinos by most American Indian tribes in Arizona. Many tribes with casino compacts have designated the profits towards the betterment of their members, through utilization of casino funds for health care, housing, and so on. If casinos are indeed providing a "breeding ground" for smokers, or undermining the ability of smokers to quit, then a study is needed to determine the factors that contribute to the initiation and continuation of smoking. The Casino Board of the Desert Diamond Casino has offered their support for such a study with the goal that a program that targets those factors can be developed and implemented to moderate, if not counteract, the negative influences of smoking-related temptations in the workplace.

This study will test the hypothesis that working in a smoking environment increases smoking behavior among employees. In addition, we will determine whether individuals are affected differently depending upon their smoking history. We will compare the smoking behaviors of casino employees in four different groups: 1) those currently smoking; 2) those who smoked regularly but were not smoking at least 30 days before being employed by the casino; 3) those who tried smoking but didn't smoke regularly; and 4) those who have never smoked.

We will also explore the relationship between smoking and other factors that are either negatively or positively associated with health, including health status, perceptions of health and smoking, exercise, weight loss, and stress. Information on the knowledge and perceptions that employees have about smoking will provide the basis for a prevention program if the results of this study indicate that it is necessary.

Chow, Sherry H., Ph.D.

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

Pharmacokinetics Modeling of the Anabolites of Nucleoside Analogues

Nucleoside analogues are designed to mimic the natural nucleosides necessary for DNA synthesis. These compounds originally were synthesized and used as cancer chemotherapeutic agents but recently have been employed to develop treatments for viral infection, including HIV. The nucleoside analogues in themselves are not active; they need to be converted inside the cells by a series of enzymes to phosphorylated products in order to exert their pharmacological activities. Once the phosphorylated products are formed inside the cells, they cannot easily permeate the cell membranes and be released into the blood circulation. Conventional pharmacokinetics studies determining the drug levels in circulating blood have been unable to monitor and predict the concentrations of the active products inside target tissue. This deficiency has resulted in lack of understanding of the formation and elimination of these active products in the body and has contributed in part to failure to optimize the therapeutic use of the nucleoside analogues.

Many nucleoside analogues have been developed and used as chemotherapeutic agents in the treatment of cancers, including lung cancer. In addition, cancer remains the second leading cause of death for residents of the state of Arizona, with lung cancer being the first leading type of cancer mortality. We believe that resolving the research problem stated above should improve the use of this important class of compounds in treating neoplastic diseases which may be caused or influenced by tobacco use.

The overall objective of the proposed research is to develop a physiologically-based pharmacokinetics model (PB-PK model) of the intracellularly formed and retained active products of the nucleoside analogues. The model will be developed based on the physiological characteristics of the animals and physicochemical and biochemical information about the compound of interest. Our hypothesis is that a PB-PK model developed by linking real anatomical, physiological, and biochemical information could help predict the time course of the phosphorylated products in specific target tissues in the body. The hypothesis will be tested in mice, using a thymidine analogue, 3'-azido-3'-deoxythymidine (also known as zidovudine; AZT), as the model substrate. The specific aims of the research outlined in this proposal are the following:

Aim 1. Characterize the tissue formation and elimination kinetics of the phosphorylated AZT products in mice after single-dose administration of AZT.

Aim 2. Develop a PB-PK model of the phosphorylated AZT products based on the physiological characteristics of the animals and physicochemical and biochemical information about AZT and the phosphorylated products.

The long-term goals of our research involve evaluation of the feasibility of employing a PB-PK model to help improve the therapeutic use of nucleoside analogues and scaling up of the PB-PK model to non-human primates and then to humans.

Dembroski, Ph.D., Theodore M.

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

Biobehavioral Risk Profile of Smokers

Smokers differ in many ways, including difficulty in giving up the addiction and the degree of risk that smoking may confer for them, but most smoking cessation treatment programs offer a uniform protocol for all who seek help in cessation efforts. Problems are a) to identify different groups of smokers and attempt to design specific treatment approaches that work best for each group, and b) to determine the groups of smokers who are at especially high risk for life-threatening disease. It is important to measure and determine a) the degree to which a high risk profile is related to difficulty and success in smoking cessation attempts and b) if a special personalized treatment approach to these high risk individuals will increase their chances of successful smoking cessation. Positive findings from the proposed research will help the University of Arizona Program for Nicotine and Tobacco Research refine intervention and assessment techniques that can be communicated to other treatment programs.

The *goals* of the proposed research are a) to measure biobehavioral characteristics that together identify a group of smokers who are at high risk; b) to determine the degree to which the profile is associated with difficulty and success in smoking cessation; and c) to test the effectiveness of a new treatment approach to smoking cessation specifically designed to overcome the "it won't happen to me" belief, especially in those manifesting the high risk profile. The *objectives* are a) to demonstrate that smokers who are rated high in hostility respond with greater blood pressure (BP) and heart rate (HR) elevations while smoking a cigarette and engaging in verbal and mental activity (mild stress); b) to confirm that this high risk profile in both men and women is associated with markedly greater difficulty and less success in a organized smoking cessation program; and c) to test the hypothesis that a new intervention approach in which the individual's own BP and HR responses are used to explain how heart disease is accelerated each time he or she smokes a cigarette is more effective than when the same educational material is presented without BP and HR feedback.

Enright, M.D., Paul

University of Arizona

Award Amount FY 1998: $49,953

Spirometry for the Detection of High Risk Cigarette Smokers in Southern Arizona Primary Care Physicians' Offices

More than one of every five adult smokers develop disabling Chronic Obstructive Pulmonary Disease (COPD). Nationwide, COPD is the third most common cause for permanent Social Security Disability and the fifth most frequent cause of death. Currently, there is only one way to determine a smoker's risk of developing COPD: a simple 5 minute breathing test called spirometry. The results of the ten center Lung Health Study showed that the 5000 adult cigarette smokers who had abnormal spirometry test results were at high risk of developing COPD (an excessive loss of lung function) during the next five years, and that smoking cessation intervention, including a stern physician's message to quit smoking and the use of nicotine chewing gum, reduced the risk of COPD in these smokers. Personalizing the risk of smoking in causing emphysema and the benefits of freedom from smoking (improved lung function) for each individual was thought to contribute to the excellent biochemically confirmed long-term smoking cessation rates achieved during the study. However, the study was not designed to determine exactly which of the many smoking cessation techniques employed were the most effective. Since two-thirds of primary care physicians in the U.S. do not even own a spirometer, starting in 1997, the National Institutes of Health is funding a program called the "National Lung Health Education Program" to encourage primary care physicians to provide spirometry test for their patients who smoke.

The primary objective is to determine the incremental benefit of office spirometry in a program to encourage smoking cessation by primary care physicians in southern Arizona. The 30 physicians randomized to the special intervention group will agree to provide a spirometry test for all of their adult cigarette-smoking patients who come for an office visit during the one-year period. As in the Lung Health Study, the physician will use the patients spirometry results to briefly discuss the health benefits of smoking cessation and then refer them to the Arizona Smokers Helpline. Six months after each patient is tested, we will contact them and ask them about their smoking status. The smoking cessation rate of the 2000 patients who received spirometry tests will be compared with the cessation rate of the 2000 patients whose physicians did not use a spirometer.

Grimes, Ph.D., William J.

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

HLA & p53, An *In Vitro* Study of Peptide Immunotherapy in Human Lung Cancer

The regulatory protein p53 is known to be mutated as an early event in lung cancer, leading to progressing tumor growth and contributing to failure of radiations and chemotherapy. We propose to study how altered p53 protein could be a target for immunotherapy of cancer. Immuno-reactivity depends on presentation of antigenic peptides by cell surface HLA antigens. We will select HLA antigens which are representative of Caucasians, African Americans, Hispanics, and Native Americans. We will use techniques developed in our laboratory to determine if we can increase the potency of peptides derived from altered p53 as antigens. We will also study the ability of *in vitro* methods to assess patient immuno-reactivity to p53 protein. Our goal is to develop new immunotherapies for lung cancer, a disease where 85% of patients die within 5 years. These are:

A. To use peptide libraries to screen for binding to HLA alleles.

B. To use peptides from mutant and wild-type p53 proteins to test for the *in vitro* stimulation of cytotoxic lymphocyte production, and determine whether increasing affinities by altering amino acids in peptides increases the immune reactivity. These studies use control and then lung cancer patients as sources of immune cells.

C. To use *in vitro* binding of T-cells to peptide-MHC complexed beads as an assay for *in vivo* CTL responses to p53 protein in lung cancer.

Hatch, M.D., Kenneth 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

Over one million cases of cervical dysplasia, the precursor lesion to cervical cancer, are diagnosed each year in the U.S. Research has definitively shown that infection with the human Papillomavirus (HRV) is the cause of most cases of cervical cancer. Although, a woman's risk for cervical cancer is 10 to 20-fold higher if she has HPV infection, HPV infection alone is insufficient to cause cervical cancer. Only 28% of women infected with HPV develop clinically significant cervical lesions. With the general agreement that HPV is the sexually transmitted infection related to cervical cancer and its precursor lesions, and the recently published data indicating that persistent HPV infection is significantly related to cervical disease persistence and progression, attention is now focused on factors which are associated with acquisition of HPV, and the factors which modify the risk for maintaining a persistent HPV infection. Co-infection with other sexually transmitted diseases, such as chlamydia, may be related to HPV cervical carcinogenesis, and is currently under investigation. A major risk factor for cervical cancer is smoking with an increase risk of cervical dysplasia of 200-400% reported. However, no studies have investigated the interaction of chlamydia, HPV, and cervical dysplasia in smokers.

We hypothesize that smoking increases a woman's risk of cervical dysplasia by facilitating HPV and Chlamydia infection and this effect will be greatest among populations of women (Hispanic) where the prevalence of HPV is higher. We propose to conduct an epidemiological study to:

1) determine differences in smoking behavior in women across the U.S. and Mexican border
2) determine prevalence of HPV, chlamydia and cervical dysplasia, and the established risk factors
3) determine if the amount of cigarettes smoked and the number of years smoked are independent risk factors for HPV, chlamydia infection and cervical dysplasia.

In the United States, the cervical cancer incidence rate is higher among Hispanics (14.8/100,000) compared with white, non-Hispanic women (8.4/100,000). U.S. Hispanic women are also at a higher risk for the precursor lesion, squamous intraepithelial lesion of the cervix (SIL). We believe that the results of our research will provide needed information about smoking habits in Hispanics which is a modifiable non-infectious factor that may be associated with chlamydia and HPV infection. It will give us information on the prevalence of chlamydia, HPV, and cervical dysplasia among smoking and non smoking women. This information will form the basis of educational outreach programs to decrease smoking and increase access to screening programs among women living along the U.S.-Mexico border, thus decreasing the number of women at risk of cervical dysplasia/cancer.

Heimark, Ph.D., Ronald L.

University of Arizona

Award Amount FY 1998: $132,713

Mechanisms of Vascular Dysfunction in Atherogenesis: Cell-Cell Interactions

Cardiovascular disease remains the chief cause of death in the United States and Western Europe, and atherosclerosis, the principal cause of heart attack and stroke, accounts for the majority of these deaths. Multiple risk factors contribute to atherosclerosis in the non-smoking population. Smoking causes a two- to four-fold increase in risk of cardiovascular disease and is synergistic with the other major risk factors - hypertension (altered hemodynamics), hyperlipidemia, and diabetes. The atherosclerotic disease process involves both cell types common to the wall of blood vessels, endothelial cells (ECS), which line the vessel and form a barrier between the blood and tissues, and smooth muscle cells (SMCs), which control blood pressure. Despite our increased understanding of the basic lesions of atherosclerosis, and the cellular events that lead to lesion development, the precise mechanisms by which risk factors contribute to atherosclerosis is unclear. It is widely believed that the risk factors initiate atherogenesis by injuring endothelial and smooth muscle cells. Manifestations of endothelial injury or dysfunction include increased permeability of blood components and cells, and accumulations of growth factors, lipoprotein and blood cells in the vessel wall. The lipoproteins and factors released by the blood cells stimulate the characteristic proliferation of smooth muscle cells that ultimately form atherosclerotic plaques. The proteins that hold cells together and allow them to communicate with each other, the junctional proteins, play a central role in determining endothelial permeability and reactivity, and in maintaining SMCs in a non-proliferative state. In this proposal, we determine how hemodynamic factors, inflammatory mediators, and hyperlipidemia alter the normal function of these junctional proteins and, thereby, contribute to atherogenesis. To therapeutically intervene in the formation of an atherosclerotic plaque, we need to better understand the mechanisms that contribute to the process of endothelial dysfunction and smooth muscle cell proliferation.

The major risk factors for atherosclerosis, cigarette smoking, elevated blood LDL levels, and hemodynamic forces contribute to initiating endothelial injury. Injury to the endothelial lining of vessels promotes sticking of inflammatory cells and subsequent migration of these cells into the intima, and release of soluble inflammatory cytokines. The injury itself and the presence of inflammatory mediators cause further endothelial dysfunction, notably increased permeability and release of growth factors. The macrophage and SMCs also secrete growth factors which, along with the (oxidized)LDL, stimulate SMCs to proliferate and secrete excess extracellular matrix materials. Effective therapeutic intervention would block this cascade of events at one or more points - ideally at early points in the cascade. A critical lack in our understanding of the pathogenesis of atherosclerosis is how risk factors regulate Endothelial Cell: Endothelial Cell and Smooth Muscle Cell: Smooth Muscle Cell interactions. Our central hypothesis is that expression and function of intercellular junctional proteins are altered by the early events of atherosclerosis and consequently contribute to atherogenesis. Intercellular communication in the vessel wall plays a central role in endothelial permeability, growth control and regulation of vascular tone. The experiments proposed in aims I, II, and III are designed to examine risk factors in atherosclerosis, shear stress, inflammation and oxidized LDL and their regulation of cell-cell interaction. Products from cigarette smoking are synergistic with these risk factors increasing the chance of cardiovascular disease four-fold. Specific Aim I examines the role of hemodynamic shear stress in the alignment of endothelial cells with flow and the maintenance of intercellular junction integrity. Specific Aim II evaluates the

mechanisms underlying endothelial and smooth muscle cell response to the inflammatory cytokines. Specific Aim III assesses the mechanisms underlying oxidized-LDL's effects on endothelial and smooth muscle cells.

Hoyer, Ph.D., Patricia B. University of Arizona

Award Amount FY 1998: $49,990

Mechanisms of Ovarian Follicular Cell Death Initiated by Polycyclic Aromatic Hydrocarbons

Loss of ovarian follicles can cause ovarian failure (menopause in women). Menopause is associated with a variety of female health problems, such as ovarian cancer. Early onset of menopause has been associated with osteoporosis and depression. Therefore, there may be increased health risks in women who experience early menopause. Age at menopause is significantly accelerated in women who smoke cigarettes (or live with smokers). Because polycyclic aromatic hydrocarbons (PAH, contaminants in cigarette smoke) are known to destroy small pre-antral follicles in rats and mice, this provides a logical link with the early menopause seen in women smokers. In our studies, daily dosing of female rats with the occupational chemical 4-vinylcyclohexene diepoxide (VCD) destroyed small follicles via physiological cell death (apoptosis). This poses the question, is this type of follicle damage also initiated by ovotoxic PAHs? We will investigate this question in the proposed studies. Apoptosis occurs without producing detectable signs in surrounding tissue. Therefore, this 'silent' mechanism to toxicity could occur progressively in exposed females and result in extensive ovarian damage that has gone unnoticed until reproductive function is prematurely destroyed (early menopause). Further, the evidence in animal studies for congenital ovarian damage from these chemicals suggests this might also occur in daughters of mothers who smoke during pregnancy. Because of the large population of elderly women in Arizona, there is an increase concern about health problems associated with menopause. Thus, it is important to evaluate and predict the impact of life-long smoking on this population of females and their daughters.

The *hypothesis* to be tested is: Low dose exposure to ovotoxic polycyclic aromatic hydrocarbons (PAH) can be shown to induce follicular cell death in rats and mice by a common mechanism, apoptosis, and this requires repeated dosing. The studies proposed here will determine whether a) a low dose for each PAH can be identified that specifically produces ovotoxicity in rats and mice, b) these ovotoxic chemicals can uniformly demonstrate follicle loss via apoptosis, and c) repeated dosing is required for this effect. These studies will extend our combined *in vivo* and *in vitro* model of ovotoxicity, and will utilize an integrated morphological, biochemical, and molecular approach. The study will specifically test the PAHs, benso[a] pyrene (BaP), 7, 12-dimethyl-[a]benzanthracene (DMBA), and 3-methylchloranthrene (3-MC). The specific *aims* are to determine for each chemical 1) a dose that produces selective ovotoxicity in rats and mice, 2) the day of daily dosing that demonstrates the earliest evidence of impending follicular destruction, and 3) the mechanism of cell death involved in follicle destruction. From these results, a distinct comparison of the relative potency (risk factor; ovotoxic index) of each chemical will be compared with that which we have demonstrated for the occupational chemical, 4-vinylcyclohexene diepoxide, VCD. This will enable us to predict the potential impact of chronic exposure to combinations of these chemicals in cigarette smoke and in the environment.

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

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 basic pathophysiology and mechanism of one of the most common syndromes associated with lung cancer, that is, a condition of high serum calcium level (called "hypercalcemia") that is due to excess production of hormone made by the lung cancer cells. This hormone, called parathyroid hormone-related peptide, similar to the endogenous parathyroid hormone, has major effects on calcium mobilization from bone, calcium excretion and reabsorption in kidney. It was estimated that this debilitating "paraneoplastic" syndrome may occur in up to 20% of patients with lung cancer, especially the squamous cell type, a most common type of lung cancer that is associated with cigarette smoking. This syndrome is one of the worst conditions for patients with lung cancer. It causes marked dehydration, renal failure, conscious disturbance which frequently lead to coma and death. Unfortunately, therapies for hypercalcemia are troublesome and not always effective. This project is to try to understand the way kidney handles calcium excretion, especially the mechanisms that are regulated by parathyroid hormone and parathyroid hormone related peptide. The results from this research may have significant impact on the therapy of hypercalcemia and may lead to the development of new drugs that can be used to treat hypercalcemia more effectively. This research will:

a) Find out the proteins that are involved in parathyroid hormone- and vitamin D-regulated calcium transport in kidney. *Hypothesis:* Parathyroid hormone can activate the initial calcium entry pathway (from urinary side back to blood). Vitamin D can markedly enhance this activation, possibly through generations of proteins that may interact with the entry pathway. We will use one of these proteins (calbindin D-28k) to "fish out" the proteins mediating the calcium entry using modern molecular biology and biochemical methods.

b)Study the hormonal effects on these protein to protein interactions, focusing on the effect of parathyroid hormone. *Hypothesis:* Parathyroid hormone binds to its receptor on the cell membrane and activates the calcium reabsorption by modifying these intracellular protein to protein interactions.

c) Find out the cellular and subcellular locations of these proteins that control the calcium transport in kidney using molecular imaging techniques. *Hypothesis:* These calcium entry pathway proteins are expected to locate near or on the kidney cell membrane of the urinary side. These proteins are expected to be present in the kidney cells that also respond to parathyroid hormone and vitamin D.

d) Study the functions of these calcium entry pathway proteins using electrophysiological methods. *Hypothesis*: These calcium entry pathway proteins are expected to have properties of calcium channels that can be regulated by parathyroid hormone and vitamin D.

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

University of Arizona

Award Amount FY 1998: $47,954

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

This proposal will look at the effects of cigarette smoke (CS), and all too often environmental contaminant, and Trichloroethylene (TCE), a contaminant of ground water found in the state of Arizona and worldwide.

It has been documented through research that smoking affects the permeability of the lung tissues, thus making them more susceptible to other inhaled chemicals. It is felt that inhaled TCE will then more readily cross the lung barriers and enter the maternal system. TCE has been shown to cause an increase in the number of heart defects in fetal rat pups when the mothers drank TCE-contaminated water during pregnancy. CS is well documented as to its effect on the development of the fetus.

Secondary smoke exposure has been well documented, and its effects are extremely important. TCE, a common industrial contaminant, has been found in contaminated ground water and also in chlorinated municipal waters (as a result of chlorination in the presence of the organic mater), and is, thus, a world wide environmental problem. As marked by the Superfund Hazardous Waste site located in South Tucson, from the dumping of waste material into the ground and subsequent contamination of drinking water wells, TCE is a specific problem for the state of Arizona.

By demonstrating the increased number of heart defects that result from TCE exposure (drinking water and inhalation) when combined with CS exposure [secondary exposure to cigarette smoke through exposure to side stream cigarette (SSCS)] we will show that the acceptable level of TCE exposure needs to be even less than anticipated with solitary exposure to TCE, which is unlikely, except in the research setting.

The hypothesis of the proposed research is that the combination of Trichloroethylene (TCE) and cigarette smoke (CS) will greatly increase the number heart defects in fetuses born to mothers that are exposed to both in a period just prior to and during pregnancy, as compared to the normal number of expected congenital heart defects (those that are considered spontaneous) in a similar population. The objective is to test combinations to TCE (drinking water and inhaled) and CS [side stream cigarette smoke (SSCS)] exposure in the pregnant rat model and determine the effects on the developing heart through a combination of various routes, which include: 1) SSCS inhalation and TCE inhalation; 2) SSCS inhalation and TCE in drinking water; 3) CS inhalation, TCE inhalation, and TCE in drinking water. A series of exposure levels to TCE will be utilized to determine if a dose effect occurs.

The goal is to determine, evaluate, and confirm the detrimental effects on the developing fetal rat heart as a result of combined exposure to the common environmental contaminants TCE and CS during pregnancy.

Kay, M.D., Marguerite M. B.

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

Tobacco and Free Radical Damage to Brain Anion Transporters During Aging and Neurological Disease: Tandem MS-MS Spectrometry and Cellular Studies

This proposal will determine the contribution of free radicals generated by cigarette smoke to neurological disease during aging. A marker protein band 3, a major anion transporter, will be used to monitor damage to the brain and the immune system. Our studies on aging and neurological disease suggest that band 3 is a pivotal protein. Our previous studies indicate that changes occur in peripheral blood cells and the immune system that parallel changes in brain band 3.

Antioxidants may play an important role in preventing free radical damage associated with aging by interfering directly in the generations of radicals and/or by scavenging them. In another study, we investigated the effects of a high vitamin E and/or 6-carotene diet on aging of the anion transporter, band 3, in lymphocytes and brain. Results showed that vitamin E protected from free radical damage to both the central nervous and immune systems. Previous studies in other laboratories have indicated decreased blood levels of antioxidants in smokers and in Alzheimer's disease, but the two have not been studied together in the same study. In this proposed study, we will monitor antioxidant and band 3 status in non-smokers both control and with Alzheimer's disease, and new onset stroke. In addition, we will use the most advanced physical-chemical technique currently available ("time-of-flight tandem ms-ms spectrometry and fast atom bombardment (FAB) spectrometry") to compare effects of tobacco on brain band 3. This will provide concrete evidence of the damage that tobacco does to living tissue and irreversible changes induced by tobacco that lead to vegetative states and death.

In this study, we propose to investigate changes in band 3 protein, anion transporters, in blood cells and brain to determine whether smoking causes accelerated aging of the brain and/or accelerates the specific changes observed in age associated dementias such as Alzheimer's disease (AD). Results will be compared for age-matched non-smokers and smokers. We hypothesize that smoking will accelerate aging changes in band 3 transporters, and that damage caused by tobacco smoke will cause changes observed in age-associated dementias such as AD prematurely. This hypothesis is based on the observations that free radical damage accelerates aging of band 3 transporters; band 3 transporters exhibit definite damage changes during aging and in AD; and free radicals and oxidation are involved in the cause of AD. We are focusing on the protein band 3 which crosses and recrosses the cell membrane many times and, is therefore, particularly vulnerable to free radical damage. Band 3 is a "canary" protein that detects and reflects alterations in the bodies' environment early, and is a biomarker for brain aging and disease.

Band 3, the anion transport protein, is the most heavily used ion transport system in the body. It is responsible for respiration\breathing by all cells and tissues. It maintains water balances. Our ongoing studies indicate that anion transporter activities in lymphocytes and brain declines with age, and that this decline is caused, at least in part, by free radical damage. Smoking creates free radicals which enter all tissues by circulating with the blood. Band 3 anion transporter is extremely sensitive to free radical damage and is one of the first proteins to be injured. With aging, the brain and the immune system are most severely affected. Prevention of damage to these two systems would prolong productive lifespan and, potentially, keep people from being confined to nursing homes. In this study, we will examine the effect of smoking on the immune system and the brain in order to develop strategies to block free radical damage.

Kling, M.D., Pamela J.

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

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

Pregnant smokers give birth to infants with increased prematurity and a greater risk of mortality. Yearly, 8500 (12.3% of) pregnant women giving birth in Arizona smoke, with low birth weight almost 3 times more common in women who smoke. Premature low birthweight infants experience more complications than term infants. Yearly, 825 such infants are born in Arizona (30,000 infants in the U.S.). Premature infants have decreased antioxidant status and experience increased red blood cell turnover, one of the manifestations of the anemia of prematurity. Premature infants with anemia must be exposed to oxidants, such as transfusions and supplemental oxygen. The body normally balances the toxic and beneficial effects of oxygen-induced free radicals, but this balance is impaired in premature infants. Many of the complications of prematurity relate to an imbalance of oxidants and free radical damage.

Mothers who smoke also commonly feed infants human milk. Human milk composition is altered with smoking, and its intrinsic antioxidant capacity may also be impaired. Mothers who smoke and breast feed are encouraged to stop smoking, but because human milk is so beneficial to infant nutrition, smoking is not prohibitive to breast feeding. Previous studies which examined the role of mothers smoking and breast feeding utilized infants also exposed to passive inhalation of smoke. Therefore, we sought to analyze, based on mother's smoking status, the isolated effects of human milk feeding of premature infants in a non-smoking hospital environment.

It is known that all of smoking mothers have more complicated pre- and postnatal health status and that infants fed human milk receive nutrition and are better protected from infections in the newborn period. However, it is not know whether the beneficial effects of supplying human milk to premature infants is reversed when the mother smokes. The hypothesis to be tested is that human milk feeding is unable to compensate for and may be detrimental to the antioxidant and metabolic status in infants born to women who smoke.

To test the hypothesis, we will address three specific aims:
Specific Aim I will retrospectively compare, based on mother's smoking status, duration of jaundice (red blood cell turnover), degree of anemia and number of transfusions in premature infants fed human milk *vs.* formula. Specific Aim II will compare at hospital discharge, based on mother's smoking status., blood oxidants, anemia and number of transfusions in premature infants fed human milk *vs.* formula. Specific Aim III will compare, based on maternal smoking status, milk-bone levels of antioxidants, growth factors and proteins that supply iron.

Lai, Ph.D., Li-Wen

University of Arizona

Award Amount FY 1998: $50,000

Enhancement of Non-Viral Gene Transfer in the Lung

Smoking is one of the major environmental hazards, causing a variety of pulmonary diseases including emphysema, chronic bronchitis, asthma, and lung cancers. It is not only hazardous to smokers, but people exposed to secondary smoke are also at risk of developing these pulmonary diseases. Arizona, one of the western states, has a high smoking rate, as well as a high incidence of the aforementioned pulmonary diseases. Genetic studies indicate that in addition to environmental factors, there are genetic factors predisposing people to the development of pulmonary diseases. Many of these genes have been identified, for example, the cystic fibrosis gene. Therefore, gene therapy targeted to pulmonary tissues is a promising approach for treating or preventing pulmonary diseases. Unfortunately, up to now, most of clinical trials of gene therapy in the lung have failed because expression of the gene is transient. Therefore, enhancing gene delivery and prolonging gene expression are the major challenges in this area of research.

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, the investigator will use two new strategies: 1) using polycationic peptides, that is, peptides carrying multiple positive charges. These peptides may condense DNA (which carry negative charge) through electrical charge interaction, thus enhancing gene delivery. These peptides may also be capable of directing genes to the nucleus of the cell; 2) using fusogenic peptides, that is, peptides capable of disrupting endosomes, which are small intracellular vesicles that engulf genes once they enter cells and destroy them. By disrupting endosomes, genes can be released to enter the nucleus of cell to perform their function. The investigator will first test the modified gene delivery systems in cultured lung epithelial cells. The systems found to be effective *in vitro* will then be tested in normal mice via intravenous injection using a reporter gene which can produce β-galactosidase, an enzyme which can be detected chemically. Lastly, these gene delivery systems will be tested on a strain of mice which retain CO_2 due to a genetic defect of an enzyme named carbonic anhydrase II. The carbonic anhydrase II gene will be given to these mice and effectiveness of the treatment will be measured by reduction of blood CO_2 content and duration of gene expression. The side effects due to gene therapy will be systemically monitored. The information obtained from this study will be valuable for future development of gene therapy for patients with pulmonary diseases.

Lake, Ph.D., Douglas F.

University of Arizona

Award Amount FY 1998: $49,941

Genetic Immunization Against Mutant P53 for Lung Cancer

For Arizona, over 2,800 people are projected to be diagnosed with lung cancer in 1996. Ninety percent of these cases are due to cigarette smoking and sixty percent of those diagnosed will die within a year. Despite state-of-the-art health care and anti-smoking educational efforts, tobacco use continues in our society with no clear indication of cessation. Clearly, new treatments and therapies for lung cancer are urgently needed. There is abundant evidence that an immune response to cancer exists. Lymphocytes (white blood cells) removed from a person with cancer and grown to large numbers have been shown to directly destroy that person's tumor cells *in vitro* (outside the body). Unfortunately, the concentration of these anti-tumor lymphocytes *in vivo* (inside the body) is low and factors secreted by the tumor inhibit lymphocyte functions.

Recently, by-products of tobacco smoke have been shown to directly affect a protein, called p53, which controls cell growth. If p53 becomes *mutated*, uncontrolled cell growth can occur resulting in tumor formation; the cause of many tumors is mutated p53. For this study, we will employ a novel genetic vaccination strategy to induce an immune response against tumor cells bearing mutated p53.

To address the need for new treatments and therapies for lung cancer, we are targeting immune recognition of mutated p53 on tumor cells. We hypothesize that cytotoxic T lymphocytes (CTL) (white blood cells which can destroy tumor cells) will be induced by vaccination with mutated p53 DNA. Genetic vaccination by particle bombardment is a new and novel technique in which microscopic gold beads coated with DNA (mutated p53 DNA, in this case) are propelled into skin cells by helium pressure. Once inside cells, the DNA can begin to produce mutated p53 protein which the immune system will recognize. The objectives of this study are to vaccinate mice with DNA encoding mutated p53 and then test whether CTLs specific for tumor cells bearing mutated p53 were generated. Then, we will define the exact specificity of the CTLs which are elicited by the DNA vaccination. Another objective is to optimize the vaccination strategy by co-administering mutant p53 with another gene which attracts specialized immune cells to the site immunization, boosting anti-tumor activity. We will then determine exactly how these specialized immune cells activate CTLs. The results of this investigation will define unknown immunological parameters of genetic vaccination and position us to develop a research program in this new area.

Lebowitz, Ph.D., Michael D.

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

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

Pulmonary diseases are the third major cause of death and one of the two leading causes of disability in Arizona and the U.S.A. Cardiovascular diseases are the leading cause of death and disability. We know that tobacco smoking is a major risk factor for pulmonary and cardiovascular diseases, and for cancer, but not all smokers develop and die of these diseases. It is still unclear what factors make individuals susceptible or resistant to the effects of tobacco in the development of emphysema, other chronic obstructive pulmonary diseases, (COPD), asthma, lung cancer, and cardiovascular diseases. These factors may be heritable (genetic/familial) and/or related to environmental exposures that affect the immune and/or physiological system. Early childhood health problems that result from heredity-environmental interactions may also influence susceptibility/resistance to tobacco smoking, and such problems and early adult exposures play major roles in the development of these diseases in adult life. Thus, a major research need is to determine what factors are associated with susceptibility and resistance. Epidemiologically, it is accepted that one requires a longitudinal population study of disease development and death to properly evaluate these factors.

This study will continue to try to determine the factors related to the development of emphysema, other COPD and asthma (together called airway obstructive diseases [AOD]), and to cardiovascular diseases and lung cancer. The primary area of interest in this study is the determination of susceptibility/resistance to tobacco smoking and intermediate health problems related to the interaction of tobacco smoking with other risk factors. There are several relevant factors which require further specific analyses to satisfy the specific aims: 1) the importance of parental smoking in the familial aspects of diseases status; 2) the importance of smoking on immunologic status, childhood respiratory histories and acute respiratory illnesses; 3) the importance of interactions between pulmonary and cardiovascular diseases); 4) the attributable influences of smoking, and its joint influence with occupational and environmental exposures.

We are fortunate to have a unique longitudinal study in Arizona, the Tucson Longitudinal Epidemiological Population Study, to address these issues. This study of over 5500 individuals in the course of its 25 years has collected a unique set of relevant data not elsewhere available.

Manseau, Ph.D., Lynn J.

University of Arizona

Award Amount FY 1998: $47,416

Analysis of DrtGEF, A Regulator of Rho-type GTPase Signaling

In many cases, tobacco-induced lung cancer appears to be caused by mutations in a protein called ras. Ras works through a second protein called rho to cause cancer. If rho is absent, then ras is unable to cause cancer. Thus, understanding what rho does and how it does it is important to understanding the process by which tobacco-induced cancer arise. Rho proteins are cellular switches that can be turned off or on to control processes within the cell, including whether a cell divides unchecked as in cancer. We have identified a new fruit fly gene that encodes a protein that regulates whether rho proteins are switched on or off. A closely related gene is also found in humans, suggesting that its analysis in fruit flies will be relevant to understanding carcinogenesis in humans. We propose to use this gene to study how rho works and to ask what other proteins in the cell work with rho. Identification of these proteins that work with rho may identify additional targets for drug therapy in lung cancers caused by mutations in ras.

As described above, we have identified a new protein that regulates whether rho, a protein required for ras to induce cancer, is switched on or off. Many smoking-induced cancers are caused by mutations in ras. Thus, we would like to understand how rho allows ras to induce lung cancer. The first two goals of this proposal are to determine what happens in the cell if the rho protein is permanently switched on and what happens in the cell if the rho protein is permanently switched off. This will help us understand what processes the rho protein is usually regulating in the cell. One possibility is that rho regulates the state of the cell skeleton, so we will ask whether the cell skeleton looks different when rho is switched on *versus* when it is switched off. In addition, we will use two different approaches to identifying what proteins work with rho in the cell. This will also help us understand how rho works, but has the additional potential of identifying proteins that might be useful in drug treatment of tobacco-induced cancer.

Marchalonis, Ph.D., John J.

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

Analysis of Autoantibodies to T-Cell Receptors in Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic, usually progressive inflammatory disorder of joints that has a prevalence of approximately 5% in the Tucson area due to the influx of individuals suffering from the disease and the high percentage of native Americans. High levels of RA-associated autoantibodies termed rheumatoid factors (RF) correlate with poor long-term prognosis. A strong correlation between levels of these autoantibodies and smoking has been documented in males. Adverse lung complications occur in ~20% of RA patients. Smoking causes a more rapid progression of RA-associated lung disease and significant increases in need for clinical intervention. We have found that approximately 70% of individuals suffering form RA have increased levels of autoantibodies (Aabs) directed against the recognition molecules on their own thymus-derived lymphocytes. Our data indicate that these are novel recognition Aabs distinct from the classical rheumatoid factors. Furthermore, these new Aabs can modulate the T-cell arm of the immune system. We initially detected these Aabs using a novel synthetic immunopeptide approach developed here and were able to generate monoclonal autoantibodies from B-cells of RA patients. Our ability to generate these molecules gives us the unique opportunity to determine the gene usage in the generation of these molecules and to study their biological function in interaction with T-cells and in interfering with the immune response. 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 distinct disease-related populations that use different immunoglobulin variable region genes and are more destructive in the pathogenesis of RA. This approach offers new possibilities for diagnosis and potential therapy for this prevalent and crippling autoimmune disease.

Our central hypothesis is that the initially occurring autoantibodies to autologous TCR that we discovered in RA patients arise from an over expression of naturally occurring immunomoulatory IgM Aabs. With the course of progressive disease, we hypothesize that later Aabs are qualitatively distinct from the initial regulatory Aabs due to selection and recruitment. We have generated monoclonal autoantibodies (mAabs) directed against defined epitopes of TCRs and propose that these can be used to analyze human T-cell populations both in health and disease. We hypothesize that idiotypic markers (Ids) will be shared among these IgM Aabs, and that such idiotypes will also be found among anti-TCR of other isotopes, e.g., IgG. We propose that gene probes characteristic for the V_H or V_L structures used by B-cells of Ra patients can be used to monitor the B-cell autoantibody repertoire in progressive autoimmunity and by comparison with healthy individuals. We hypothesize that smoking will have an effect on the generation of IgM Aabs to TCR parallel to its effect on the production of rheumatoid factors. The specific aims are: 1) Analysis of the autoreactive B-cell repertoire by the production of monoclonal B-cell lines secreting IgM or IgG mAAbs and molecular characterization of the variable regions used. 2) Characterization of the binding and functional activity of the mAabs, including analysis of binding to recombinant molecules, intact T-cells and synthetic peptide epitopes and analysis of *in vitro* effects on the modulation of T-cell function. 3) Analyses of idiotypes expressed by the mAabs will be carried out. We will prepare polyclonal and monoclonal anti-Id antibodies and determine the structural factors involved in Id expression. We will use the anti-Id Abs to analyze the numbers and distribution of Id-bearing B-cells in blood and synovial tissue and to quantitate serum and synovial immunoglobulins

expressing the appropriate specificities. 4) The autoantibody gene repertoire in RA patients at early and late stages will be assessed using probes derived from Aab-V region genes. This will be done by a combination of polymerase chain reaction and Southern Blot analysis of mRNA expressed by B-cells.

Montfort, Ph.D., William R.

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

Structural Studies of Human Thioredoxin: Target for Anticancer Drug Design

A clear link between smoking and lung cancer has been established. We are studying human thioredoxin, a protein that is synthesized at a rate higher than normal in lung and colon tumors. Addition of thioredoxin to cultured breast cancer cells results in a high rate of growth for these cells, and provides greater cell-growth stimulation than some other growth factors. Over-expression of thioredoxin in these cells causes them to be more tumorigenic in mice. Inhibition of thioredoxin in these cells, either through mutation or by the addition of the thioredoxin inhibitor IV-2, reduces and, in some cases, eliminates tumorigenicity. Thus human thioredoxin is a promising target for anticancer drug design, and compound IV-2 a promising lead for such drugs. Unfortunately, IV-2 exhibits some toxicity and improvements are needed before treatment of human tumors can proceed. In addition, the role of thioredoxin in tumor growth is not yet clear.

The long-term goal for this research is four-fold: 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. 3) To understand the catalytic mechanism of thioredoxin. And 4) to understand the role of homodimer formation in thioredoxin, a feature of the protein that we discovered. Central to all of these goals is the three dimensional structure of thioredoxin, which we have just determined through the use of X-ray crystallography, and the way in which inhibitors bind to the protein, which we propose to determine for compound IV-2 and other inhibitors. This information can then be used to design improved inhibitors (called structure-assisted drug design). The *hypothesis* on which our work is based is that X-ray crystallographic studies of wild-type, mutant, and inhibited forms of thioredoxin will: a) provide important information concerning thioredoxin function; and b) allow the design of selective inhibitors of thioredoxin as potential antiproliferative agents. The *specific aims* of this proposal are: To conduct X-ray crystallographic and solution studies of recombinant wild-type, mutant, and inhibited human thioredoxins, and to begin structure-assisted design of new thioredoxin inhibitors. To achieve our long term goals, we are collaborating with two scientists that bring additional expertise to this problem: Dr. Lynn Kirkpatrick, a medicinal chemist at the University of Regina who will synthesize compounds of interest, and Dr. Garth Powis, a pharmacologist at the University of Arizona who will conduct *in vivo* tests on mutant thioredoxins and potential drugs.

Pettit, Ph.D., George R.

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

Preclinical Development of New Anticancer Drugs Necessary to Improve Treatment of Tobacco Related Human Cancer

Human cancer constitutes some 200 related diseases that continue to cause a catastrophic number of deaths accompanied by tremendous personal and economic disasters. Of the nearly 600,00 annual deaths from cancer in the United States, over 200,000 of these are related to tobacco use, and the overall total corresponds to about 21% of all mortality. The total medical and economic loss estimates this year from cancer are expected to be over 120 billion dollars and that indicates combined medical costs and economic loss in Arizona of well over one billion dollars per year! Only 48% of cancer patients can now be treated curatively and that number will not increase until more effective and curative anticancer drugs are discovered and developed. Truly important advances in improving human cancer treatment are quite dependent upon discovery and development of new and curative anticancer drugs. The ADCRC Tobacco Tax Research Funding will be sharply focused on that objective.

The Arizona State University Cancer Research Institute (ASU-CRI) is completely committed to pursuing research directed at the discovery and development of new and effective anticancer drugs for improving human cancer treatment. Acceleration and expansion of this vigorous research program directed at discovery and development of new anticancer drugs for those types of cancer arising from tobacco use will be continued. Further development toward clinical trials of our most promising and advanced anticancer drugs, such as spongistatin 1, dolastatin 15, auristatin PE, auristatin PYE, dolastatins 16-18, combretastatin A-4 and A-1 prodrugs, cephalostatins 1 and 7 and the pancratistatin prodrug has been proposed for ADCRC contract support. In addition, we will continue to isolate and/or synthesize sufficient quantities of these exciting anticancer drugs to ensure adequate supplies for U.S. National Cancer Institute (NCI) preclinical research necessary to make clinical decisions and further development beyond our Institute resources. The ADCRC research will also include continuing the synthesis of vitally important clinical supplies of our anticancer drug discoveries for the NCI such as that now in progress for dolastatin 10.

Powis, D.Phil., Garth

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

Interdisciplinary Basic Science Program Project

Tobacco-related cancers represent one of the most common causes of death in Arizona. Cancers, such as lung cancer, cancer of the head and neck and gastrointestinal tract (such as colon cancer) have been causally related to tobacco use, and are of rising incidence and mortality. While prevention of tobacco use is an essential strategy to limit development of such cancers, there is a critical need to understand at the molecular and cellular level, the basic mechanisms leading to the development, progression and spread of such cancers. The information will be invaluable to developing novel strategies to prevent and treat tobacco-related cancers and may have applicability to other nontobacco related cancers. The Arizona Cancer Center is at the forefront of basic research into the mechanisms of cancer causation, development, prevention and treatment, and in translation of this research into the clinic. This research proposal is an interdisciplinary program of basic research into the mechanisms of the causation and development of the tobacco-related cancers, i.e., lung, oral and colon cancer.

The goal of this program is to conduct interdisciplinary basic research into the molecular and cellular mechanisms of the causes, progression and fate of human cancer that will synergize with clinical treatment and prevention programs at the Arizona Cancer Center. The 3 objectives of the program are:

1) To study redox mechanisms relating to lung cancer growth.
2) To conduct studies of gene mutations and susceptibility to smoking related colon cancer.
3) To study matrix metalloproteinases as determinants of oral cancer invasion and metastases.

Reaves, Ph.D., Scott K.

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

Regulation of Human Tumor Suppressor p53 Gene Expression By Zinc Status

The tumor suppressor gene p53 is mutated in more than half of all human tumors (Hollstein et. al., 1991; Greenblatt et. al., 1994). Damage to cellular DNA induces p53 expression causing either cessation of cell growth (cell cycle arrest), to allow DNA repair, or apoptosis (a controlled type of cell death). Loss of normal function of p53 results in the loss of cell cycle arrest after DNA damage, thereby possibly allowing the propagation of genetically damaged cells. Interestingly, p53 mutations occur at high frequencies in tobacco-related cancers (Iggo et. al., 1990; Sakai, 1992; Zhang et. al., 1994; Bartek, 1990) and smoking has been shown to increase p53 expression in some types of tumors (Curiglian et. al., 1996). Identifying other factors or nutrients that also alter normal p53 expression may help to promote environmental and/or dietary practices that mediate optimal p53 expression as a protective means against the development of cancer. Since p53 is a zinc-containing protein, and zinc status may be altered in certain forms of cancer, normal function of p53 may be compromised directly or indirectly during diminished levels of cellular zinc. Moreover, the induction of esophageal cancer by nitrosamine, a component of tobacco smoke, was found to be enhanced by zinc deficiency (Fong et. al., 1978). In view of the prevalence of zinc deficiency in certain populations (Hambidge et. al., 1985; Prasad et. al., 1993; Sandstead 1995) and the protective role of p53, these studies will determine if compromised zinc status may diminish the ability of p53 to exert its normal tumor suppressor capabilities.

The ultimate goal is to establish ways to promote the optimal p53 tumor suppressor capabilities. In order to accomplish this goal, we need to fully understand the molecular mechanisms responsible for the regulation of p53 promoter activity by cellular zinc status. The effect of zinc on p53 gene expression will be examined by: a) The determination of p53 protein and mRNA levels in human hepatic cell lines maintained in zinc-deficient and zinc-supplemented conditions; b) The use of p53 reporter gene constructs to identify specific elements within the promoter that confer zinc-responsiveness; and c) The determination of which DNA-binding factors are affected by zinc status. These objectives will be accomplished by : 1) Using RNASE protection and Western Blot analyses to detect changes in p mRNA and protein levels; 2) Transient transfections of HepG2 and SK-HEP-1 cells with various p reporter genes designed to examine the contributions of different responsive elements to the overall p53 promoter activity; 3) The use of gel shift analysis to examine the effects of cellular zinc status on the binding of nuclear factor -kB (NfkB), activator protein-1 (AP-1), and p to their responsive elements within the p promoter; 4) Characterizing factors that bind the p promoter South-Western analysis and UV-crosslinking studies. The above objectives are designed to test the following hypotheses: 1) The increase in cellular p is the result of enhanced p synthesis due to an increase in p mRNA abundance; 2) The elevation in p mRNA is a new steady state level established by enhanced promoter activity induced by zinc deficiency; 3) The increase in promoter activity results from an increased abundance of nuclear factors binding to responsive elements of the p promoter; and 4) ZN deficiency reduces tissue ZN-CU superoxide dismutase and subsequently elevates cellular reactive oxygen species (ROS) which are known to induce the activation of NfkB and AP-1. The enhanced binding of NfkB and AP-1 to the p promoter increases p promoter activity and up-regulates p gene expression.

Roeke, M.D., William R.

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

Regulation of the Human Delta Opioid Receptor

Smoking has been implicated in several forms of cancer including that of the lung. This has significant health consequences for the residents of Arizona as a 1990 survey indicated approximately 25% of the state's population smoked. Lung cancer takes a terrible toll on Arizonans being responsible for 44 deaths per 100,000 population in the period of 1986-1990. Despite advances in treatment, lung cancer is incredibly deadly with only a 13% survival rate 5 years after diagnosis. These statistics suggest that much of the care provided these patients is, by necessity, supportive and often designed to reduce the pain associated with lung cancer.

Lung cancer pain is generally controlled through a combination of nonsteroidal anti-inflammatory drugs and opioid drugs. While extremely effective, opioid drugs such as morphine, levorphanol and methadone have a variety of undesirable side effects such as breathing problems, constipation, nausea and withdrawal. Over the last 10 years a new class of opioid drugs known as δ-opioid-receptor agonists have been developed that are effective in pain relief but show fewer side effects than the opioid drugs currently in use. However, this new drug class also displays tolerance in animal models. The work outlined in this proposal will provide the mechanistic basis to develop strategies for the use of δ-opioid-receptor agonists in the treatment of lung cancer pain without the induction of tolerance or the side effects associated with current opioid therapy.

The effect of δ-opioid-receptor agonists are mediated through surface proteins on nerve cells known as δ-opioid receptors. By analogy with other receptors, tolerance is believed to be induced by the loss of receptor function from the cell surface (down-regulation) or by the loss of receptor function over a matter of minutes (desensitization) or hours (subsensitivity). We hypothesize that extended treatment of cells with δ-opioid-receptor agonists induces phosphorylation of the human δ-opioid receptor leading to receptor desensitization, down-regulation and subsensitivity. Our major goal is to identify regions of the human δ-opioid receptor and specific sites within the receptor that are involved in the induction of tolerance, as well as identify intracellular proteins that phosphorylate the receptor leading to its regulation. Our objective is to remove sections of, and change specific sites in, the δ-opioid receptor to determine which parts of the receptor are involved in regulatory mechanisms believed to cause tolerance. We will also block the activity of known intracellular proteins that phosphorylate receptors to elucidate the mechanisms of receptor phosphorylation. As desensitization, down-regulation and subsensitivity modulate tolerance to chronic drug treatment these studies will enhance our understanding of the mechanisms responsible for δ-opioid-receptor agonist-induced tolerance. As δ-opioid-receptor agonists are already known to have fewer side effects than opioids currently used to treat cancer pain and the studies outlined here will be performed with the human δ-opioid receptor, our finding should provide information that will aid in the development of pain relieving agents that act via δ-opioid receptors without inducing tolerance or troublesome side effects.

Salmon, M.D., Sydney E.

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

Developmental Treatment of Smoking-related Cancers: A Program Project

Tobacco-related cancers represent one of the most common causes of death in Arizona. Cancers, such as those of the head and neck, lung and gastrointestinal tract (e.g., of the colon and pancreas) have been causally related to tobacco use, and are of rising incidence and mortality. While prevention of tobacco use is essential strategy to limit development of related cancers, there remains a major need to develop effective therapy for patients who develop tobacco-related cancers, as current therapy is unsatisfactory. The Arizona Cancer (ACC) is at the forefront in the translation of new research from the laboratory into cancer treatment. This research proposal delineates our strategy for developing more effective therapy for some of the major tobacco-related cancers.

The goal of this project is to develop effective treatment for tobacco-related cancers via closely integrated laboratory projects and clinical treatment research at the Arizona Cancer Center. Our five objectives for the project are as follows:

1) To use in vitro (test tube) cell culture methods with human cancer cells to identify potentially useful anticancer agents.
2) To conduct studies of promising new anticancer agents against human tumors grown in mice as a necessary step towards clinical trials.
3) To develop treatments that prevent new blood vessels from forming in tumors and, thereby, prevent their growth.
4) To apply chemical methods to measure levels of new drugs in animals and patients with caner to aid in drug development.
5) To conduct clinical trials of new anticancer therapies in patients with tobacco-related cancers.

Skibo, Ph.D., Edward B.

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

Antitumor Agents Targeting Topoisomerase II

The proposed research involves the development of new inhibitors of the enzyme topoisomerase II. The division of cancer cells rely on this enzyme, which plays a role in "unpacking" the chromosomal DNA for replication. The unpacking process involves the relaxation of highly coiled DNA by double strand cleavage followed by rejoining the strands after relaxation is complete.

This rapidly dividing cancer cell is obviously very dependent on the presence of this enzyme. Indeed, the cancer drug etoposide, which is one of the drugs used in the treatment of lung and ovarian caneer, inhibits topoisomerase II. This research will involve the continued development of new topoisomerase II inhibitors discovered in this laboratory and could lead to new chemotherapeutic agents for the treatment of lung cancer. Preliminary results indicate that these agents are also effective against melanoma, which is a common cancer in Arizona.

The pyrrolobenzimidazoles represent a new class of quinone-based antitumor agents possessing anticancer activity *in vitro* and *in vivo*. There are three structural types belonging to this class of antitumor agent: the 6-aziridinyl derivatives (PBIs) which alkylate and cleave DNA upon bioreduction, the 6-acetamido derivatives (APBIs), and the corresponding imino analogues (imino-APBIs). The latter two structural types are cytotoxic as the quinones and bioreduction results in inactivation. Currently, six pyrrolo-benzimidazoles, with all three structural types represented, are undergoing *in vivo* trials at the National Cancer Institute. These compounds are two decisions from Phase I trials and there is confidence that some compounds will eventually enter these trials. This confidence is based on the fact that many of the compounds have already been screened and shown to possess antitumor activity. In the past year, enough *in vivo* results were accumulated to draw conclusions concerning pyrrolobenzimidazole antitumor activity. The APBI analogues, which were found to inhibit topoisomerase II relaxation of DNA, appear to be the most effective antitumor agents based on percent increase in life spans, activity at tumors distant from the drug injection site, and low toxicity. Over the next three years, the specific aims of the proposed research will, therefore, center around the further development of the APBIs and related antitumor agents. These specific aims will be: 1) The design of new APBIs based on the structure activity relationship recently obtained in this laboratory; 2) The design of highly electron-deficient analogues of the APBI rings system which should be effective DNA intercalating agents and toposiomerase II inhibitors; 3) The design of a new class of topoisomerase II inhibitors capable of cross-linking DNA to the enzyme. Since these specific aims have been guided by *in vivo* results, carrying out these aims will no doubt result in agents possessing *in vivo* activity.

St. John, Ph.D., Paul A.

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

Nicotine Receptors in the Spinal Cord

Nicotinic acetylcholine receptors (nAChRs) are found on the surfaces of many types of cells throughout the nervous system. nAChRs receive and translate signals carried from electrically active nerve cells by the chemical neurotransmitter, acetylcholine. Interaction between acetylcholine and nAChRs triggers electrical activity of cells on which nAChRs are expressed. Thus, acetylcholine and nAChRs 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 1 million Arizonans who regularly use tobacco products, nAChRs also are principal biological targets of nicotine from tobacco. Nicotine acts acutely, as does acetylcholine, to activate nAChRs and affect nerve cells and circuits, but effects of chronic nicotine exposure, as occurs with habitual use of tobacco products, are less well understood. nAChRs in the spinal cord have been postulated to play important roles in physiological functions ranging from sensory processing to control of movement. However, our understanding is poor about the kinds of spinal cord cells that make nAChRs. nAChRs exist as a family of similar but distinctive molecules that can have significantly different functional properties. However, it is not known which of these nAChRs subtypes are found on cells in the spinal cord. The project will address these research problems.

The first goal of the project is to determine what kind(s) of cell(s) (neurons/glia) in the developing and adult rat spinal cord express nAChR . It is hypothesized that nAChR are expressed not only by Renshaw cells, but also by spinal motoneurons and neurons involved in sensory processing, and, furthermore, that neurons nAChRs at important stages of prenatal and postnatal development. The second goal is to identify the nAChR subtypes (family members) that are made by specific kinds of rat spinal cord cells. It is hypothesized that nAChR containing $\alpha 4$ or $\alpha 7$ subunits are expressed by Renshaw or spinal motoneurons, respectively, but that these nAChr subtypes are also expressed by other spinal cells.

These studies will fill significant gaps in our knowledge about targets and effects of nicotine action in the adult and developing spinal cord. The results will provide insights into possible effects of nicotine exposure *in utero* on spinal cord cell circuits in developing fetuses or infants and their relationship to documented delays in development of the children of smoking mothers.

Ulreich, Ph.D., Judith B.

University of Arizona

Award Amount FY 1998: $102,844

Arizona Liver Transplantation Research: Optimizing Organ Replacement in Tobacco Related Liver Disease

Liver disease is caused in large part by the use of tobacco. The risk of cirrhosis of the liver and liver cancer is many times greater in smokers that in non-smokers and is exacerbated by the combined use of alcohol and tobacco. Since combined use approaches 70-80% of smokers, a large portion of the population is affected. It is an especially serious problem in Arizona where the incidence of alcoholism, thus smoking, is greater in some native populations. Liver transplantation is a viable, but limited, resource for patients with end-stage liver disease. There are, at present, 42,393 patients awaiting all forms of solid organ transplantation in the United States. Of these, over 5,000 are waiting for a liver. Unfortunately, 10-25% of patients die while waiting. Arizona is in Region 5 of the UNOS list of patients awaiting transplants. Twenty percent of the people waiting for livers are in the region, one of 11 in the US. The need for organs is highest on Region 5 among all regions in the country. There are, at present, 7,398 people awaiting organs in our region alone. We propose that by developing methods for preserving livers for longer times and by developing methods whereby organs for non-heart-beating donors, which are not generally used, can be preserved in good enough conditions to be used, we will provide the residents of Arizona with a greater supply of livers which are much needed for transplantation. We propose to accomplish this through the use of a new organ preservation solution under development at the University of Arizona, by developing a pulsatile perfusion system for use in preserving the livers for longer periods of time than possible under the static preservation method in use at present; and, most importantly, by "rescuing" livers from non-heart-beating donors. In a small pilot study, we have identified an agent, dimethyl sulfoxide, which, when given to rats prior to the non-heart-beating state, appears to maintain the viability of the tissue. The research we propose may make this method of preservation accessible to humans.

Specific Aim 1: To "rescue" non-heart-beating donor liver for use in transplantation by pretreating the animals with dimethyl sulfoxide (DMSO). *Hypothesis:* DMSO maintains the integrity/viability of the liver because of its antioxidant activity which protects against free radical injury induced by ischemia and allows nutrients/preservation solutions/gases to permeate the organ more readily because of its ability to rapidly penetrate tissue.

Specific Aim 2: To determine whether addition of DMSO to preservation solutions aids in maintaining liver viability during storage. *Hypothesis:* DMSO will improve the maintenance of viability in non-ischemic donor livers but will not improve the status of non-heart-beating donor livers not pretreated with DMSO since "the damage has been done" during the ischemic period.

Specific Aim 3: To determine the mechanisms by which DMSO exerts its beneficial effect on liver viability. *Hypothesis:* Despite its effect of raising intracellular calcium ion concentrations, DMSO, because of its antioxidant properties, prevents the damage generally associated with elevated calcium levels in the cell.

Specific Aim 4: To determine the molecular sequelae to isolated hepatic sinusoidal endothelial cells (EC) cultured *in vitro* by preservation in solutions containing DMSO. *Hypothesis:* That improved viability of EC following preservation in solutions containing DMSO will lead to reduced PMN binding *in vitro* as well as *in vivo*.

Specific Aim 5: To conduct the ultimate test of the efficacy of over preservation by transplanting

preserved rat livers into recipients. *Hypothesis*: Livers from animals pretreated with DMSO will have a greater success rate post-transplantation.

Specific Aim 6: To quantitate induction of heat shock proteins (HSP) in preserved EC, liver slices and whole preserved liver containing DMSO in the preservation solution. *Hypothesis*: HSP induction protects the cells/tissue and results in greater viability and metabolic activity post-preservation.

Specific Aim 7: To compare viability, metabolism and HSP content in precision-cut liver slices from static preservation of isolated NHBD livers on ice with tissue from continuous or pulsed perfusion on NHBD livers on ice. *Hypothesis*: 1) Perfusion of organs with preservation solutions leads to better preservation than static preservation since metabolic products/free radicals are removed, acidosis is controlled and provision is made for supplying nutrients and oxygen to the tissue. 2) Pulsed perfusion leads to better organ preservation than continuous perfusion because the higher pressure used reaches more vascular beds and there is less washing away of cellular components.

Specific Aim 8: To correlate findings in the *in vitro* systems with results of similar studies in whole isolated perfused liver (sometimes followed by orthotopic liver transplantation in the rat) in order to assess the adequacy of the *in vitro* models to predict *in vivo* outcomes of various preservation methods. *Hypothesis*: *In vitro* models accurately predict the outcome of organ preservation in transplantation.

Vaillancourt, Ph.D., Richard R.

University of Arizona

Award Amount FY 1998: $48,510

Intracellular Signal Transduction Pathways Activated By Nicotine

It is widely recognized by the scientific and lay communities that nicotine is the driving force that motivates individuals to use tobacco products. Unfortunately, repeated exposure to tobacco and tobacco smoke exposes the individual to numerous carcinogens, which leads to cancer in many cases. Thus, there is a tremendous effort to reduce and eliminate the use of tobacco products, especially among children. Due to the addictive nature of nicotine, it is much more difficult to stop using tobacco products, particularly when individuals begin using them at a young age. Nicotine replacement therapy, in the form of chewing gum containing nicotine, has become the most effective method to help individuals stop using tobacco products. Our understanding of the biochemical events that occur in the brain after the administration of nicotine is limited. One of the well characterized effects of nicotine is the release of the neurotransmitter, dopamine, which stimulates nerve cells in the "pleasure center" of the brain. Subsequently, when dopamine levels decrease in the brain, the individual seeks more of the stimulating agent, nicotine. Thus, the biochemical basis for nicotine dependence is low levels of dopamine. In addition to causing dopamine secretion, nicotine activates enzymes in nerve cells that are involved in the dopamine biosynthetic pathway. This proposal will characterize the enzyme pathways that are activated by nicotine. The rationale for studying these events is that understanding the action of nicotine in nerve cells will further elucidate the biochemical basis for nicotine dependence.

The objective of this proposal is to characterize the intracellular signaling pathways that nicotine activates, in particular, the pathway for dopamine biosynthesis, since the biochemical basis for nicotine dependence is mediated by pathways that regulate the biosynthesis of dopamine. The objectives of this proposal are to determine the *expression, function, and regulation* of the serine/threonine kinase, MEKK1, 2, 3, and 4. How these proteins are activated by nicotine will be emphasized. The reason for characterizing the activity of MEKK proteins is that these proteins regulate the 14-3-3 family of proteins, which are the key regulatory proteins for tyrosine hydroxylase, the rate-limiting enzyme in the dopamine and catecholamine biosynthetic pathway. The mesolimbic dopamine system in the brain is the site of increased neuronal activity whose stimulation results in the nicotine-seeking behavior that is associated with nicotine dependence. Thus, the overall goal of this proposal is to characterize the biochemical signaling pathways that are the physiological basis for nicotine addiction.

The specific aims of this proposal and relevant questions that will be addressed are:

1. To determine the expression of MEKK.
2. To determine the function of MEKK proteins.
3. To determine how MEKK proteins are regulated.

Wang, Ph.D., Yi Ran

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

Apo A-I is the major protein of high density lipoproteins (HDL). Plasma levels of apo A-I and HDL are inversely correlated with the risks of cardiovascular disease (CVD). Cigarette smoking is a risk factor associated with lower apo A-I and HDL levels [Haffner et. al., 1985]. The adverse effect of smoking for CVD has been speculated to be mediated by a lipoprotein mechanism. In the state of Arizona and the U.S., CVD is a major cause of premature death. Thus, new approaches are needed to increase plasma levels of apo A-I as a therapeutic strategy for the reduction of the prevalence of CVD. In order to accomplish this goal, we need to understand the tissue-specific mechanisms controlling the regulation of apo A-I gene expression in liver and intestinal cells because in humans, apo A-I gene is expressed predominantly in liver and intestine. The promoter elements that control liver expression of apo A-I gene are located at nucleotide -256 to -41, whereas the region up to -2052 is essential for expression in intestine. Recent studies have demonstrated that a common apo A-I polymorphism, with a frequency of 10-20%, is created by a point mutation---a single nucleotide substitution at -78 position of the apo A-I gene promoter region. This common mutation is associated with elevated plasma apo A-I. However, this association was found to be counteracted by smoking in two population studies. In this project, studies will be performed to establish the functional significance of this apo A-I polymorphism and to expand our understanding of the overall regulation of apo A-I promoter activity and gene expression in the liver and intestine.

The ultimate goal is to establish ways to increase plasma apo A-I levels to counter the adverse apo A-I lowering effect of risk factors (including cigarette smoking) for the reduction of CVD risks. In this project, the structure-function of the human hepatic and intestinal apo A-I gene promoter will be examined by: a) the determination of molecular mechanisms responsible for the elevated plasma apo A-I level observed in a common apo A-I gene polymorphism, with G to A substitution at -78 position of the apo A-I promoter; and b) the evaluation of the importance of a responsive element (site c) of apo A-I promoter for maximal promoter activity. These objectives will be accomplished by : 1) co-transfection of cDNAs encoding enhancer (hepatocyte nuclear factor 4 - HNF4) and repressor (apolipoprotein regulatory protein 1-ARP-1) nuclear proteins with various apo A-I reporter gene constructs, with or without the point mutation, in a human liver cell line (hep G2) and a colon carcinoma (Caco-2) cell line; 2) the use of footprinting analysis to locate the binding sites within the apo A-I promoter region for nuclear proteins and to determine possible displacement of these sites in the apo A-I gene polymorphism; and 3) the use of gel shift assays to establish the interaction of nuclear enhancer (HNF 4 and retinoic acid receptor α-RXRα) and repressor (ARP1) proteins with DNA fragments of the apo A-I promoter, with or without the mutation. 4) Nuclear factors binding to new binding site created by point mutation will be purified by DNA affinity chromatography and characterized. The above objectives are designed to test two specific hypothesis: a) the point mutation resulted from apo A-I polymorphism enhances apo A-I promoter activity by the creation of a new binding site or by the modification of the adjacent site C's responsiveness to nuclear factors; and b) site C is responsive to the binding of ARP1, HNF4 and RXRα in the modulation of apo A-I promoter activity.

Principal Investigators, Continuing Contracts

Principal Investigators, New Awards