National Institute of Dental and Craniofacial Research
Division of Intramural Research

ANNUAL REPORT SUMMARY

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Fiscal Year 1998 Annual Report

National Institute of Dental and Craniofacial Research

Introduction and Overview

Henning Birkedal-Hansen, Scientific Director

THE DIVISION OF INTRAMURAL RESEARCH
ANNUAL REPORT 1998
INTRODUCTION AND OVERVIEW

The National Institute of Dental and Craniofacial Research witnessed several major changes in FY98. The Institute changed its name (formerly the National Institute of Dental Research) to more accurately reflect its continuously evolving mission and also in the same period celebrated its 50th anniversary. Since the NIDCR was originally entirely intramural, FY98 also marked the 50th anniversary of the Institute's in-house research program. The Division helped celebrate this monumental milestone in many ways. It co-organized two major scientific symposia, one at the American Association of Dental Research Annual Meeting in Minneapolis, MN (March, 1998), and another at the Natcher Conference Center, NIH in Bethesda, MD (June, 1998). A brief account of the history and highlights of the "first fifty years" was published in the Journal of the American Dental Association (Birkedal-Hansen, H.: JADA 129, 702-710, 1998).

Continuing Reorganization and Restructuring

Pursuant to the reorganization plan for the intramural research programs initiated in FY97, a number of additional steps were taken to align the structure and function of the DIR to more closely reflect the letter and the spirit of the NIDCR Strategic Plan as detailed below.

The Division reaffirmed its commitment to empowerment of independent Senior Investigators (SIs) by flattening the Branch structure and by adopting an annually negotiated SI-based resource allocation process. The ultimate goal, which will be achieved in FY2000, is a fully implemented project- (rather than investigator-) based budget negotiation process, resource allocation process and annual progress report process. For the first time, this establishes a direct link between resource allocation and scientific project progress as detailed in the annual report.

The reorganization of the Oral Health Promotion, Risk Factors and Molecular Epidemiology Branch (OHPRFMEB) is continuing. The Health Program Section was moved to the Office of the Director. In addition, the conduct and management of national surveys is being transferred to the Office of the Director. To provide for a seamless transfer and to assist with the preparation of the Surgeon General's Report on Oral Health, Dr. Deborah Winn was unofficially "detailed" for one year to the Office of the Director. Dr. Henning Birkedal-Hansen served as Acting Branch Chief, while Dr. Scott Diehl served as Deputy Acting Branch Chief and managed the Branch on a daily basis. As a consequence of the progressive reorganization of OHPRFMEB and the needs of the Institute, an NIDCR Biostatistics Core Facility was established to provide expert consult on biostatistics, experimental design, data management, analysis, and related services. Dr. Albert Kingman, NIDCR Chief Statistician, was appointed Core Director. Organizationally, the Core represents an entirely new concept as it straddles all Divisions/Offices within the organization without definitive boundaries.

The Division funded a second round of "Emerging Opportunities" proposals aimed at providing support for particularly novel and imaginative research ideas. An ad hoc group of outside experts reviewed all proposals and identified the most meritorious, which were subsequently funded.

The program has been quite successful in providing financial support for particularly innovative science, and has as such fully served the purpose for which it was intended.

In recognition of his many important contributions to the leadership of the Division, Dr. Jack London was named Assistant Scientific Director. In addition to his role in daily divisional leadership functions, Dr. London has assumed responsibility for issues related to space, the research core facilities, and advisory committees.

Trans-NIH Initiatives

Areas of interest shared widely on campus, such as Pain, Head and Neck Cancer, and Skeletal Diseases, increasingly have become the foci for efforts to build campus-wide, multi-institute programs and initiatives. (i) The NIDCR, in partnership with NINDS, conducted a two-day "Pain Research Think Tank" aimed at considering the feasibility of and mechanisms for establishing a campus-wide pain research program (see the "Report of the Pain and Neurosensory Research Think Tank"). The workshop culminated in development of a plan for building a world-class, comprehensive pain research program on the NIH campus with NIDCR and NINDS serving as leads and participation from several other institutes. NIDCR and NINDS are currently engaged in discussions aimed at finding common ground for creation of this program. (ii) Under the leadership of Dr. Silvio Gutkind, the NIDCR is exploring with NCI and NIDCD the possibility of building a joint bench-to-bedside program or initiative in head and neck cancer research. This planned initiative has allowed us to partner with the Cancer Genome Anatomy Project (CGAP), and to work toward a joint clinical research program in head and neck cancer. (iii) The NIDCR is continuing efforts to explore and partner with other ICs to build a state-of-the-art skeletal diseases research program. Dr. Pamela Gehron Robey continues to play an important leadership role in these efforts.

NIDCR Clinical Research

During the past year, the Division's clinical research activities grew significantly. Presently all seven Branches are engaged in clinical research, albeit to different degrees and at different stages of development. As an example, the Craniofacial and Skeletal Diseases Branch (CSDB) developed its three first protocols last year. This research will enable Branch scientists to rapidly move their basic research findings in skeletal biology to the clinic.

The Pain and Neurosensory Mechanisms Branch (PNMB) was reviewed by the Board of Scientific Counselors in June of 1998. The Branch received many positive and encouraging comments for the significance and uniqueness of its research, as well as some constructive critique for enhancing its program. In order to meet a need often expressed by the members of the Board, the December meeting was dedicated to providing a comprehensive overview of the DIR's research activities. Each Branch and single standing Unit presented and discussed its research at a meeting convened on December 3, 1998. The BSC also conducted a highly successful tenure track mid-term review of Dr. Ashok Kulkarni. We were pleased that three members of the National Advisory Dental Research Council, NADRC, (Dr. Harold Morris,

Dr. Caswell Evans, Dr. Michael Rethman) were also able to join us and learn more about our scientific programs.

Scientific Collaborations

During the past year NIDCR scientists enjoyed extensive interactions with the extramural community by presenting their research findings at scientific meetings, universities and private industry. Many also served as advisors to industry, government and academia and contributed to the advancement of science through participation in professional and scientific organizations, including editorial board memberships and editorships, etc. All of these activities are detailed in the document "Interactions with Scientific Community." Moreover, the Division hosted over 50 outstanding scientists from the US and abroad to NIH as seminar speakers and consultants.

Training

Pursuant to the Institute's commitment to creating a learning environment as outlined in the NIDCR Strategic Plan, the Division established an Office of Education. The Office coordinates and administers all divisional programs that relate to training and education and serves as a liaison to any and all related Institute- and NIH-wide activities, agencies and programs. The Office also serves as a conduit to all national and international activities that relate to training issues. Following a national search, Dr. Sharon Gordon was selected as Director of the Office of Education.

FY98 was a very active and, in many respects, innovative year for NIDCR student research programs. It marked the third successful year of the nationally competitive "NIDCR Summer Dental Student Award." In addition, the Division hosted a Howard Hughes Fellow (Mr. Mark Berkman, Ohio State University), and a participant in the Clinical Center Clinical Research Program (Mr. Clifford Davis, UCLA). To further expand these opportunities, the Division established a new, one-year research program for dental students based on the Howard Hughes experience (Mr. Michael Palante, New Jersey). The Division was also successful in attracting three Hispanic students for short-term training under the provision of the Hispanic Association of Colleges and Universities program. In order to help expand efforts to recruit young underrepresented minority scientists, this year the Division took an important first step toward establishing personal connections and liaisons with predominantly Hispanic institutions and with Native American institutions. In 1998 teams representing the Division and the Office of the Director visited three institutions in New Mexico and Puerto Rico to introduce young, future scientists to the potential opportunities available in the NIDCR.

In another initiative to more effectively recruit young scientists among traditionally underrepresented minorities, the Division established an incentive program that provides partial support for two years from the Office of the Scientific Director to Senior Investigators who recruit Hispanic, African-American or Native American fellows. The program has already been successful in helping expand DIR outreach programs for fellows and students.

Planning Activities, Retreats and Workshops

To more specifically review and assess future clinical research opportunities in the NIH environment, the DIR convened a group of outside experts from NIH and from the extramural community on May 18-19, 1998. A subgroup of this panel also reviewed and critiqued recent progress made on DIR clinical protocols. This one-day workshop produced many constructive and thoughtful ideas that are likely to further strengthen and energize the NIDCR clinical research agenda. (Details of the recommendations may be found in the "Clinical Research Opportunities Workshop Summary").

The Division leadership and the Senior Investigators participated in a two-day retreat at Harbourtowne Conference Center, St. Michaels, Maryland, September 24-25, 1998. The meeting provided a valuable opportunity to assess recent DIR organizational changes, to evaluate progress toward implementation of the NIDCR Strategic Plan, and to identify overall program priorities for the future. Additional details of the immediate and future program priorities may be found in the "DIR Five Year Plan."

Renovation Program in Building 30 and Building 10

During the past year the Division embarked on a much needed and long overdue renovation program in Building 30 and in Building 10. Many of our laboratories are out-moded and incommensurate with the state-of-the-art research being conducted in the DIR. The Division plans to allocate a significant portion of its future budget growth to the renovation process.

Scientific Highlights and Accomplishments

While the administrative and organizational changes of the past year required a significant amount of effort and energy from our staff, I am very pleased that our investigators remained highly productive and widely shared their research findings in publications; in presentations at meetings and seminars; and in talks at institutions across the country. These many scientific accomplishments are detailed by Branch in a separate document and the highlights are summarized in the Division Summary immediately following this introduction.

Other activities of the year, and important background information, may be found in these DIR or NIDCR documents:

"Shaping the Future. The NIDR Strategic Plan"
DIR Reorganization Plan: "The Division of Intramural Research, November 15, 1996"
Clinical Research Opportunities Workshop Summary
Report of the Pain and Neurosensory Research Think Tank
The DIR Five Year Plan: "Scientific Opportunities for Growth and Expansion"
Article in JADA by H. Birkedal-Hansen
DIR "Interactions with Scientific Community"

Craniofacial Developmental Biology and Regeneration Branch

Kenneth Yamada
Hynda Kleinman
Yoshihiko Yamada

CRANIOFACIAL DEVELOPMENTAL BIOLOGY AND REGENERATION BRANCH 1998

Our Branch continues to generate a variety of exciting research advances and to receive international recognition. We also continue to place a high priority on the training of young scientists to become independent leaders in academia and industry. In addition, we provide extensive service and citizenship activities on behalf of NIDCR, NIH, and our research fields. Our Branch mission spans the range from basic research through clinical. Our goals focus on creating research breakthroughs (a) to understand the mechanisms of normal and abnormal craniofacial development and function at the genetic, molecular, and cell biological levels, (b) to discover and refine new biologicals and biomimetics relevant to diagnosis, repair, and therapy, and (c) to develop creative, biologically based methods to regenerate craniofacial tissues that are defective or damaged. Our Branch continues to explore important fundamental questions in development, regeneration, and related fields, including the molecular and cell biological mechanisms of morphogenesis, structure and function of extracellular matrix and its receptors, tissue organization, signaling from the cell surface to the nucleus for novel gene induction, cellular differentiation, and the mechanisms of cancer cell growth and metastasis. Discoveries and ongoing innovations in basic research will provide the basis for novel translational and patient-oriented applications.

Members of CDBRB were again invited this year as featured speakers at a variety of international meetings and symposia. Examples of meeting presentations from this past fiscal year included H. Kleinman at a conference on "Cellular, Molecular and Genetic Aspects of Aging" in Israel and K. Yamada as a Plenary Lecturer at the Third Congress of the Asian Pacific Organization for Cell Biology in Japan.

Members of the Branch also continue to serve on the editorial boards of a number of leading journals including J. Cell Biology (H. Kleinman on the board, and K. Yamada as an Associate Editor), J. Biological Chemistry (H. Kleinman), J. Cellular Physiology (K. Yamada, Editor), J. Cell Science (K. Yamada), and Cancer Research (H. Kleinman). H. Kleinman also serves on five other journal boards including J. National Cancer Institute and Angiogenesis, and K. Yamada serves on seven other boards including Current Protocols in Cell Biology and J. Craniofacial Genetics and Developmental Biology. Members also serve on NIH and U.S. Army study sections (H. Kleinman) and on the Council of the International Society for Matrix Biology (K. Yamada). Members of our Branch also provide extensive service on more than 30 NIH and NIDCR committees, including the NIH Biomedical Research Service Policy Board, NIH Diversity Council, NIH Scientific Conduct and Ethics Committee, Search Committee for Director of the NIDCR Division of Extramural Research, and NIDCR Tenure and Promotion Committee.

Our Branch distributes its research products extensively by licensing materials, donating them to repositories, and providing numerous gifts to research colleagues. Products generated by members of the Laboratory that are currently being licensed by companies include Matrigel and invasion substrates (Collaborative/Becton Dickinson and Sigma) and monoclonal antibodies against integrins (Becton Dickinson). The Branch has donated the EHS sarcoma to ATCC along with approximately 500 cDNA clones. We will have dozens of new, formal Material Transfer

Agreements with non-NIDCR researchers this year. Members of the Branch have also received support from outside organizations. Significant support for research on proteoglycans was received from Seikagaku. NASA provided funds to study salivary gland cell differentiation in microgravity. Non-NIH salary support for postdoctoral members of the Laboratory has come a wide variety of sources including the Japan Society for the Promotion of Science, the Dutch Cancer Society, the French CNRS, the German government, the Japanese Ministry of Education, and the Spanish government. The Branch also has a Cooperative Research and Development Agreement (CRADA) with the biotechnology company Trevigen, focusing on novel molecular approaches to wound repair.

Researchers in the Craniofacial Developmental Biology and Regeneration Branch have made substantial research progress and exciting scientific breakthroughs during the past year, and our annual report bibliography lists over 80 publications. A variety of arbitrarily selected research advances are highlighted below. The project reports from each Section provide more comprehensive summaries of the major new findings in our Branch.

New research initiatives in all three sections are identifying and characterizing proteins and genes important for tooth and craniofacial development and cancer. Hundreds of novel genes (i.e., genes never previously described) have been identified by partial sequencing of rodent tooth and embryonic craniofacial unidirectional cDNA libraries, as well as of human salivary gland subtraction libraries. Selected novel genes show interesting and quite distinctive mRNA expression patterns in developing tissues; several genes are expressed preferentially in early mouse embryos. A contract is currently underway to generate antibodies against murine gene products of interest. Branch members are also the contract officers of a major new contract to be awarded shortly to discover and catalogue expression patterns of human craniofacial genes that are active during early development. All clones and antibodies will continue to be made freely available to dental scientists and to other qualified investigators to promote research in the area. The first protein to be characterized by the CDBRB using these approaches was ameloblastin, discovered to be a novel tooth-specific, developmentally regulated gene product associated with enamel formation and linked to the congenital disorder amelogenesis imperfecta. Other genes can be used similarly as candidates for the identification of genetically linked diseases and disorders of oral and craniofacial tissues.

New transcription factors and mechanisms of gene regulation essential for normal development are being characterized, such as a novel Kruppel zinc finger protein found to be essential for normal tooth development. The Molecular Biology Section has also characterized the enhancer of the link protein gene and protein factors that selectively inhibit enhancer activity of two types of cartilage-specific collagen genes. This Section has also identified a 6 bp sequence in intron 1 of the $\alpha 2$(XI) collagen gene critical for cartilage-specific transcription of the gene. The Developmental Mechanisms Section has characterized craniofacial and other sites of expression of the zinc finger protein termed Slug, shown to be essential for an initial step in the morphogenetic process of epithelial-to-mesenchymal transformation. Our knowledge about new genes and regulatory mechanisms is being used to examine pathology in animal models and in human diseases. For example, the Molecular Biology Section has created gene knockout mice to study the biological roles of link protein and perlecan.

Laminin and laminin peptides have been implicated by Branch members in angiogenesis, neurite outgrowth, and tumor growth and metastasis. Over 700 overlapping laminin peptides spanning the entire laminin molecule are being tested in a variety of biological processes by the Molecular and Cell Biology Sections. Over 40 of these peptides have cell-type specific effects on cell adhesion, growth, angiogenesis, or salivary gland differentiation; several are highly potent. Another peptide can switch on the metastatic phenotype to help study this process. Comparisons of the functions, cell-type specificity, receptors, and signaling mechanisms of these peptides are in progress. These studies should lead to the development of new therapeutic reagents.

The interaction of cells with extracellular matrix via integrin receptors induces altered signaling, cytoskeletal organization, gene expression, growth, and differentiation. The newly described tumor suppressor termed PTEN/MMAC1, which is mutated in 10-45% of various types of human tumors, was discovered by the Developmental Mechanisms Section to be a potent down-regulator of integrin-mediated functions, including cell migration and spreading. It specifically dephosphorylates key proteins such as focal adhesion kinase and $p130^{Cas}$. It also appears to target MAP kinase signaling, which is a crucial cellular regulator of growth and differentiation. New technologies are being developed by this Section to study these signaling processes. One approach has adapted synthetic leucine zipper domains for the formation of specific inter-molecular hybrids to provide a method to the localization of any protein in a cell. Another is the use of molecular chimeras to examine the roles of transmembrane clustering of cytoplasmic molecules in signal transduction.

Novel extracellular regulators of cell migration and tumor metastasis have been identified by the Cell Biology Section. Ongoing studies on thymosin $\beta 4$ and thymosin $\alpha 1$ have established roles in endothelial cell migration, and acceleration of wound repair in a rat skin biopsy model, suggesting a potential application in promoting human wound healing. A novel function for osteonectin was also identified in supporting prostate cancer cell metastasis to bone and induction of proteases. Several patent applications were filed for potential clinical use of thymosin $\alpha 1$, thymosin $\beta 4$, and osteonectin.

The specific molecules and stages of cellular responses to matrix are being characterized in human salivary gland (HSG) cells and in fibroblasts by the Cell Biology and Developmental Mechanisms Sections. When salivary gland cells are placed on extracellular matrix proteins in cell culture, they show large changes in gene expression and protein biosynthesis. More than two dozen genes were identified as induced by integrin-mediated adhesion of salivary cells to collagen or fibronectin, many of them completely novel. On a basement membrane extract, salivary gland cells can differentiate and form mini-glands. This process of differentiation involves an 8-amino acid sequence in laminin whose cellular receptor has been found to be syndecan-1. Growth factors needed for differentiation are also being characterized, and other genes induced after adhesion to basement membrane substrates are being sought. These studies in cell culture systems are building the knowledge base necessary to develop creative therapeutic approaches for the repair or replacement of salivary glands and other tissues. For example, CDBRB is collaborating with the Gene Therapy and Therapeutics Branch toward the ambitious goal of developing an artificial salivary gland, and a joint invention report was filed describing

this concept. These and other exciting advances are described in the following list of publications and in reports on specific projects from each Section.

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Craniofacial and Skeletal Diseases Branch

Pamela Gehron Robey
Edward David Eanes
Larry W. Fisher
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CRANIOFACIAL AND SKELETAL DISEASES BRANCH
1998

During the past fiscal year, the Branch has continued to focus its efforts on determining the processes by which skeletal elements are modeled during embryogenesis, remodeled and maintained in the post-natal organism, and how these processes are altered in disease states that affect the skeleton. This focus is fostered through the coordinated efforts of senior investigators working in the areas of developmental biology, cell and molecular biology, and protein and mineral chemistry. Furthermore, the translation of these efforts into clinical studies and applications has been, and remains a major area of emphasis in the Branch. A national search for a chief of this Skeletal Clinical Studies demonstrates the commitment of the Branch to the latter area. Two highly qualified candidates were identified, however, the position remains unfilled. Nonetheless, efforts in translational and clinical research are progressing rapidly through the addition of clinically trained junior staff, a continuation of the Georgetown University Orthopaedic Residents Training Program, establishment of collaborations with other NIH Institutes, institutions around the world, and the use of outside consultants. In addition, the Branch has joined forces with members of the Molecular Genetics Branch of NHGRI to establish the Skeletal Genome Anatomy Project (SGAP) which is designed to aid in gene discovery and to determine changes in the pattern of gene expression of skeletally derived cells as a function of developmental age and of disease processes. The Branch has made major advances in several areas, as is highlighted below, which represents not only progress within each Senior Investigator (SI) group, but a great deal of interaction between SI groups and collaborators at NIH and around the world.

Developmental Biology

This program focuses on the signaling molecules involved in skeletal development and joint morphogenesis. Although the primary investigator of the group, Dr. Frank Luyten, left the NIDR in the fall of 1997, Dr. John Terrig Thomas, a Visiting Associate, has remained to complete several ongoing projects. Previously, the group had isolated two new members of the TGF-beta superfamily, CDMP-1 and CDMP-2, which are expressed in the cartilage condensations and in the interzone region of future joint spaces during limb development. From the study of three different inherited chondrodysplasias, it was demonstrated that CDMP-1 plays a critical role in the development of the appendicular skeleton. Having isolated both mouse and human *cdmp-1*, transient transgenic experiments are underway to determine the location of the cartilage and joint-specific regulatory elements in CDMP-1. Another protein, Frzb (pronounced "frisbee") was initially isolated from purified cartilage extracts and shares homology to the cysteine rich domain (CRD) of *frizzled*, a Wnt receptor. Wnt proteins are secreted signaling molecules having numerous developmental functions, including skeletal development, as well as dysfunction in oncogenesis. Frzb is able to bind to and inactivate Wnt activity leading to speculation for a potential therapeutic in modifying Wnt induced developmental and oncogenic events. It has been shown that Frzb is temporally and spatially expressed during skeletal and craniofacial development and the present aim is to determine the exact function of Frzb during skeletogenesis using the Cre-LoxP recombination system, whereby the effect of loss of Frzb function at various stages of development can be observed. Chimeric "floxed" mice have been obtained and

germline transmission is currently being confirmed. The generation of "floxed"-*frzb* mice will provide a powerful tool to study the role of Frzb, and thus indirectly, Wnt signaling, in any organ system in which Frzb displays an interesting expression pattern either during development or postnatally. In addition, the regulatory elements of *frzb* are being identified which will provide useful information as to the control of its temporal and spatial expression pattern.

Skeletal Biology

The Skeletal Biology program, headed by Dr. Pamela Gehron Robey, has focused on biochemically characterizing osteogenic cells, including bone marrow stromal cells (BMSCs), a heterogeneous population of clonogenic cells that give rise to osteoblasts/chondrocytes, adipocytes and hematopoiesis supportive stroma. Studies were designed to test the hypothesis that different members of the stromal cell population represent pluripotential cells, while others are more committed to particular phenotypes. This hypothesis was tested by determining the ability of individual clones of human BMSCs to form a bone/bone marrow organ by using a newly developed in vivo transplantation system. It was found that 54% of the clones studied supported bone formation, but only half of these clones supported bone formation and hematopoiesis. These results prove that some, but not all, of the members of the stromal cell population maintain their ability to form bone, hematopoiesis supportive stroma and associated adipocytes. The differences between non-bone forming clones, clones that form bone were further investigated by examining their expression of receptor tyrosine kinases (RTKs), a family of receptors that has been implicated in the proliferation and differentiation of many connective tissue cells. PDGF-receptor beta, EGF receptor, FGF receptor-1 and Axl were identified in the multi-colony derived population of BMSCs. Clonal populations of BMSCs were found to express varying levels of these four RTKs. Although not absolutely predictive of a particular clone's ability to support bone formation in the in vivo transplantation assay, it was found that there is relatively high levels of PDGF-R (beta) in bone-forming clones, and relatively high levels of EGF-R in non-bone forming clones, and that the rate of proliferation of bone-forming clones is positively correlated with the amount of bone formed in vivo. Members of the program also collaborated with members of the Molecular Biology of Bones and Teeth program and outside investigators in the establishment of immortalized BMSCs from a patient null for the expression of the estrogen receptor (alpha), the identification of SHOX/PHOG, a transcription factor implicated in skeletal growth in BMSCs, and identification and characterization of human cementoblasts by using an in vivo transplantation system.

Molecular Biology of Bones and Teeth

The objective of the Molecular Biology of Bones and Teeth program, headed by Dr. Marian Young, is to study the function and regulation of matrix proteins found in bones and teeth using a combination of in vitro and in vivo analysis. These proteins play key roles in the metabolism of these tissues. The genes that have been studied are biglycan (BGN) and bone sialoprotein (BSP), both of which are highly expressed in bones and teeth. A major impediment for the study of human gene function and regulation has been the extreme inefficiency of DNA transfer into human, non-transformed cultured cells. In order to devise new methods to overcome this hurdle, two adenoviral-based procedures were tested using bone marrow stromal cells and trabecular

bone cells, both derived from human skeletal material in collaboration with members of the Skeletal Biology program. Adenoviruses containing beta-galactosidase recombinant genes showed substantial gene transfer (10,000x over control) indicating that adenovirus based methods are ideal for these unique cell types. Adenoviruses additionally modified with poly-lysine were also tested and it was found that they transferred genes into multilayered, highly differentiated non-dividing bone cells, also with high efficiency. This latter procedure has numerous advantages in that it is rapid, simple, and does not require incorporation of transgenes into the viral genome. To examine the function of matrix proteins in vivo, *bgn*-deficient mice were generated, and characterized in collaboration with other members of the Branch. While apparently normal at birth, these mice display a phenotype characterized by a reduced growth rate and failure to achieve peak bone mass due to the absence of *bgn*. This is the first report that deficiency of a non-collagenous extracellular matrix protein leads to a skeletal phenotype that is marked by low bone mass which becomes more obvious with age. These mice may serve as an animal model to study the role of genetic factors that control peak bone mass and extracellular matrix composition and function in osteoporosis.

Matrix Biochemistry

The Matrix Biochemistry program, headed by Dr. Larry Fisher, has focused on structure-function studies of the non-collagenous proteins of bones and teeth, with particular emphasis on matrix protein-protein or matrix protein-cell interactions. It is highly likely that both the assembly of the matrix and its subsequent mineralization is controlled by cells via the use of some of these non-collagenous proteins. A study was completed showing that the small proteoglycan, decorin, and very likely its homolog, biglycan, is endocytosed by cells using the Leu125-Val230 portion of the protein(s). Additional work on the biglycan promoter has lead to hypothesis that the transcription factor, c-Krox, is important in the regulation of this important molecule, and the phenotype of the biglycan knockout animal was also characterized in collaboration with other members of the Branch. Another abundant protein in the mineralized matrices of bones and dentin is a protein discovered by this group several years ago, bone sialoprotein (BSP). This phosphorylated, sulfated glycoprotein binds to integrins, particularly the vitronectin receptor, through its RGD tripeptide. Although BSP in adults is generally limited to the skeleton, work with collaborators has shown that this molecule is likely to be a very good marker for the appearance of many osteotropic cancers including breast, lung, thyroid and prostate. Current efforts are aimed at developing useful BSP assays for these cancers. Working with members of the Skeletal Biology and Skeletal Clinical Studies programs, the group has contributed to the genetic mutation analysis of human McCune-Albright Syndrome marrow stromal cells and their role in the development of fibrous dysplastic bone. Collaborative work with the enamel matrix protein, tuftelin, has continued with the description of the human protein and gene. This highly conserved protein is on chromosome 1 and is currently a candidate gene for some cases of amelogenesis imperfecta.

Mineral Chemistry and Structure

The Mineral Chemistry and Structure program, headed by Dr. David Eanes, continued to focus its research effort on the study of calcium phosphate salts, with particular emphasis on their

chemistry and structure, on in vitro model systems designed to more full elucidate the various physicochemical factors that control their formation in vivo, and on developing uses for these salts in dental and biomedical materials applications. Previous work suggested that methacrylate-based composites with ACP as filler could possibly find uses as mineralizing agents in clinical dentistry. It was found that the mechanical strength of ACP-filled methacrylate composites was comparable to that of commercial cavity lining materials. These findings, together with the unique ability of ACP composites to release calcium and phosphate ions that possibly could stimulate the formation of reparative dentin, suggest that ACP composites may make excellent lining materials for protecting the pulp against restoration invoked chemical irritations. Also, methods were developed for synthesizing by hydrolysis-condensation reactions of trialkoxyorganosilanes, a variety of oligomeric organofluorosilsesquioxanes. These highly fluorinated organofluorosilsesquioxanes have potential use in dentistry as well. Another major effort is being directed toward delineating the effects that solution state has on the formation, growth, and textural properties (size, shape) of calcium phosphate salts, especially apatite. Findings revealed that carbonate-induced proliferation of new crystals could be a significant factor in controlling the size of apatite crystals in skeletal tissues. Fluoride and magnesium ions were found to partially offset the repressive effect carbonate had on primary crystal growth. Fluoride ions also directly affected crystal growth. In other studies, the calcium salt of glutarylbisphosphonate (GIBPD), a new bisphosphonate that has potential clinical use in treating calcium-related bone disorders, was characterized. The near-perfect lattice matches found between the CaGIBPD crystal faces and the major crystal faces of hydroxyapatite indicate that epitaxial growth of CaGIBPD can occur on the apatitic surface. This may be a mechanism by which GIBPD could clinically affect biomineralization processes.

Skeletal Clinical Studies

A major goal of the Skeletal Clinical Studies program, currently directed by Dr. Pamela Gehron Robey, is to elucidate the role of osteogenic cells in the generation of a variety of skeletal dysplasias. Under Protocol #97-DK-0055, it was found that the metabolic activity of bone-forming cells is altered by a number of known mutations. In fibrous dysplasia of bone (FD) and in McCune-Albright Syndrome (MAS) which presents with severe fibrous dysplasia, there are missense mutations of the G protein, Gs alpha, leading to overproduction of cAMP. It was found that this protein is dramatically upregulated as bone marrow stromal cells (BMSCs) mature into osteoblasts, and the effects of the mutations are manifested by abnormal cell-cell (hyperosteocytic bone), cell-matrix interactions (cellular retraction), and the formation of an abnormal bone matrix. A spectrum of bone lesions associated with such mutations was identified and recognizable as three primary, but distinct, histological patterns. The data emphasize the non-random (site-specific) variability of FD histopathology in patients carrying activating mutations of the Gs alpha gene, and provide additional evidence for the occurrence of Gs alpha mutations in cases of FD other than typical MAS. Using an in vivo transplantation system, it was found that populations containing mutated BMSCs leads to the recapitulation of FD formation in immunocompromised mice, representing a novel model system for determination of the pathophysiology of the disease and development of new therapeutic strategies. Furthermore, the study demonstrated the need for both normal and mutated cells (somatic mosaic) for formation of a FD lesion. From these studies, several clinical protocols for

the study and treatment of patients with FD have been approved (98-D-0145, 98-D-0146 and 99-D-0003) and patient recruitment is about to begin. Another goal is to examine the ability of ex vivo expanded BMSCs (obtained under Protocol 94-DR-0188), to regenerate normal bone tissue. Full thickness osseous defects (5 mm) were prepared in the cranium of immunocompromised mice and were treated with gelatin sponges containing murine alloplastic bone marrow stromal cells. Cultured bone marrow stromal cells transplanted within an appropriate vehicle resulted in osteogenesis that repaired greater than 99.0% ± 2.20% of the original surgical defect within two weeks. A pre-IND application for use of this technology in human patients with calvarial defects has recently been reviewed by FDA, and final approval is pending "proof of principle" studies in dogs, which are due to be analyzed in the winter of 1999.

CRANIOFACIAL AND SKELETAL DISEASES BRANCH
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Gene Therapy and Therapeutics Branch

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GENE THERAPY AND THERAPEUTICS BRANCH
1998

The Gene Therapy and Therapeutics Branch (GTTB) has 5 principal investigators (PIs), each with a unique research focus, who cooperatively provide a bench to clinic continuum focusing on questions related to salivary gland dysfunction. Indu Ambudkar is Chief of the Secretory Physiology Section (SPS), and is generally studying signal transduction mechanisms operative in salivary glands with a primary interest in Ca^{2+} entry pathways of non-excitable cells. Jim Turner is Chief of the Membrane Biology Section (MBS), and is generally addressing ion transport pathways leading to transcellular salt gradient formation in salivary acini, with a main focus on the secretory isoform of the Na^{+}-K^{+}-$2Cl^{-}$ cotransporter. Bruce Baum is Chief of the Gene Transfer Section (GTS), and has primarily used adenoviral-mediated gene transfer in strategies to repair irradiation-damaged salivary glands and to direct the secretion of transgene products for systemic use. Brian O'Connell is Chief of the Gene Regulation and Expression Unit, (GREU), and is studying the cellular factors and genomic elements modulating salivary cell gene expression in addition to developing a gene transfer strategy to manage azole-resistant mucosal candidiasis. Phil Fox is Chief of the Clinical Investigations Section (CIS), which is focused on the management of patients with primary Sjogren's syndrome (SS). We are a group which believes significant advances in clinical care will only come from understanding biological mechanism. We focus on salivary glands, secretory epithelial tissues with a complex, highly regulated biology.

Neurotransmitter regulation of fluid secretion in salivary glands primarily depends on the activation of Ca^{2+} influx. This Ca^{2+} influx mechanism in salivary gland, and other non-excitable, cells is as yet largely uncharacterized. For many years the SPS has focused its efforts on identifying the molecular component(s) mediating and regulating this Ca^{2+} influx process. During this fiscal year SPS studies concentrated in two areas. The first involved a candidate molecule for this pathway, termed Trp, which was recognized initially in drosophila studies. The SPS has demonstrated the presence of two Trp isoforms in salivary epithelial cells via molecular and confocal microscopic methods, and now are assessing their function. The second major study area has involved the electrophysiological characterization of Ca^{2+}- dependent K^{+} channel activity in salivary cells. Using this as a tool to study Ca^{2+} influx, SPS investigators have shown that the Ca^{2+} influx pathway is tightly regulated by the $[Ca^{2+}]$ at the site of influx, which in turn is controlled by activity of the intracellular Ca^{2+} pump.

Once a salivary acinar cell is stimulated to secrete fluid (via a Ca^{2+} mobilizing stimulus) it is the cell's job to generate a transcellular salt (NaCl) gradient to drive the secretion of osmotically obliged water. This involves the activation of several specific ion channels and transporters. The Na^{+}-K^{+}-$2Cl^{-}$ cotransporter is considered to be a key component of this process. It is thought to be the major Cl^{-} entry pathway in acinar cells and thereby the principal factor in driving the acinar Cl^{-} secretion which ultimately leads to salt and water secretion. The MBS has over the years focused considerable effort in better understanding the functioning and regulation of this cotransporter in salivary acinar cells. Studies during the past year have been directed at understanding the phosphorylation event associated with upregulation of cotransporter activity in response to cAMP mobilizing stimuli. This phosphorylation site has now been localized to an N-

terminal peptide obtained by CnBr digestion. Interestingly, this peptide does not contain a consensus site for cAMP-dependent protein kinase A, suggesting that the signaling cascade following intracellular cAMP production is more complex than originally thought. Additional experiments carried out over the past year indicate that phosphorylation at this site is required for functional activity. Much of the MBS's future efforts will be geared towards understanding the structure/function relationships of the cotransporter and its homologues. To this end they have recently established that the cotransporter exists as a homodimer in the acinar cell membrane and their results suggest that this is due to an interaction between the hydrophobic membrane spanning domains of the protein. In addition the MBS has succeeded in expressing the cotransporter in yeast as a first step in obtaining sufficient quantities of protein for more detailed biochemical and biophysical studies.

Research in the GTS and GREU continued to be directed at clinically-relevant applications of gene transfer to salivary glands. Two specific clinical situations have been targeted by the GTS; the repair of irradiation-damaged salivary glands and growth hormone (GH) deficiency. The GTS has continued to make progress in both projects during this reporting period. For example, we previously showed that administration of a recombinant adenovirus (AdhAQP1) encoding the water channel aquaporin-1 leads to increased fluid secretion from irradiated rat submandibular glands. During this year GTS scientists have shown that AdhAQP1 can be administered to irradiated primate parotid glands without untoward local (salivary) or systemic effect. Further, GTS investigators showed that hAQP1 can be delivered to mouse salivary glands in vivo via a recombinant adeno-associated virus (AAV). This offers the possibility that recombinant AAV, which is less immunogenic and allows longer-lived transgene expression than recombinant adenoviruses, can be used clinically with these glands. In the second focus area, the GTS has developed a recombinant adenovirus encoding murine GH. With a dwarf mouse model, this virus (AdCMVmGH) leads to a doubling in animal size within 30 days after a single intramuscular injection and to therapeutic levels of the hormone after a single administration to the submandibular glands.

One of the main deficiencies of in vivo gene transfer is the difficulty in achieving stable gene expression. Previous work in the GREU has focused on the attenuation of immunologic responses to adenoviral vectors. Now the GREU has made considerable progress in the development of a novel integrating viral vector. Sequences from a retrovirus were incorporated into a replication-deficient adenoviral vector backbone. The resulting chimera was able to efficiently infect cells in vivo and in vitro. In addition, the vector demonstrated strong evidence of genomic integration and persistent gene expression. Such a vector would have broad applicability for in vivo gene transfer and gene therapy in a variety of dividing and quiescent cell types. The GREU has continued the characterization of salivary acinar cell and ductal cell promoters. Using an in vitro model of salivary cell differentiation, studies of the effects of various growth factors and other extracellular matrix components on the induction of salivary amylase have progressed and allowed a better understanding of the pathways that lead to cytodifferentiation. Also the GREU's important studies on the salivary antifungal proteins, histatins, have proceeded in several directions. Adenovirus-mediated expression of histatin 3 in rabbit submandibular saliva was achieved and the recombinant protein was found to be functional in vitro. Laboratory experiments with histatin 3 have revealed a specific binding site

for the protein on the C. albicans membrane. Binding and translocation of histatin 3 was shown to be necessary for cell death to occur.

During this year, the CIS continued its landmark studies on the natural history and pathogenesis of primary SS, as well as initiating two important clinical trials. The first of these is examining the efficacy of dehydroepiandosterone (DHEA) to improve the autoimmune status of patients with primary SS, and has achieved >50% enrollment. The second study protocol was just approved and looks at the utility of thalidomide in managing primary SS. Enrollment is set to begin. CIS scientists have also continued to examine immunopathologic mechanisms involved in SS by studying differential cytokine gene expression in subpopulations of cells found in the minor salivary glands (acinar, ductal, lymphoid) of patients and controls. As reported last year, the approach used was a cell-specific microdissection technique coupled with RT-PCR and Southern hybridization. Results have shown that there is abundant cytokine gene expression by epithelial cells in salivary glands of both patients and controls, suggesting their implication in normal salivary gland homeostasis.

Overall, FY98 has been a highly productive year for the GTTB. We continue to make progress bringing innovative clinical management tools, grounded in solid basic science, to benefit patients with salivary gland disorders.

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Oral Health Promotion, Risk Factors, and Molecular Epidemiology Branch

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ORAL HEALTH PROMOTION, RISK FACTORS, AND MOLECULAR EPIDEMIOLOGY BRANCH 1998

The Oral Health Promotion, Risk Factors, and Molecular Epidemiology Branch (OHPRFMEB), has continued its scientific focus on molecular genetic epidemiology, analytical epidemiology, and oral health promotion research. In keeping with the NIDCR Strategic Plan, the Branch has been in transition over the past year. Dr. Henning Birkedal-Hansen served as the Acting Branch Chief, and Dr. Scott R. Diehl and Dr. Lawrence Furman served as Acting Deputy Branch Chief during this period of planned restructuring.

The staff of the Health Promotion component of the branch provided special leadership and staffing to the newly evolving Office of Science Policy and Analysis located within the Office of the Director. Dr. Deborah Winn, leader of the Analytical Epidemiology component of the branch, assumed responsibilities in the Office of the Director to help establish the organizational infrastructure for future assessment of the magnitude and distribution of dental, oral and craniofacial diseases and conditions. Over the past year, she and her staff have continued to design and implement of Oral Health Component of the Fourth National Health and Nutrition Examination Survey (NHANES IV) scheduled for field implementation in March of 1999.

The Molecular Genetic Epidemiology component of the branch under the direction of Dr. Scott R. Diehl has continued to conduct family based molecular genetic clinical studies of cleft lip and palate, periodontal disease, Kartagener syndrome, and oral and nasopharyngeal cancers. Strategies include linkage analysis and transmission disequilibrium gene mapping. Dr. Diehl and his staff have continued to provide innovative leadership in large-scale genotyping for highly polymorphic DNA markers including the development of new methods of database management and of improved automated methods for the analysis for complex human genetic disorders.

Dr. Albert Kingman has continued to serve in his capacity as NIDCR's Chief Statistician, conducting data analyses for the NIDCR Amalgam Study, an epidemiological investigation of the potential health risks of exposure to dental amalgam (mercury vapor). As part of his expanded interest in statistical genetics, he began a part time sabbatical with senior researchers in the National Human Genome Research Institute (NHGRI), focused on developing improved methods for conducting linkage analyses of data from genome screens. As part of the transition of the Branch, Dr. Kingman has assisted in formulating and implementing plans for a Biostatistical Core within the Division of Intramural Research that will provide support to all components of the NIDCR.

At the close of this transitional year, the staff and programs of the original Division of Epidemiology and Oral Disease Prevention Program have been successfully assimilated into the Division of Intramural Research and the Office of the Director. The new streamlined DIR Branch will consist of a Molecular Genetic Epidemiology component directed by Dr. Diehl, an Analytical Epidemiology component, directed by Dr. Winn and the Biostatistical Core and related independent research directed by Dr. Kingman. The Oral Health Promotion component and the National survey and related activities previously associated with the branch are being transferred to the Office of the Director. With the refocusing of program emphasis on intramural

research, the OHPRFMEB will be officially renamed in the coming year, and a full time chief recruited for leadership of the branch.

Molecular Genetic Epidemiology

The Molecular Genetic Epidemiology component under the direction of Dr. Scott R. Diehl is conducting studies of oral, dental and craniofacial disorders using strategies such as linkage analysis and transmission disequilibrium gene mapping. Most studies involve molecular assays such as marker polymorphisms or mutation analyses of candidate genes, but some projects are more classical in nature and investigate familial aggregation of diseases and disease-related phenotypes without incorporation of molecular data. Analyses include key behavioral risk factors such as diet, smoking and alcohol consumption, and these are treated both as covariates to disease risk and as genetically heritable phenotypes of interest themselves. Dr. Diehl directs a laboratory engaged in large-scale genotyping for highly polymorphic DNA markers and a statistical and computer group responsible for data management and statistical analyses. Research team members are also responsible for designing and conducting the field studies required for subject recruitment, implemented through collaborations with clinicians throughout the world. Current projects include several studies of oral cancer using both case control designs and families; studies of nasopharyngeal carcinoma using both simplex and multiplex families; studies of non-syndromic cleft lip and palate using both simplex and multiplex families (complemented by a mouse model); studies or early onset periodontitis; a study of immotile cilia syndrome with chronic sinusitis and bronchiecstasis. Continuation and completion of the major studies currently in data collection phases will occupy much of the section's available efforts for the next couple years. New clinical initiatives are currently being planned including clinical molecular genetic studies of adult onset periodontitis, and sensitivity and perception of pain. In addition, Dr. Diehl's laboratory will continue to very actively pursue new molecular technologies such as array-based single nucleotide polymorphism (SNPs) for further molecular analyses of the large samples of study subjects currently being recruited, in recognition of the potential for adding substantial power to detect genetic effects for complex disorders that these approaches may provide.

Analytical Epidemiology

The staff of the Analytical Epidemiology component, under the direction of Dr. Deborah Winn, has continued a program of scientific accomplishment and professional service. Their Intramural research includes studies of periodontal health and of oral and pharyngeal cancer. Early onset periodontitis is a disease characterized by a progressive loss of the tooth supporting tissue in adolescents and young adults. A study of adolescents with early onset periodontitis and a control group of adolescents were identified within a population of 14,000 pupils in grades 8 to 12 examined by a national survey of the oral health of US children conducted by NIDCR during the 1986/87 school year. Repeat oral examinations were conducted six year later on these subjects. Discoveries this year include identification of several bacterial species that are more strongly associated with severe EOP than others: P. gingivalis and T. denticola, and the development of an enhanced clinical classification system for early onset periodontitis. In addition, it was discovered that Beta-glucoronidase in the gingival crevicular fluid of young persons in the study

may be a valuable marker of individuals at higher risk of the generalized form of the disease. Plans are being developed collaboratively with Dr. Diehl to study the FMLP receptor and other genetic markers, to establish a bacterial profile for cases and to examine local and systemic immune factors, associated with this disease.

Oral and pharyngeal cancers are characterized by a) alcohol and tobacco etiology, b) low proportion of tumors identified at an early stage, c) racial differences in stage at presentation, d) racial disparities in survival even controlling for stage, and e) approximately 50% survival rate. These ongoing interrelated studies have as their goal the identification of risk factors for oral and pharyngeal cancer and identification of factors that influence detection of oral cancer or of persons at highest risk of these cancers. One project is the Puerto Rico Oral and Pharyngeal Cancer study in which patients identified through a cancer registry are compared to the general population. The purpose is to identify behavioral, medical, familial, and genetic factors involved in the etiology of this disease. Using the Puerto Rico study population, collaborative investigations with the National Cancer Institute (NCI) are underway to determine how behavior (e.g., tobacco and alcohol consumption, dietary habits), medical and oral conditions, and other factors interact to increase risk of these cancers. The study will help elucidate oral cancer etiology as well as suggest potential opportunities for prevention. Key findings this year include the first evidence that oral and pharyngeal cancer risks decrease with cessation of alcohol consumption, although risks of these cancers remains elevated for up to 20 years after cessation of tobacco or alcohol use. The alcohol dehydrogenase type 3 genotype for slow metabolism of alcohol to acetaldehyde was found to substantially increase the risk of ethanol-related oral and pharyngeal cancer in heavy drinkers. This finding provides further evidence for the carcinogenicity of acetaldehyde.

The SEER/Medicare Linkage Project is focused currently on a determination of patient medical care contacts occurring in the year prior to diagnosis of oral and pharyngeal cancer and the reasons for those contacts. It is hypothesized that relative to persons without cancer, oral cancer patients have more contacts and for more conditions due to the morbidity consequences of high alcohol intake and tobacco use. Answering this question may help suggest approaches to identify the cancers earlier. In a related analysis, we have recently discovered a striking increase over time in the incidence of in-situ carcinomas of the oral cavity, pharynx and larynx.

Health Promotion Research

Activities undertaken by Health Promotion Research staff during the year cover a broad spectrum of oral health related issues and research areas. Research projects focused on: 1) significant but often neglected diseases; for example, early childhood caries and oral cancers, 2) the effects of socioeconomic status and differences in oral health care on the oral health status of racial and ethnic minorities among children, adolescents and adults, 3) limitations of poor/non-poor comparisons of inequalities in oral health status, 4) a study of the prevalence and extent of periodontal conditions in previously diagnosed Type II diabetics as compared with persons not having diabetes, 5) ways of improving approaches to oral health education and health promotion directed at health care professionals and the public, and 6) differential patterns in dental caries by age and implications for the use of dental sealants.

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Oral Infection and Immunity Branch

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ORAL INFECTION AND IMMUNITY BRANCH
1998

The Oral Infection and Immunity Branch (OIIB) experienced a highly productive year with new collaborative efforts to build on our strengths and to integrate our diverse research portfolio. A new clinical endeavor, the Clinical Investigations in Infectious and Autoimmune Diseases Program, was initiated, underscoring our commitment to the transition from our basic through translational to clinical research. In a short time our inaugural clinical protocol received IRB approval and began to accrue patients.

Our multiple interactions with the extramural community have enabled members of the Branch to advance science and share our research findings through participation in national and international meetings, invitations to speak, chair and organize meetings and symposia throughout the world, and serve on boards of national/international foundations and organizations (Society of Leukocyte Biology, FAES, National Disease Research Interchange, Paul Ehrlich Foundation, March of Dimes Foundation). Beyond participating in NIDR's 50thAnniversary Celebration, OIIB members were invited to represent NIDR at the 10th Anniversary of the Dental Research Program in Llubljana, Slovenia and at the 50thAnniversary of the School of Dentistry, University of Alabama at Birmingham. Editorial board responsibilities on Journal of Immunology, Journal of Experimental Medicine, and other prestigious journals further attest to the stature of our Senior Investigators. In additional recognition, substantial outside funding rewarded OIIB's productive programs in anthrax biology and diabetes research and two NIDCR, DIR Emerging Scientific Opportunities Grants have been awarded.

Biweekly meetings of the Branch Chief and Senior Investigators maintain open lines of communication, providing a forum for administrative and programmatic issues and to facilitate Branch activities. In our continuing efforts to facilitate interactions of the Branch at all levels, monthly poster sessions with refreshments, social and scientific interchange were held, followed by a Meet the Senior Investigators Lecture Series, all orchestrated by the OIIB Seminar Coordinating Committee together with the OIIB Quality of Life Committee.

Senior Investigators in the OIIB have made important research progress, including exciting breakthroughs in the past year, and our FY 98 Bibliography lists nearly 100 publications. Among these are reports in Science, Proceedings of the National Academy of Science, Journal of Biological Chemistry, Journal of Clinical Investigations, EMBO, Journal of Experimental Medicine, and Neuron. Our research portfolio continues to focus on the molecular and biochemical diversity of infectious pathogens, on characterizing innate and adaptive immune responses, and on dissecting pathogen-host interactions, and several of these areas are highlighted in this Annual Report.

The establishment and persistence of bacterial microcommunities is dependent on specific interactions between different organisms that inhabit the biofilm. Co-aggregations, microbial cell-to-cell interactions among genetically distinct partner strains, are mediated by complementary cell surface components on the participating bacterial partners. Bacteria also may recognize host epithelial cells by some of these same surface components. Oral streptococci

play a critical role in the normal microbial ecology of the human oral cavity by functioning as primary colonizers of hard tissue surfaces. Members of this group also are the leading cause of subacute bacterial endocarditis and dental caries. Characterizing biofilms formed with oral streptococci and other early colonizers continues to aid in understanding the biofilm community structure that forms in advance of destructive periodontal disease and serves as a loading dock for other organisms. In this regard, OIIB investigators provided the first evidence that the *sca* operon in *Streptococcus gordonii* encodes a high-affinity Mn2+ transporter that appears to be necessary both for growth of cells in low Mn2+ environments and for DNA-mediated transformation. The putative Mn2+ binding lipoprotein ScaA, which is inducible under Mn2+ - limiting conditions, would thus provide a vital mechanism for the acquisition of Mn2+ by streptococci for growth and survival in the human host. Mutagenesis studies of another operon, the *dlt* operon, of *S. gordonii* revealed that D-alanylation of lipoteichoic acid was critical in adherence, genetic exchange and cell septation. Continued characterization of the streptococcal structurally-related cell wall polysaccharides, recognized by adhesins of other bacteria, involves collaborations with TIGR and will be greatly facilitated by the *S. gordonii* genomic data base. The prevalence and increase in numbers of microorganisms which bind to streptococci, particularly the Gram-negative anaerobic fusobacteria, are hallmarks of the progression of gingivitis and other periodontal diseases. Fusobacteria, in turn, may provide a binding site and reservoir for *H. pylori*, which is then transported to other mucosal sites. In *S. pneumoniae*, genetic mutations provide the basis for the evolution of multi-drug resistance organisms. Trimethoprim resistance was traced to an altered chromosome-encoded dihydrofolate reductase and optochin resistance is the result of a single amino acid mutation of the ATPase gene.

In important new studies, investigators in OIIB have shown that the major virulence factor of *Bacillus anthracis*, the lethal factor protein, is a metalloprotease. Lethal factor which is released as *B. anthracis* spores germinate, cleaves and inactivates mitogen activated protein kinase kinase (MAPKK) to disrupt a central cellular signal transduction pathway involved in cell growth and differentiation. These findings provide a new focal point to develop rational strategies to block lethal factor proteolytic activity and inhibit anthrax toxicity.

Another virulent microbe, HIV, employs other unique genetic strategies to cause infection and disease in its hosts. HIV can influence expression of its requisite co-factor, the CCR5 receptor, in host cells of monocytic lineage and also takes advantage of the ability of *Mycobacterium avium* to enhance the CCR5 receptor. *M. avium* is a frequent opportunistic infection (OI) in immunosuppressed individuals and has recently been shown by OIIB staff to activate the transcription factor, NF-κB. NF-κB mediates the transcription of multiple pro-inflammatory molecules, at least two of which, TNFα and CCR5, increase host cell vulnerability to HIV. Thus, there appears to be a reciprocal regulation between HIV and opportunistic infections such as *M. avium*, emphasizing that treating OI may not only inhibit OI-induced pathology, but also limit the viral burden.

How the host responds to these and other mucosal pathogens as well as to other environmental and endogenous stimuli is the focus of several Senior Investigators in the Branch. Fascinating new studies delineating signaling circuits in the olfactory system have emerged as prototypes for signal transduction pathways, in general. In this regard, efforts have focused on characterization

of the newly identified multigene family of pheromone receptors in the vomeronasal organ of rodents. This family contains as many as 100 genes, and these putative pheromone receptors have related large N-terminal extracellular domains that appear to be the site of ligand binding. Receptor genes are expressed at high levels in small sub-populations of vomeronasal neurons and the one neuron-one receptor model provides a molecular logic for the discrimination of pheromones. However, different sub-families have distinct but overlapping expression patterns within the vomeronasal epithelium, indicating a link between the choice of which receptor is expressed by a vomeronasal neuron and the location of that neuron within the epithelium. The neurons in the vomeronasal epithelium are unusual in that they turnover throughout life, being replaced by division, differentiation of stem cells, and continuous migration of neurons towards the center of the epithelium. These observations emphasize a need for coordinate expression of receptor sub-families and migratory guidance molecules.

The inflammatory response involves the accumulation of extracellular matrix proteins at sites of injury where leukocytes migrate and release an array of mediators. Among these leukocytes, basophils and mast cells have surface adhesion receptors (integrins) that are involved in their binding to other cells or to the extracellular matrix. Such integrin mediated adherence of cells to fibronectin stimulates cell spreading and generates intracellular signals, including tyrosine phosphorylation of proteins such as the focal adhesion kinase, $pp125^{FAK}$ (FAK), with enhanced histamine secretion. FAK also becomes tyrosine phosphorylated in response to the activation of various receptors including the high affinity IgE-receptor (Fc$\in$RI). Transfection of FAK into FAK-deficient mast cells reconstituted their defective secretion and histamine release. As the signaling pathways initiated by immune receptors on lymphocytes and mast cells are related, FAK may play a similar role in their signal transduction. In RBL-2H3 mast cells, tyrosine phosphorylation of several proteins occurs after aggregation of Fc$\in$RI, although the receptor itself has no kinase activity. New insights into the role of several of the protein tyrosine kinases involved in this intracellular signal transduction have been revealed this past year. Besides FAK, pyk2 and Syk appear critical. In Syk-deficient cells, aggregation of Fc$\in$RI induced no histamine release and no detectable increase in total cellular protein tyrosine phosphorylation. By transfection, stable expression of Syk signaling was restored. Syk also appears to be critical for cytoskeletal and shape changes.

Autoimmune diseases, often associated with dysregulated signalling events, are characterized by loss of self-tolerance and may lead to destruction of host tissues. Although genetically based, it is difficult to predict the emergence of these often devastating diseases. Important new studies in the OIIB have identified predictive markers for the development of insulin dependent diabetes mellitus (IDDM). Starting with a cDNA subtraction library, 5 new genes were identified, two of which have particular significance in IDDM. IA-2 and IA-2β which are protein tyrosine phosphatases are major autoantigens and induce the formation of autoantibodies which can be detected years before the development of clinical disease. Individuals with antibodies to IA-2 together with glutamic acid decarboxylase (GAD) have a 50% or greater likelihood of developing IDDM within 5 years. New clinical assays are being developed in conjunction with CDC and WHO to screen the general population for susceptible individuals which will provide opportunities for intervention.

In addition to predicting autoimmune disease, efforts in the Branch have focused on approaches for the treatment of autoimmune and infectious diseases. Taking advantage of an experimental model of arthritis triggered by Group A streptococcal cell wall (SCW) peptidoglycan-polysaccharide complexes, investigators have provided the first evidence that erosive arthritis can be suppressed through TGF-β gene therapy. Intramuscular administration of plasmid DNA encoding TGF-β prior to the administration of arthritogenic SCW, but also after the onset of clinical symptoms, dramatically altered the course of disease. In other studies, the potent immunosuppressive activities of TGF-β were also found to be instrumental in the mediation of oral tolerance. In these experiments, which provide the first evidence that a non-self antigen, in this case, SCW, can induce therapeutic oral tolerance, arthritis could be blocked even when oral administration of the antigen was not initiated until later in the disease. The timing was critical, however, providing important information as to delivery kinetics of oral antigens in human disease.

Infectious disease remains the world's leading cause of death and as the OIIB continues to integrate its research portfolio and to develop its clinical program, the Branch will address the challenges of emerging and re-emerging infections, antibiotic resistance, the potential inappropriate use of micro-organisms, infectious links to autoimmunity and therapeutic interventions.

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Oral and Pharyngeal Cancer Branch

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ORAL AND PHARYNGEAL CANCER BRANCH
1998

Cancer of the head and neck area is the sixth most common neoplastic disease in the developed world, representing a very serious health problem based on annual morbidity and mortality rates. The molecular and etiological factors involved in the development of head and neck tumors, including oral cancers, are still largely unknown. Members of our Branch work on several complementary aspects of cancer cell biology, in an effort to understand the molecular basis for malignant transformation as well as to use this knowledge to develop molecular markers of disease progression and novel therapeutic approaches for oral malignancies.

During the current reporting period, we have strategically initiated programs and recruited staff in areas which are relevant to oral cancer research. This includes the appointment of Dr. J. Silvio Gutkind as the Branch Chief, OPCB, the recruitment of Dr. Wendy Weinberg as a Staff Scientist to head a new workgroup focused on keratinocyte biology, and the expansion of Dr. Myung Hee Park, Dr. Frank Robey, and Dr. Gutkind's research programs, initiating several new projects in the areas of oral epithelial cell proliferation, differentiation, and neoplastic transformation. This new direction will afford us a better understanding of normal epithelial cell biology, and will likely broaden the horizon on developing potential oral tumor markers and treatments. Furthermore, our Branch has been playing a leading role in a newly formed NCI/NIDCR/NIDCD Inter-Institute Consortium in Head and Neck Cancer, whose goal is the development of a comprehensive clinical research program in head and neck cancer at the NIH, based on the interdisciplinary expertise of the intra- and extramural components of the three institutes.

Our Branch has been highly productive during this year: our annual report lists over 40 publications. We have made substantial contributions to the field, providing new concepts and shedding new light on questions of fundamental importance for cancer biology. We have also developed a large number of novel reagents, such as new genes, expression vectors, cell lines, antibodies, and bioactive peptides of value to biomedical research, and provided them to hundreds of investigators in the U.S. and abroad. Another tradition of our Branch has been our high priority on the training of postdoctoral investigators to become independent leaders in the field; we have tried to continue and strengthen this commitment. During the current reporting period, progress has been made in a number of research efforts at the OPCB. A variety of arbitrarily selected research advances are highlighted below. The progress report for each project provides a more comprehensive description of the major findings in our Branch.

We have recently initiated a new drug evaluation effort at the NIDCR, whose goal is to develop novel therapies aimed at improving the quality of life and life expectancy of oral cancer patients. As part of this project, we have recently evaluated newly identified drug candidates for their effectiveness in squamous cell carcinomas as a collaborative effort with the Developmental Therapeutic Program, NCI. It was found that one of the tested new drug candidates, flavopiridol, potently inhibits the proliferation of human squamous carcinoma cells and dramatically reduces tumor growth *in vivo*, thus establishing flavopiridol as a suitable drug candidate for treatment of head and neck carcinomas.

We have recently teamed up with other ICs to establish the Head and Neck Cancer Genome Anatomy Project, a collaborative joint effort with the NCI's Cancer Genome Anatomy Project. This involves the generation of cDNA libraries from squamous cell carcinoma lines and from microdissected cells derived from oral cancer tissues. After random sequencing, expressed genes are being catalogued and compared with those from normal corresponding tissues, and clones and data are being made available to qualified investigators through a new Web site. This work is expected to help identify gene products involved in the neoplastic process, as well as novel molecules representing clinically useful markers of oral carcinogenesis.

Acquired immunodeficiency syndrome-associated Kaposi's sarcoma (AIDS-KS) is the most common malignancy in human HIV infection. The oral cavity is frequently involved in AIDS-KS and may represent one of the most frequent initial sites of this malignancy. Kaposi's sarcoma associated herpesvirus (KSHV/ HHV 8) is a newly characterized γ-2 herpesvirus implicated in the pathogenesis of Kaposi's sarcoma. As part as a collaborative effort, we have recently found that a G protein-coupled receptor (GPCR) encoded by the open reading frame 74 of KSHV (KSHV-GPCR) displays constitutive (agonist-independent) activity, and is able to stimulate signaling cascades linked to cell proliferation and expression of angiogenic growth factors, such as VEGF.

Receptors coupled to heterotrimeric G proteins can effectively stimulate growth promoting pathways in a large variety of cell types, and if persistently activated, these receptors can also behave as dominant-acting oncoproteins. Consistently, activating mutations for G proteins of the $G\alpha_s$ and $G\alpha_{i2}$ families have been recently found in human tumors; and we have shown that members of the $G\alpha_q$ and $G\alpha_{12}$ families are fully transforming when expressed in murine fibroblasts. In an effort aimed to elucidate the molecular events involved in proliferative signaling through heterotrimeric G proteins we have focused recently on gene expression regulation. We have recently demonstrated that G protein coupled receptors, $G\alpha_{12}$ and the small GTP-binding protein RhoA are components of a novel signal transduction pathway that leads to the transcriptional activation of the c-*fos* Serum Response Element (SRE), and to cellular transformation. Furthermore, we have shown that a novel PDZ-domain containing guanine nucleotide exchange factor links heterotrimeric G proteins of the $G\alpha_{12}$ family to Rho. In turn, the small GTP-binding proteins of the Rho family control a variety of biological activities, including organization of the actin cytoskeleton, regulation of gene expression and cellular transformation. Using effector loop mutants of Rho, we have recently established that cytoskeletal changes are not sufficient to induce the transformed phenotype, and that Rho-effector molecules regulating the actin cytostructure are distinct from those signaling to the nucleus and subverting normal growth control.

We have investigated the growth and differentiation properties of normal human gingival keratinocytes (NHGK) and found that the $[Ca^{++}]$ optimum for culture and the squamous envelope composition of oral keratinocytes are different from those of normal human skin keratinocytes. We have also examined known markers of differentiation and found major differences in the expression of transglutaminase (TGase) I, TGase II and cytokeratins between NHGK and several head and neck cancer cell lines. In order to evaluate the roles of various proto-oncogenes and activated oncogenes in oral carcinogenesis, we introduced various oncogenes into immortalized

human gingival keratinocytes (IHGK). Immortalization of NHGK was accomplished by transfection with the pLXSN vector or the pBabe-Hygro vector carrying HPV16 E6/E7 genes, and immortalized cell lines were established after continued passage (>50) in vitro. IHGK express a similar pattern of cytokeratins as do NHGK and appear to retain the ability to induce TGase I and terminal differentiation after reaching post-confluence in media containing Ca^{++} at 0.15 mM. Various oncogenes implicated in head and neck squamous cell carcinomas (SCC) were individually transfected into IHGK. Oncogene-transfected cells were isolated and their growth and differentiation characteristics and tumorogenicity are currently under investigation.

Patients suffering from head and neck squamous cell carcinomas may have significant deficiencies in natural cellular and humoral immunity. Particularly, severe cellular immunosuppression can be detected early in the disease, and the cause of this is not clear yet. We have been building on our knowledge of immunosuppression caused by the envelope protein from HIV, gp120, as a template for studying HNSCC-induced immunosuppression. We have found that detergent extracts of solid tumors of HNSCC contain factors that are capable of binding to recombinant soluble CD4 in a fashion that is very closely related to the way gp120 binds to CD4. The same was found to be true of proteins secreted by cell cultures containing HNSCC. Studies using fluorescently labeled CD4 and HNSCC showed extensive labeling throughout the cells in culture and this indicates perhaps that natural proteins in the tumor, upon expression on the surface of the tumor or upon release from the tumor, can bind cell surface CD4 and induce CD4-mediated immunosuppression in T cells Additionally, whether the HNSCC-derived proteins related to bone proteins such as osteopontin and BSP are involved in immunosuppression is a topic of investigation at the present time.

We have utilized keratinocytes of murine epidermis and both *in vitro* and *in vivo* approaches to dissect the stages of differentiation and neoplastic progression. Current efforts involve studies aimed at elucidating the function and mechanism of action of the p53 tumor suppressor gene product in keratinocyte biology and in squamous carcinogenesis. Particularly, we have explored the role of the WAF1 gene product in multistep carcinogenesis, and using both *in vivo* and *in vitro* models have demonstrated that loss of the p53 mediated gene WAF1 is not sufficient to explain the malignant phenotype of p53 null tumors; thus, other features of p53 beside transcriptional activation of the WAF1 gene are necessary for its tumor suppressor function.

With the assistance of our NIDCR Gene-targeting Core Facility, we have recently generated transgenic mice carrying the tetracycline-inducible system (*tet*-on receptor) targeted to the basal layer of stratified epithelium using the cytokeratin 5 promoter. Transgenes of interest, including candidate oncogenes, will be then expressed under the control of a tetracycline-responsive promoter, in a tetracycline dependent manner. Using this and other biologically relevant animal models, we are now positioned to test the transforming potential *in vivo* of activated alleles of a number of newly discovered signaling molecules, alone or upon co-expression with dominant interfering mutants of a variety of tumor suppressor genes.

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Pain and Neurosensory Mechanisms Branch

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PAIN AND NEUROSENSORY MECHANISMS BRANCH
1998

The Pain and Neurosensory Mechanisms Branch (PNMB) conducts a multidisciplinary research program aimed at improved understanding and treatment of pain. Studies range from evaluating molecular responses to tissue injury and elucidating the mechanisms of peripheral tissue inflammation, including subsequent changes within the nervous system, to evaluating novel drugs and clinical hypotheses about pain and its control in human models of acute and chronic pain. The hallmark of the Branch's research program is the integration of basic and clinical research which permits not only a rapid transfer of new findings from the laboratory to the clinic, but also fosters basic research based on clinical problems. This integrative approach provides an optimal environment for training clinicians and basic researchers in the principles and methods of pain research across a spectrum spanning basic molecular mechanisms to the clinical management of pain. In addition, the Branch's senior investigators participate widely in speaking, writing, and collaboration with professional organizations and academic institutions to transfer emerging scientific information to the training of clinicians and the treatment of patients.

The independence and challenge presented by the NIDCR reorganization and the opportunities for PI-initiated research have increased scientific vigor and productivity in the Branch. The mean number of publications per investigator (N=8) during fiscal year 1998, an indirect measure of scientific productivity, exceeds the number of publications per investigator during fiscal year 1995 (N=7), the last year under the old organizational structure. The Branch continues to operate a large clinical research program, the Pain Research Clinic, in the Magnuson Ambulatory Clinical Research Facility under the scientific direction of Drs. Max, Dionne and Gracely. Research conducted in this clinic is based on observations made in the Branch's basic laboratories as well as using novel and prototypic drugs to test emerging scientific hypotheses in man, representing a true 'bench to bedside' continuum. Numerous publications in respected peer-reviewed scientific journals attest to the Branch's continued scientific impact. Highlights of research findings by Branch investigators during the past year are presented below.

Selective Neurotoxins and Gene Transfer Mechanisms of Analgesia

Demonstration of the internalization of the substance P receptor led to the hypothesis that this mechanism could interrupt pain transmission by selectively lesioning substance P receptor expressing cells in the spinal cord. Drs. Iadarola and Caudle coined the word "nocitoxins" to describe this concept of administering a ligand combining a toxin with substance P which when taken up into a SP secreting cell, results in cell death. They evaluated a compound composed of a substance P-diphtheria toxin fusion protein in the chronic constriction injury model. Administration of the fusion protein resulted in a partial, bilateral loss of SP receptor in the superficial dorsal horn which was accompanied by reduced mechanical and thermal hyperalgesia, but preserved normal nociceptive sensitivity in the side opposite the constriction injury and motor function in general, suggestive of selectivity for suppressing pathologic pain while maintaining normal protective sensory function.

Dr. Iadarola's lab also initiated an in vivo gene transfer program as a potential new means to treat chronic pain. Initial attempts with plasmids proved unsuccessful but direct infusion of adenoviral vectors was effective for transferring and expressing a reporter gene to spinal cord neurons and glial cells. The expression cassette is composed of a nerve growth factor leader sequence fused to beta-endorphin and is designed to secrete beta-endorphin. Injection of this vector into rat cerebrospinal fluid resulted in gene transfer to the cells covering the spinal cord and a substantial increase in cerebrospinal fluid beta-endorphin content. In an inflammation model of persistent pain, administration of the vector attenuated inflammatory hyperalgesia but had no effect on basal nociceptive responses. These data demonstrate a gene transfer approach to treatment of chronic pain disorders or cancer pain and will be further developed over the next year for possible clinical use.

Neonatal Persistent Pain Produces Plasticity in Nociceptive Neuronal Circuits

Plasticity in the nervous system is increasingly recognized as a potential mechanism of chronic pain which persists after the initial stimulus or injury has been removed. Work in the Cellular Neuroscience Section this year provides insight into how this process may occur. Spinal nociceptive neural circuits develop during both embryonic and postnatal times when painful stimuli are normally absent or limited. Pain represents a unique stimulus when pathways are programmed to develop without nociceptive stimuli. A study conducted by Drs. Ruda and Ling examined the effect of early postnatal persistent pain on development of spinal pain pathways as a model for the effects of persistent pain on plasticity in the nervous system. On postnatal day one rats received an injection of complete Freund's adjuvant, a chronic irritant, unilaterally into a hindpaw followed by administration of a horseradish peroxidase conjugate at eight weeks of age as a marker for small diameter primary afferent nerve fibers. Spinal cord segments and dorsal root ganglion were later examined for labeling of these small diameter fibers in comparison to large myelinated fibers. Neonatal injection of the hindpaw resulted in a different pattern of staining in various laminae of the dorsal spinal cord as compared to the untreated paw. No difference was seen in labeling of the large unmyelinated axons in the right and left sciatic nerve. These data demonstrate a dramatic alteration in spinal pain pathways after neonatal persistent pain. This plasticity in nociceptive neuronal circuits may result in differences in the response to painful stimuli in the adult.

Imaging Pain Pathways in Humans

The human functional brain imaging program conducted by Drs. Iadarola and Coghill continues to define the neural networks that subserve pain, the interaction between these networks, and the functional abnormalities induced by chronic pain in select pain populations. One study completed this year documents that acute pain induces a decrease in global cerebral blood flow that is likely attributable to activation of the sympathetic nervous system. This novel finding suggests a previously unidentified sympathetic regulation of the cerebral vasculature in acute pain/stress states and may explain pain-related syncope. A second investigation explored the relationship between painful heat stimuli, subjects' rating of pain intensity and unpleasantness, and regional brain activation. A significant relationship was observed between pain intensity and activation of a diverse array of brain regions providing a new level of insight into the

organization of brain mechanisms of pain processing. Two general schemes are evident: a distributed, parallel mechanism that is closely coupled with information about pain intensity, and a hierarchical mechanism involved in processes related to pain, but secondary to pain intensity. The distributed processing of pain intensity within the human brain ensures that this critical ability to detect tissue injury can be spared in the face of central nervous system damage while the hierarchical aspects of pain processing provide a basis for cognitive modulation of pain.

Another study conducted in collaboration with Dr. Gracely compared three qualitatively different pain sensations: pain evoked by brief painful heat stimulation of the skin, prolonged deep pain produced by exercising an ischemic limb, and the dysesthetic sensations that accompany reperfusion of the limb after 25 minutes of blood flow occlusion. The results showed a profound difference in the inferred patterns of supraspinal processing to these qualitatively different sensations. Brief heat pain sensations were accompanied by a large number of activations that have been observed in previous studies. The prolonged pain of ischemia resulted in fewer activations but with a marked increase in scalp blood flow by 20 minutes. The dysesthetic sensations were accompanied by a dramatic increase in activity in the somatosensory cortex, thalamus and insula. This result emphasizes the negative impact of dysesthesias, which are commonly observed in neuropathic pain conditions. The differences in activation with three equally painful conditions challenges the concept of a general pain-related pattern of supraspinal activity and suggests possible mechanisms which will be addressed in future studies.

Antihyperalgesic Effects of an AMPA/kainate Receptor Antagonist in Humans

A studied conducted by Dr. Max and colleagues in the past year involved the first reported administration of an antagonist for the AMPA/kainate receptor in humans. The study determined the maximally tolerated dose followed by a randomized, double-blind crossover study comparing the maximally tolerated dose, 1/3 the dose or placebo on capsaicin-evoked pain, mechanical hyperalgesia and allodynia. There was a dose-related reduction of pain, hyperalgesia and allodynia with the 1/3 maximally tolerated dose producing about a 50% reduction. While the maximally tolerated dose produced transient visual obscuration in most subjects, the lower dose was well tolerated. These data suggest possible clinical utility to developing this class of drugs in favor of other types of NMDA receptor antagonists that produce a high incidence of adverse effects along with their anti-hyperalgesic effects.

Postoperative Pain Contributes to the Development of Central Hyperalgesia in Humans

It has been previously demonstrated in several clinical models that intraoperative nociceptive barrage contributes to the development of central hyperalgesia manifesting as increased clinical pain at later time points. A clinical study concluded this year evaluated the role of postoperative pain to the development of central hyperalgesia in humans. Subjects were randomly allocated to receive local anesthetic blockade of painful input either prior to oral surgery conducted under general anesthesia or prior to the onset of postoperative pain, both in comparison to placebo anesthetic. Blockade of the relatively brief duration of intraoperative nociceptive barrage could not be differentiated from patients receiving placebo. In contrast, blockade of postoperative nociceptive input significantly lowered the amount of pain reported at 48 hours, suggestive of

attenuation of the development of central hyperalgesia. These observations demonstrate the role of postoperative nociceptive input in the development of central hyperalgesia and suggest the need for preventive measures prior to pain onset to block pain during the postoperative period.

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Functional Genomics Unit

Ashok Kulkarni

FUNCTIONAL GENOMICS UNIT
1998

Our Unit continues to generate exciting research advances in the pursuit of basic scientific knowledge in dissecting pathways relevant to the disorders that affect craniofacial and dental systems as a result of genetic abnormalities and inflammation. The Unit's primary research approach is centered on functional genomics. Since its inception, the key research work of the Unit has concentrated on the following studies:

Molecular Genetics of Development

In the first set of studies, we have begun to delineate precise roles of Cyclin dependent kinase-5 (Cdk5) in neuronal phosphorylation to gain insight into its role in abnormal phosphorylation observed in a number of neurodegenerative disorders. Following cloning of Cdk5 gene, we generated mice deficient in Cdk5 expression which exhibit perinatal mortality associated with gross lesions in the brains and spinal cords. Normal stratification of neocortical neurons in the cerebral cortex was absent in Cdk5(-/-) mice. These lesions included abnormal corticogenesis with a lack of neuronal, cerebellar defoliation, accumulation of neurofilaments in the neuronal cell bodies and ballooned motor neurons. Further studies revealed a typical inverted cortex in these mice indicating a special role of Cdk5 in neuronal migration. These findings prompt us to analyze in the future the key components of the neuronal cytoskeleton in the Cdk5 "conditional" knockouts.

In the second project, we chose to work on Fabry disorder because of its unique nature as a painful and fatal metabolic disorder and the challenges it presents in developing much needed therapeutic approaches. Following the cloning of the murine gene of alpha-galactosidase A, the gene involved in Fabry disease, we generated null which exhibit lipid inclusions in the target organs typically seen in Fabry patients. In collaboration with the Laboratory of Cell Biology in NCI, using gene therapy approaches we have demonstrated correction of the deficiency of enzymatic activity and the clearance of accumulated material in the cells derived from these mice. We are currently evaluating the progressive phenotypic changes in the older mice.

In the third project, we have begun to delineate the in vivo role of dentin sialophospho protein gene (dspp) in dentinogenesis. We have cloned and begun to characterize the structure, regulation and functions associated with the dspp gene products. We have developed a transgenic animal model with a reporter gene (ß-galactosidase) under the control of 5.7 kb 5' flanking dspp sequences for further characterization.

Molecular Genetics of Inflammation

In this category of projects, we have begun to analyze the autocrine and endocrine roles of transforming growth factor beta-1 (TGF-ß1) and macrophage migration inhibitory factor (MIF) in immune dysregulation and inflammation. The initial findings from studies indicate ameliorating effects of MHC-1 deficiency in inflammatory responses in the absence of TGF-ß1 and also involvement of MIF beyond the immunology domain.

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Immunopathology Section

Larry Wahl

IMMUNOPATHOLOGY SECTION
1998

The focus of the Immunopathology Section is on defining the factors and signal transduction pathways involved in the modulation of human monocyte functions that may contribute to the immunopathology associated with various disease states. Connective tissue destruction is associated with many diseases in which the monocyte/macrophage is a prominent cell. A major emphasis has been placed on how matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) are regulated, since these enzymes and inhibitors are believed to play a major role in the destruction and remodeling of connective tissue.

Cytokine regulation of monocyte MMPs and TIMP-1

We have shown that the individual addition of TNFα, GM-CSF, or IL-1β to monocytes enhanced MMP-9 and TIMP-1 but failed to induce MMP-1. However, when these cytokines were added together, particularly TNFα and GM-CSF, they stimulated the synthesis of MMP-1 and synergistically enhanced MMP-9 and TIMP-1. IFN-γ has been shown to inhibit MMP production by Con A or LPS activated monocytes but the effect of this cytokine on these mediators differs in the presence of other cytokines. IFN-γ inhibited the enhancement of MMP-9 and TIMP-1 by TNFα or IL-1β, but increased GM-CSF induced levels of MMP-9. Moreover, GM-CSF blocked the ability of IFN-γ to inhibit TNFα or IL-1β induced MMP-9. Of particular interest was the induction of MMP-1 when IFN-γ was combined with GM-CSF. IFN-γ also enhanced MMP-1 production induced by the combination of GM-CSF and TNFα but decreased MMP-9 and TIMP-1. These results indicate that regulation of monocyte MMPs and TIMP-1 at an inflammatory site is complex and depends, in part, on the specific cytokines present.

Signaling pathways involved in the differential regulation of monocyte MMPs

Our recent studies on the signal transduction pathway leading to MMP production by LPS stimulated monocytes have revealed that p42 MAP kinase is predominately involved in the regulation of MMP-9 whereas MMP-1 and prostaglandin H synthase-2 are mainly controlled by p38 MAP kinase. Inhibition of p38 MAP kinase activity significantly reduced the LPS-induced MMP-1 and phosphorylation of AP-1, CREB, and NFκB. This inhibition was reversed by the addition of PGE2, a known modulator of monocyte MMPs. However, inhibition of p42 MAP kinase activity did not block LPS-mediated activation of AP-1 and CREB, but did irreversibly suppress the phosphorylation of NFκB. Taken together, these findings indicate that p42 MAP kinase activation appears to be linked to the preferential regulation of MMP-9 through NFκB, whereas the phosphorylation of AP-1 and CREB by p38 MAP kinase is involved in the regulation of monocyte MMP-1 and prostaglandin H synthase-2.

Monocyte MT1-MMP

The recently described membrane-type metalloproteinases (MT-MMPs) form a family of membrane-bound enzymes that have as one of their functions the activation of MMP-2 (gelatinase A). We have shown that activation of monocytes results in the induction of MT1-

MMP mRNA and protein through a prostaglandin dependent mechanism. Monocyte MT1-MMP activated the MMP-2 produced by human marrow stromal cells and some cancer cell lines. These findings suggest that the MT1-MMP expressed on monocytes may have an important role in bone metabolism and in cancer progression.

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Matrix Metalloproteinase Unit

Henning Birkedal-Hansen

MATRIX METALLOPROTEINASE UNIT
1998

The research of the Matrix Metalloproteinase Unit explores mechanisms which enable cells to dissolve and degrade the extracellular matrix. Specifically we are seeking to define the role of matrix metalloproteinases (MMPs) and their inhibitors in this process.

A body of evidence suggests that MT1-MMP, TIMP-2 and Gelatinase A play important roles in mediating or regulating collagen degradation. Using gene targeting approaches we observed that disruption of the MT1-MMP gene resulted in a severe skeletal and soft tissue phenotype indicative of major defects in extracellular matrix metabolism including dwarfism, severe runting, osteopenia, deficient enchondral and intramembraneous bone formation, abnormal craniofacial development, and premature death. The MT1-MMP phenotype is the most severe of any MMP "knock-out." Disruption of the TIMP-2 gene by deletion of exons 2 and 3 gave rise to seemingly minor phenotypic changes, but further analysis unveiled significant and profound differences between wildtype and mutant mice. In a murine mammary tumor virus (MMTV) model, TIMP-2 mutant mice had the essentially the same tumor burden but much lower levels of (spontaneous) lung metastasis than wildtype mice. We also noticed that while Gelatinase A is partially activated in extracts of normal lungs, comparable extracts from homozygous TIMP-2 mutant mice contain Gelatinase A in its pro-enzyme form, lending the first in vivo support for the MT1-MMP activation of Gelatinase A through TIMP-2 bridging.

In search of enzymes and inhibtiors which regulate and catalyze the metabolic degradation of extracellular matrix, we pursued a strategy which allowed us to monitor the dissolution of a proteolytically resistant, single-component matrix (collagen type I fibrils) by live cells. Using this model, we examined the function of MT1-MMP and TIMP-2 deficient mice using cells derived from homozygous mutant mice. Cells deficient in MT1-MMP show greatly reduced ability to activate Gelatinase A and to dissolve collagen fibrils. Cells derived from TIMP-2 deficient mice showed reduced ability to activate Gelatinase A (MMP-2) and failed to degrade collagen fibrils in some model systems, lending support to the notion that (at least some) pathways leading to dissolution of collagen fibrils are mediated by a TIMP-2/Gelatinase A-dependent mechanism. Transfection experiments further confirmed the requirement for both MT1-MMP and Gelatinase A for expression of a collagen-degrading phenotype.

To better understand MMP activation reactions at the molecular level, we also explored the function of the "Cysteine Switch." The switch is the structural and molecular basis for catalytic latency and activation of MMPs. Using a site-directed mutagenesis approach, we learned that the propeptide is held in place in its docked position by coordinate bonding to the active site Zn (Cys-Zn bond) and by a number of secondary molecular interactions along the propeptide. Disruption of either of these, by any of a number of disparate means, results in displacement of the propeptide and opens up access to the active site (i.e. activation).

The MMP unit also conducted studies on enamelysin (MMP-20) which appears to be the only MMP specific for tooth development. During the past year we isolated, and completed the characterization of, the 65 kilobase mouse enamelysin gene. A targeting vector for disruption of

the gene by homologous recombination has been constructed and chimeric mice generated. These studies are being conducted in collaboration with Dr. John Bartlett (Forsyth Dental Center, Boston, MA) and Dr. James Simmer (University of Texas Health Science Center, San Antonio, TX).

MATRIX METALLOPROTEINASE UNIT
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Molecular Structural Biology Unit

Dennis Torchia

MOLECULAR STRUCTURAL BIOLOGY UNIT
1998

The principal research goal of the Molecular Structural Biology Unit (MSB) is to elucidate the structure and dynamics of proteins, RNAs and associated molecules at the atomic level in order to provide a basis for understanding function. The main research tool used in this work is high resolution multidimensional nuclear magnetic resonance (NMR) spectroscopy. Three projects are currently active: (1) HIV-1 protease, as either the free enzyme or bound to a high affinity protease inhibitor; (2) the RNA binding protein S4, either free or bound to a target RNA molecule; (3) MAP-30, an anti HIV protein. Following is a summary of the progress the MSB has been made in characterizing the molecular structure and dynamics of these systems as well as the prospective research for the coming year.

HIV-1 protease

The molecular dynamics of a fully active, but stable protease mutant (Q7K, L33I, L63I) has been studied in order to understand how substrates and inhibitors are able to enter and exit the protein's active site. Novel relaxation experiments developed in the past year in the Unit show that residues G48 through I54 (in the flaps of the protein) undergo slow conformation exchange. We suggest that these slow motions reflect the dynamic equilibrium between the semi-open and fully open flap conformations. It is these slow conformational fluctuations of the protease flaps that permit entry and exit of substrates, products and inhibitors from the active site of the protein. The goal of future work is to characterize the dynamics of the protein side chains that make up the substrate/inhibitor binding site of the protease.

Structures of the ribosomal proteins S4-d41 and S4

We have solved the solution structure of S4-d41, the C-terminal domain of the RNA binding ribosomal protein S4 (S4-d41, 159 residues, S4, 200 residues). The relative orientation of the subdomains of S4-41 is loosely defined, and because the likely RNA binding site consists of side chains from each subdomain, relative flexibility of the subdomains could explain how the protein binds to two very distinct RNA target molecules. However, limited NOESY data could limit the precision of the solution structure. Therefore, we are measuring dipolar couplings to better determine the relative orientation of the subdomains of S4-d41 in solution. We have also begun work on determining the structure of intact S4, which binds to mRNA about ten-fold better than S4-d41. Our goal will be to determine how the N-terminal 41 residues affect (a) the structure of the protein and (b) its binding to its target mRNA.

Structure/function study of the anti-HIV protein, Map30

Using multidimensional NMR methods, we have obtained nearly complete signal assignments of this 263-residue protein. Based upon these assignments, ca. 4000 distance, angle restraints and dipolar restraints have been used to derive the solution structure of the protein. Comparison of the backbone coordinates of the average MAP30 solution structure and crystal structures of homologous proteins Ricin A, tyrchosanthin, byrodin and momordin show that all these proteins

have very similar overall structures. This observation raises the question as to how MAP30 can have several distinct activities not shared by ricin A and other RIPs. Preliminary results suggest that the anti-HIV integrase activity reported for MAP30 results from MAP30 binding to DNA substrates, rather than from a direct interaction with integrase.

The PI was invited to speak at the 27'th International Conference on NMR in Biological Systems in Tokyo, and serves as Associate Editor of PROTEINS. The PI participated as a major reviewer on the site visit of the NMR facility of the National Magnetic Lab, reviews ca. 50 proposals/scientific papers per year and maintains research collaborations with researchers in major institutions in this country and abroad. He was appointed to the SBRS in 2/98.

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