Research Within CareGroup: Summary of Projects at the New England Baptist Bone and Joint Institute Basic Science Laboratory

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INTRODUCTION

The Orthopaedics Department at Beth Israel Deaconess Medical Center, as a part of CareGroup, has begun a new collaboration with the New England Baptist Bone and Joint Institute Basic Science Laboratory. This laboratory is located at the Harvard Institutes of Medicine, Harvard Medical School. It occupies over 8,000 square feet of research space that includes a Genomics Core (that serves as the Beth Israel Deaconess Medical Center Genomics Center) and a Molecular Pathology Core. There are nine principal investigators who are working with a team of thirty-three post-doctoral fellows, graduate students, and laboratory technicians investigating more than two dozen studies. These projects are funded by over $5 million from the NIH, the Arthritis Foundation and also from private industry support. One of those projects, the NIDDK Biotechnology Center Grant, is one of only ten awarded in the country by the National Institutes of Health.

PRINCIPAL INVESTIGATORS: CURRENT PROJECTS

Steven R. Goldring, M.D. (Director of Research)

Steven R. Goldring, M.D. is the Director of Research for the laboratory. He is a Professor of Medicine at Harvard Medical School and is the Chief of Rheumatology at the Beth Israel Deaconess Medical Center and the New England Baptist Hospital.

Cellular Responses to Inorganic Particulates

The aims of this project are to: (1) test the hypothesis that particle surface chemistry and crystal structure are critical determinants of the pattern and magnitude of cell responses to inorganic particulate wear debris associated with total joint replacements, (2) test the hypothesis that lipopolysaccharide (LPS) "contamination" accounts for a component of particle-induced cell responses, and (3) test the hypothesis that the molecular pathways by which particles regulate the IL-1ß and TNF-α genes differ and that particle-mediated effects involve LPS-dependent and independent signal transduction systems. These experimental approaches will permit the dissection of the molecular mechanisms and signaling pathways by which foreign particulate materials modulate cell responses and will provide important insights into the factors responsible for the adverse cellular and tissue reaction to particulate implant wear debris.

Calcitonin Receptor Gene Expression

The aims of this project are to: (1) characterize the structural and functional properties of the osteoclast calcitonin receptor, (2) identify the phenotypic relationship between bone resorbing cells in physiological and pathological remodeling, (3) determine if there are osteoclast "subtypes" without calcitonin receptors and if so, what controls their development, and (4) determine the cellular and molecular mechanisms regulating calcitonin receptor gene during osteoclast differentiation.

Philip Auron, Ph.D.

Accessory Cell Activation in the Immune Response

The major goals of this project are elucidation of the mechanism for immediate early gene induction in activated monocytes using the IL-1 gene as a model.

IL-1 Receptor Mediated Signal Transduction

The major goals of this project are to understand the mechanism of IL-1 receptor signal transduction, focusing on both ligand-receptor interactions and cytoplasmic domain recruitment of signaling molecules, focusing especially on the mechanism of phosphatidylinositol 3-kinase activation of NF-κB.

Jason, Boch, D.M.D., D. Sc.

Osteoclastogenesis and RANK signaling to NF-kB via TRAF6

Considerable evidence in animal models and in humans indicates that bone loss in periodontitis is produced by osteoclasts. The origin of osteoclasts and the factors responsible for their recruitment, differentiation, and activation are not well defined. A newly described, required factor for the differentiation and activation of osteoclasts is Receptor and Activator of NF-κB Ligand (RANKL) which mediates its effects via a specific cell surface receptor, Receptor and Activator of NF-κB (RANK). This receptor is a member of the TNF receptor family, and is expressed on osteoclasts and osteoclast precursors. With RANKL binding to its receptor, a cascade of signal transduction events occurs to ultimately culminate in the activation of transcription factors, which effect expression of target genes important for osteoclast differentiation. NF-κB is one such transcription factor that is known to be activated and required for osteoclastogenesis. The signaling pathway from receptor to activation of NF-κB is not understood. The intracellular domain of RANK is linked to downstream signaling molecules

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by adapter proteins of the TNF Receptor Associated Factor (TRAF) family, which have no intrinsic catalytic properties, but are able to associate with molecules that do. One member, TRAF6, associates with RANK, and links it to a MAP3 kinase named TGFβ Activated Kinase 1 (TAK1), which is involved in NF-κB activation. The studies outlined in this proposal will examine how TRAF6 functions to activate TAK1 and induce NF-κB activation and how these events lead to osteoclast differentiation and activation. A more detailed understanding of the basic regulation of osteoclastogenesis may permit development of potent novel therapeutics for treating periodontal disease and other inflammatory disorders associated with bone loss.

Deborah Galson, Ph.D.

Regulation of the Murine Calcitonin Receptor Gene

The aims of this project are to: (1) clone and characterize the murine CTR gene and identify the transcription start site(s) in osteoclasts and other mCTR-expressing cell-types, (2) define the specific and potentially unique regulatory sequences responsible for expression of mCTR in osteoclasts and other mCTR-expressing cells and tissues and identify the putative trans-acting transcription factors that bind to these sequences, (3) investigate the developmental regulation of the mCTR gene, and (4) determine the effect of targeted disruption of the mCTR gene on osteoclasts and other mCTR-expressing tissues, and on mouse development.

Mary Goldring, Ph.D.

Signaling Pathways in Chondrocyte-Specific Gene Expression

The major goal of this work is to define the cellular and molecular mechanisms by which catabolic cytokines suppress differentiated phenotype in chondrocytes. The hypothesis is that cytokine- and dedifferentiation-induced downregulation of chondrocyte phenotype occurs by both distinct and convergent signaling pathways.

Regulation of Matrix Gene Expression in Human Chondrocytes

The major goals of this project are to: (1) determine factors that maintain and enhance chondrocyte phenotype in immortalized human chondrocyte culture models *in vitro*, (2) identify molecular mechanisms involved in induction and maintenance of differentiated chondrocyte phenotype by direct analysis of regulatory sequences of the cartilage-specific type II collagen gene (COL2A1), and (3) examine factors influencing cartilage formation *in vivo*.

Ellen Gravallese, M.D.

(Director of Molecular Pathology Core)

Pathogenesis of Bone Erosion in Rheumatoid Arthritis

Considerable evidence indicates that focal bone erosions in rheumatoid arthritis are produced by cells expressing phenotypic features of osteoclasts. The origin of these osteoclast-like cells and the factors responsible for their recruitment, differentiation, and activation are not well defined. An essential factor for the differentiation and activation of osteoclasts is receptor-activator of NF-kB ligand (RANKL). The balance between RANKL and its decoy receptor, osteoprotegerin, an inhibitor of RANKL activity, is a critical determinant of osteoclast differentiation in normal bone remodeling. The major goals of this project are to test the hypothesis that cells present in rheumatoid arthritis synovium at sites of bone invasion provide a source of RANKL that contributes to pathologic focal osteoclast mediated bone resorption. An increased understanding of the pathogenesis of bone erosion in rheumatoid arthritis will lead to new therapeutic strategies for preventing the disabling bone destruction in this disease.

Martha Gray, Ph.D.

(Co-Director of the Harvard-MIT HST Program)

In vivo MRI of Cartilage Glycosaminoglycan Content

The major goal of this project is to develop a technique for monitoring articular cartilage glycosaminoglycan content *in vivo*. The specific aims are to: (1) establish a protocol for the delivery of $Gd(DTPA)^{2-}$ to cartilage *in vivo*, (2) quantify the levels of glycosaminoglycan *in vivo*, and (3) validate the interpretation of the *in vivo* method as providing a quantitative measure of cartilage glycosaminoglycan content.

Clinical and Non-Invasive Mechanical and Physico-chemical Analysis of Hip Dysplasia

The specific aims of this project are to: (1) characterize glycosaminoglycan density in articular cartilage, using MRI, in acetabular dysplasia. Correlate these MRI findings with clinical symptoms and conventional radiographic measures as well as load density profile obtained using CT analysis, and (2) determine if periacetabular osteotomy is capable of restoring the articular cartilage integrity in dysplasia hips. If the articular cartilage integrity is restored, does it correlate with restoration of normal hip joint mechanics and does it correlate with resolution of symptoms?

MRI of Cartilage Mechanical Properties

An MRI technique to image the glycosaminoglycan content of cartilage has been demonstrated that can serve as a surrogate for biochemical and histological evaluation of the tissue glycosaminoglycan. The main challenge being addressed by this proposal is to obtain pilot data to determine whether this MRI technique can be used to predict the *mechanical* properties of the tissue.

Towia Libermann, Ph.D.

(Director of BIDMC Genomics Center Research Projects)

Function of ELF-1 and a Novel Ets Factor NERF in B Cells

The major goals of this project are to determine the role of ELF-1 and NERF in regulation of blk gene expression and to determine effect of targeted disruption of the NERF gene on B cell development and blk gene expression

Role of New Ets Factor, ESE1, in Epithelial Cells

The major goals of this project are to determine the biological role of a novel epithelial cell-specific transcription factor, ESE-1, during epithelial cell differentiation and to explore its role as a down-stream target of cytokines involved in the pathogenesis of inflammatory disorders such as rheumatoid arthritis.

NIDDK Biotechnology Center

The goal of the NIDDK Biotechnology Center is to build

a comprehensive integrated microarray facility that will allow researchers to create and analyze customized and commercial expression arrays as tools to gain new insights into disease pathogenesis and mechanisms. Furthermore, this comprehensive integrated microarray facility will also permit the characterization of the functional and regulatory pathways of disease related genes.

Role of the Novel Prostate-Specific Transcription Factor, PDEF, on Prostate Cancer

The major goal of this project is to determine the role of PDEF, a member of the Ets transcription factor/oncogene family, in prostate cancer. Due to direct implication of Ets factors in human cancers, PDEF is expected to play a role in the proliferation and metastatic spread of prostate cancer.

Peter Oettgen, M.D.

The Role of the Ets Factor NERF in Vasculogenesis

Vasculogenesis is the formation of blood vessels and a primary vascular network in the developing embryo. Several growth factors including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), Angiopoeitin, and their receptors Flk-1, Flt-1, Tie1, and Tie2 have been identified as being important mediators of these events. The approach to determining the factors which are critical for blood vessel development and endothelial cell differentiation is to identify the transcription factors which regulate vascular-specific genes. The Ets genes are a family of transcription factors that regulate developmental processes and cellular differentiation. Conserved Ets sites have been identified in the promoters of the Tie1 and Tie2 genes which are necessary for vascular directed expression of these genes. In addition, a novel human Ets factor, NERF which is expressed in endothelial cells has been characterized. This Ets factor is expressed as at least three isoforms, of which the NERF2 isoform is a strong transactivator of the Tie1 and Tie2 genes. The NERF1 isoforms can down-regulate vascular specific genes, and their overexpression leads to the impaired formation of vascular tubes and channels. The hypothesis for these studies is that selected members of the Ets factor family and in particular, NERF, are critical regulators of vascular development and endothelial function.

The Role of ESE-1 in Vascular Inflammation

Inflammation, a hallmark of atherosclerosis and other vascular diseases is characterized by the activation of several genes including cytokines, adhesion molecules, cyclooxygenases, and nitric oxide synthase. Our approach to unraveling the molecular mechanisms that mediate early inflammatory responses is to identify the transcription factors that mediate these responses. The NF-kB family of transcription factors are known to be critical mediators of these events. We have determined that a novel Ets transcription factor ESE-1, is induced in response to interleukin-1(IL-1), tumor necrosis factor(TNF-α), and endotoxin in vascular smooth muscle cells, endothelial cells and monocytes. This induction is at least in part mediated by NF-kappa B. We have identified the inducible form of nitric oxide synthase (NOS-2) as a target for ESE-1. Induction of NOS-2 and consequent production of nitric oxide (NO), has a wide variety of cellular effects depending on the cell type and the amount of NO produced. The hypothesis for these studies is that the Ets factor ESE-1 is a transcriptional mediator of vascular inflammation. The goal of this study is to examine the role of ESE-1 as a transcriptional regulator of vascular inflammation and NOS-2 gene regulation. The results of these studies should provide new insights into the molecular mechanisms involved in regulating vascular inflammation and provide potential new therapeutic avenues for treatment of vascular diseases such as atherosclerosis, restenosis, and the vasculopathy.

The Role of Angiopoietin-1 in Rheumatoid Arthritis

Angiogenesis is a critical component of the inflammation associated with rheumatoid arthritis (RA). Several angiogenic growth factors including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) have been identified within the rheumatoid synovium, all of which promote the early steps of angiogenesis. Attempts to block angiogenesis by inhibiting these factors in animal models of inflammatory arthritis have resulted in partial reductions in overall inflammation, pannus formation, and the degree of angiogenesis. Recently, a novel angiogenic factor, Angiopoietin-1 (Ang-1), was identified that has the unique property of facilitating the later stages of angiogenesis. Our preliminary results demonstrate that Ang-1 is expressed in synovial fibroblasts derived from patients with RA. Furthermore, proinflammatory cytokines can markedly upregulate the expression of Ang-1 in these cells and induce Ang-1 expression in other cell types found in the rheumatoid joint including monocytes and chondrocytes. The expression of Ang-1 by synovial fibroblasts may also enhance endothelial cell migration to the growing pannus. The hypothesis of this proposal is that Ang-1 is one of the critical factors required for mediating the angiogenic response in rheumatoid arthritis. The goals of these studies are to define the role of Ang-1 in promoting the angiogenic component of inflammatory arthritis, to further define the role of NERF2 as a transcriptional mediator of Ang-1, and to examine the therapeutic potential of blocking Ang-1 during the development of inflammatory arthritis.