COMMON MACROPHAGE SIGNATURES FOR IMMUNE SUPPRESSION

ABSTRACT

Background:
The overall thematic goal of this ARC is to define signaling mechanisms engaged by infectious agents and/or host homeostasis systems that limit immune response. This specific ARC project (project 1) is designed to explore the role of the macrophage in activation and inhibition that lead to deficient immunes response to distinct pathogens. This will be achieved by bringing together five investigators with a general interest in macrophage biology and expertise in biochemistry, virology, bacteriology, molecular biology and infectious diseases with the intention of fostering new research interactions that can be leveraged for long-term funding opportunities.

Rationale: There is a broad array of regulatory mechanisms that are engaged to i) suppress immune responses to infection, ii) limit inflammatory tissue destruction during disease, and iii) promote immune mediated tissue homeostasis. Tissue resident macrohages, whose functions include resolving inflammation and repairing tissue damage, are central to preserving the critical balance between protective innate immunity and destructive inflammation. In this proposal, we hypothesize that evolutionarily distinct pathogens and inflammatory responses to these organisms share a common biological signatures in macrophages that are associated with evasion or suppression of host immune surveillance.

Specific Aim:
Project 1. Identify a common molecular signature in macrophages for pathogen-mediated immune suppression and evasion. *Microbial elicited host signatures will be discovered through analysis of candidate MAPK, miRNA, mRNA and flow cytometric markers. We will initially focus on the TNF/cachexin pathway to identify a common pathogen-independent signature, then expand our pathway analysis to define pathogen-dependent signatures.*

Significance:
The identification of pathogen dependent and independent signatures will provide ideal target(s) for drug discovery. In addition, characterizing key regulatory networks in activated macrophages will provide insights into mechanisms that contribute to a broad spectrum of inflammatory based diseases and will provide novel targets for the control of autoimmune disease.

PARTICIPATING FACULTY TO DATE

Monty Montano, PhD	Director	Assistant Professor	MED
Paul Skolnik, MD	Co-Director	Professor	MED
Andy Henderson, PhD	Co-I	Associate Professor	MED
Frank Gibson, PhD	Co-I	Associate Professor	MED
Lisa Ganley-Leal, PhD	Co-I	Assistant Professor	MED
Martin H. Steinberg, MD	Co-I	Professor	MED
Paola Sebastiani, PhD	Co-I	Associate Professor	BUSPH
Mayetri Gupta, PhD	Co-I	Assistant Professor	BUSPH

PROJECT 1

Project 1A: *MUSCLE REGENERATION – REGULATORY FEEDBACK LOOP IN MACROPHAGE RESPONSE TO INFLAMMATORY LIGANDS.*
PI: Montano, M.
Synopsis: Muscle atrophy is a generalized phenotype of multiple inflammatory states including injury, sepsis, cachexia and HIV associated wasting. During normal muscle remodeling, there is a well described early infiltration of inflammatory CCR2+GR1+ macrophages within 24 hours that declines over a few days, in association with an increase in the presence of anti-inflammatory, tissue growth promoting, CCR2-GR1+ macrophages. Using a cardiotoxin inflammatory model of injury, our laboratory has data suggesting that MAPK activity is dramatically induced 24 hours stimulation that is abruptly suppressed by day 3-4 in association with muscle repair. We speculate that MAPK activity is actively suppressed by host factors and propose to determine the role of MAPK phosphatases, as well as suppressive miRNA, specifically miR-155 and miR-

146a, in the resolution of muscle inflammation and repair. The inflammatory ligand inducers we will use include TNF, LPS, MCP-1.
ARC contribution for project 1A: Signature identification in this project will be based on flow cytometric characterization of macrophage subsets, followed by protein, RNA isolation to evaluate MAPK and miRNA profiles respectively.

Project 1B: *SUPPRESSION OF ALVEOLAR MACROPHAGE ACTIVATION BY HIV INFECTION*
PI: Skolnik, P.
Synopsis: (HIV)-positive persons are predisposed to pulmonary infections and HIV type 1 infection of macrophages impairs effector functions, including cytokine production. In our laboratory, we observed decreased constitutive and LPS-stimulated tumor necrosis factor alpha (TNF-alpha) concentrations and increased soluble tumor necrosis factor receptor type II (sTNFRII) in bronchoalveolar lavage fluid samples from HIV-positive subjects compared to healthy controls. Since TNF-alpha is an important component of the innate immune system and is produced upon activation of Toll-like receptor (TLR) pathways, we hypothesized that the mechanism associated with deficient TNF-alpha production in the lung involved altered TLR expression or a deficit in the TLR signaling cascade. We found decreased Toll-like receptor 1 (TLR1) and TLR4 surface expression in HIV-infected U1 monocytic cells compared to the uninfected parental U937 cell line and decreased TLR message in alveolar macrophages (AMs) from HIV-positive subjects. We postulate that HIV infection alters expression of TLRs with subsequent changes in mitogen-activated protein kinase signaling and cytokine production that ultimately leads to deficiencies of innate immune responses. We have data that miRNA is altered in association with these changes in the lung; specifically, we have shown altered patterns of miRNA in alveolar macrophages between HIV+ and HIV– subjects.
ARC contribution for project 1B: Signature identification in this project will be based on cytokine, cytokine antagonist, MAPK, and miRNA profiling in alveolar macrophages and peripheral blood monocytes from HIV+ and HIV– subjects in response to TLR2 and TLR4 ligands.

Project 1C: *MACROPHAGE TRANSCRIPTIONAL INHIBITORY AXIS DURING HIV INFECTION*
PI: Henderson, A.
Synopsis: Activation of macrophages and microglia cells after HIV-1 infection and their production of inflammatory mediators contribute to HIV-associated CNS diseases, but the mechanisms that initiate and maintain inflammation after HIV-1 infection in the brain have not been well studied. In our laboratory, we have demonstrated that Tat targets the receptor tyrosine kinase recepteur d'origine nantais (RON), which negatively regulates inflammation and HIV transcription. Furthermore, we have have shown that RON represses transcription by multiple mechanisms including inducing proximal promoter pausing.
ARC contribution for project 1C: Signature identification in this project will be based on mRNA profiling of TNF stimulated macrophages and mechanisms

transcriptional suppression and chromatin dynamics of specific immune gene targets by RON.

Project 1D: *BONE REMODELING – ROLE OF MACROPHAGE DURING INFECTION WITH P. GINGIVALIS*
PI: Gibson, F.
Synopsis: Published results in our laboratory demonstrate that macrophage-dependent TLR2 signaling is crucial for TNF-alpha-dependent/RANKL-independent osteoclastogenesis in response to P. gingivalis infection. Furthermore, the ability of *P. gingivalis* to induce the cell surface expression of TLR2 may contribute to the chronic inflammatory state induced by this pathogen. Very recently, we have observed distinct macrophage subsets during infection…
ARC contribution for project 1D: Signature identification in this project will be based on TLR dependent macrophage phenotyping during infection with P. gingivalis.

Project 1E: *SOLUBLE CD23 EXPRESSION BY MACROPHAGES IN S. MANSONI INFECITON*
PI: Ganley-Leal, L.
Synopsis: CD23, the low-affinity immunoglobulin (Ig)E receptor (FcepsilonRII), is widely distributed on the surface of various human cells, including B cells and Macrophages. Elevated IgE levels are often associated with resistance to reinfection in human schistosomiasis. In our laboratory, we have shown that expression of membrane CD23 (mCD23) on B cells correlated with the development of resistance against *S. mansoni*. Higher levels of plasma sCD23, the cleaved form of mCD23, also correlated with resistance and other markers of resistance to reinfection. In this project we will evaluate sCD23 production by macropahges in the context of infection with *S. mansoni*.
ARC contribution for project 1E: Signature identification in this project will be based on S. mansoni infection of macrophages and the production of mCD23, sCD23 and TNF pathway members, including cytokine-receptor pairs.

Project 1F: *MACROPHAGE SIGNATURE IDENTIFICATION*.
PI: Paola Sebastiani, PhD
PI: Mayetri Gupta, PhD
Synopsis: The identification of common MAPK, miRNA, mRNA and surface/soluble expression of ligands signatures across the inflammatory models proposed (i.e., muscle inflammation, HIV infection, P. gingivitis infection, S. mansoni infection and bone remodeling) will be achieved through the use of bayesian modeling techniques that we have developed and used in a variety of studies, in collaboration with Dr. Montano and many others at BUMC.

PROJECTS IN DEVELOPMENT

Project 2A: *MACROPHAGE ROLE IN VASO-OCCLUSIVE DISEASE*
PI: Martin H. Steinberg, MD
Synopsis: We hypothesize that vascular inflammation, marked by activated monocytes and endothelium, plays a critical role in the pathophysiology of vaso-occlusion in sickle cell disease and that disease severity modulated by these (and yet to be discovered) genes accelerate the vascular aging phenotype.
Mechanistically, we propose that monocyte activation in sickle cell disease can enhance vaso-occlusion by activating endothelium in a process that is critically dependent upon genes within this axis. SCD is associated with activation of monocytes and endothelial cells, potentially through cell-cell contact and subsequent activation of nuclear factor-kappa B (NF-kB) translocation. NF-kB activation then drives increased expression of multiple growth factors and cytokines, including TGFb and TNFa. To test this hypothesis, human endothelial cells and normal monocytes will be co-cultured in vitro, followed by measurement of endothelial activation. Signatures for monocytes will be obtained and validated against pathogen signatures in project 1.

FUTURE STUDIES

Future studies. Based on results obtained in this ARC project, we will propose funding to screen for inhibitors to a common signature identified in project 1 and evaluate small molecule candidates for their influence on infection using an array of pathogenic models. Pathway signatures with specific targets common to most pathogens tested will be used to identify inhibitory compounds using high-throughput technology. Candidate compounds will then be tested against specific infection of macrophages. However, the primary purpose of this ARC is to facilitate new collaborations and leverage independent resources with the long-term goal of establishing a center focusing on macrophages and inflammatory diseases. To realize this stretch goal, we hope to submit several multi-investigator NIH R01 proposals after the first year and by year three we expect to be in position to submit a program project related to macrophages and their role in immune suppression.