

ANCA Vasculitis Project: Summary/Aims

Anti-neutrophil cytoplasmic autoantibody (ANCA) associated vasculitides are a group of autoimmune diseases characterized by necrotizing inflammation of small blood vessels affecting multiple organs, most notably kidney, lung, joints and gastrointestinal tract. Despite a number of significant advances in recent years, the immunogenesis and etiology of ANCA disease remains poorly understood. Clearly more research in this field is desperately needed to gain better understanding of the pathophysiology these devastating diseases for the development of improved therapeutic approaches. Experimental models have provided a wealth of information on the pathogenesis of glomerular and small vessel disease; however it is widely viewed that additional economical models of ANCA disease that mimic the human condition and are amenable to large scale experimentation are needed. Genetically engineered mouse models have provided valuable tools to parse out roles for specific proteins or pathways in the pathogenesis of ANCA; however, they do not completely address the multifaceted etiology of immune-mediated processes. Classical approaches using immunization with ANCA proteins or passive transfer of antisera targeted to ANCA immunogens have also proven to be very valuable in the investigation of effector mechanisms involved in the acute vascular inflammatory phase and in the evaluation of therapeutic interventions in rodents. The above approaches however, are limited in scope, expensive, time consuming, and/or burdensome to conduct, thus may not be amenable to large scale multi-center investigation or pre-clinical testing. Specific ANCA antibodies are currently available for purposes of immunodiagnostics (immunohistochemistry, FACS, Western blotting, etc.). However, these antibodies are sold in formats that are too small in quantity and excessively costly to be used for routine experimental testing *in vivo*. This proposal focuses on the manufacture of large volumes of antisera directed against ANCA proteins that can be used to further develop passive-immune models of ANCA disease. Sheep antisera will be raised against three target ANCA proteins associated with human vascular diseases that have likely potential to induce similar disease in rodents. Antisera will be raised against ANCA antigens linked to glomerular and small vessel vasculitis including myeloperoxidase (MPO), protease 3 (PR3), and lysosomal-associated membrane protein-2 (LAMP-2). Funds from this grant will provide necessary support to permit Probetex to produce a centralized resource of bulk ANCA antisera on a scale that will provide critical tools to foster further research in acute processes in ANCA disease. Also, this project will provide important preliminary data for a NIH Small Business Innovation Research (SBIR) application designed to broaden the scope of these models to explore complex pathogenesis of ANCA disease such as “two-hit” phenomena. Our goal is to expand our product line of nephrotoxic antisera that will be used by academic, biotech and pharmaceutical companies to combat renal and vascular disease.

Specific Aims:

Aim 1. To produce sheep antisera that target relevant antigens that have qualified potential to induce experimental ANCA vasculitis: Sheep antisera will be raised against selected antigens that are shared by rodents and humans, are associated with human vascular disease, and have a high likelihood to induce similar disease in rodents. Using current technologies and established protocols, sheep will be immunized, antisera harvested, processed and characterized by *in vitro* immunoassays. Antisera will be produced targeting the following proteins associated with ANCA: myeloperoxidase (MPO), proteinase 3 (PR3), or lysosomal-associated membrane protein-2 (LAMP-2) antibody.

Milestones: Large volumes of high quality antisera will be produced that recognize each neutrophil immunogen and characterized by immunoassay, Western analysis, and immunofluorescence microscopy.

Aim 2. To assess each antibody *in vivo* to produce the salient features of its target disease model. The primary goals of this proposal are "Proof of Concept" to produce antibody that recognizes target antigens *in vivo* with the goal to replicate the salient features of ANCA vasculitis. Each antisera collection will be tested *in vivo* in rats to assure that antibody reaches its target structure and produces glomerular and/or small vessel disease. ANCA is considered as a “two-hit” phenomenon, therefore perturbation of the immune system using LPS, TNF α , or by administration of subnephritogenic doses of anti-glomerular basement membrane (anti-GBM) antibody prior to injection of ANCA serum will be tested. Efficacy of each antisera alone or blended to replicate clinical disease will be established using histopathological and functional criteria characteristic of the disease focusing foremost on glomerular injury but also on vasculature of organ systems.

Milestones: Each disease model will show characteristic histological features of glomerular and/or small vessel disease using routine microscopy, immunofluorescence, and electron microscopy and renal function tests.