David P. Basile, Ph.D.

Candidate's Statement Section

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II Candidate's Statement

I arrived at Indiana University in 2005 after spending several years at the Medical College of Wisconsin. My decision to come to Indiana University was motivated scientifically by the outstanding environment of cardiovascular and kidney disease research present on this campus in places such as the Department of Cellular & Integrative Physiology, the Division of Nephrology, the Indiana Center for Vascular Biology, and in Pediatrics. The potential interactions and environment represented exciting opportunities to build new collaborations and advance of my ongoing research program. During my time in service I have strived to build collaborations within the IUSM, publish work in quality journals, work diligently toward maintaining a funded research program, and to be a model citizen in the academic environment while promoting excellence in education. I hope that this dossier reflects progress toward these goals.

A. Research: Area of Excellence

My research program is centered on the pathophysiology of acute kidney injury (AKI), a significant clinical problem affecting up to 5% of hospitalized patients and associated with severe mortality rates. The primary focus is to understand the influence of altered vascular reactivity and structure after an acute injury on kidney function and the development of secondary chronic kidney disease (CKD). This work has substantial clinical parallels since the number of patients with AKI is increasing. Corresponding to that, the number of patients with chronic kidney disease after an episode of AKI is also increasing and it is currently being recognized as a significant contributor toward the development of End Stage Renal Disease (ESRD). Why this occurs is not known and has become the major focus of our laboratory. My laboratory has built its reputation on studying this question at a basic science level and is one of only a few laboratories worldwide that is seriously focusing on this important clinical issue.

Since my arrival at Indiana University, I have made a concerted effort to develop a research program characterized by cohesive and progressive studies and to create a distinguished, unique perspective in the field of renal pathophysiology. My work has sought to address the broad hypothesis that cellular and molecular elements of acute kidney injury are not reversible and predispose the development of chronic kidney disease. Most importantly we have focused on the role of impaired regeneration of kidney blood vessels as central to disease progression. Highlights of my published work addressing this overlying hypothesis are summarized briefly:

1) We demonstrated that acute kidney injury (AKI) resulted in a significant and permanent reduction in blood vessels and development of chronic kidney disease.
2) We demonstrated that AKI induced reductions in blood vessel loss were associated with alterations in renal oxygenation that affects the rate of chronic disease progression.
3) We demonstrated that AKI results in the development of salt-sensitive hypertension and that salt consumption profoundly hastens chronic kidney disease following AKI. We demonstrated that normal kidney sodium reflexes are impaired following AKI. We further demonstrated that AKI resulted in impairment of normal renal medullary hemodynamic responses, a feature likely to be associated with the impaired sodium handling mechanism.
4) We demonstrated that genomic tools can be used to screen biochemical pathways important in long term progressive functional impairment and identified fibrotic inflammatory, vasoactive and anti-vasculotrophic changes in gene expression in the setting of AKI.
5) We have demonstrated that supplementation with a vascular specific growth factor can preserve blood vessel density and protect against secondary chronic kidney disease.
6) We demonstrated that AKI alters the normal responses of blood vessel in the kidney and in the peripheral vasculature. We have demonstrated that this effect is likely due to the contributory effects of persistent inflammation and alterations in vascular oxidant stress.

7) We have demonstrated a rapid accumulation of renal interstitial fibroblasts in the kidney and we have further demonstrated that these fibroblasts derive in part from damaged vascular cells and this conversion may be the mechanism of vascular impairment.

Development of novel aspects of continuing research program

The research program outlined above has set the tone for continued studies geared to advance our scientific understanding of impaired vascular regeneration in the injured kidney and to generate novel approaches to alleviate chronic complications of AKI. It is hoped that these studies will set the stage for the development of translational and therapeutic modalities to address patient care. The items listed below represent novel questions planned for investigation over the next several years and will serve as basis for renewal of existing and/or novel grant applications.

Can endothelial colony forming cells (ECFC) from the kidney be studied to understand the impairment in vascular regeneration? The major question driving this research is whether the lack of regeneration in kidney blood vessels is due to intrinsic reductions in the endothelial progenitor cell population of the kidney and to determine if renal injury affects proliferative potential of ECFCs. In collaboration with Dr. Mervin Yoder (IUSM, Pediatrics), we have made significant strides to isolate renal-derived ECFCs. Initially funded with the assistance of a pilot/bridge funding program from the O'Brien Kidney Center Grant (PI, Bruce Molitoris, Nephrology) and the IU RSFG program, this work is a central component of our renewed RO-1 grant entitled "Long-term effects of acute renal failure".

Our data suggest that kidneys from adult animals possess only ECFC with low colony forming potential, while high-potential ECFC can be isolated from kidneys of neonatal rats. We are currently investigating the microRNA expression profile which may dictate the low proliferative potential of ECFCs. We will investigate if altered miRNA expression underlies reduced proliferative potential in adult kidney endothelial cells and if this represents the basis for impaired vascular regeneration. We anticipate the work on the epigenetic distinction of ECFC in the regulation or renal vascular remodeling will be primary feature of the renewal of this RO-1 for 2012.

Can endothelial colony forming cells (ECFC) be used to treat acute kidney injury, repopulate kidney blood vessels, and alleviate chronic effects of AKI? We are currently developing two significant lines of investigation related to the potential therapeutic use of ECFC.

Our first line of investigation, which is covered in our active RO1 grant, relates to the goal of promoting the homing of endothelial cells to the injured kidney. The long term goal of such studies is to provide the basis for the formation of new blood vessels in the kidney. Since, the injured kidneys contain low proliferative potential ECFC, we are attempting to use high proliferative potential cells with the goal of promoting endothelial cell regeneration.

A second line of investigation relates the use of ECFC as a therapeutic approach in the early phase of acute kidney injury. Preliminary data demonstrated that ECFC provide immediate protection from the manifestations of acute kidney injury following ischemia/reperfusion. These effects do not depend on homing of endothelial cells to the kidney, and are mediated in part by rapid effects controlling blood perfusion in the kidney, suggesting a humoral mechanism. A preliminary version of this work has been presented in abstract form (Am. Soc. Nephrol, 2009) and additional studies are ongoing to develop a complete manuscript.

This latter approach represents a novel research direction that is distinct from our current RO-1 application and represents work for new funding opportunities with direct clinical

implications. A short-term goal will be submit a small business technology transfer (STTR) application for the potential commercial development of ECFC in the setting of AKI.

What is the basis of altered vascular responsiveness following AKI in both kidney and peripheral blood vessels? What is the role of inflammation in altering renal and non renal vascular control? Interest in these questions follow from our published observations that peripheral vascular reactivity is altered in post-ischemic animals, that peripheral blood vessels have increased oxidant stress, and that treatment with an inhibitor of lymphocyte activity blocks the development of salt sensitive hypertension. Unpublished data from our laboratory suggest that injury to a single kidney can have effects on the salt-sensitivity of the contralateral kidney. We hypothesize that lymphocyte dependent inflammatory processes contribute to post-AKI salt sensitivity by influencing local renal hemodynamics. An initial version of this study was submitted for funding to the American Heart Association and scored in the 15th percentile, but was not funded due to budgetary limitations. In response, I intend to expand the scope of these studies and submit a joint Co-PI application with Dr. Hamid Rabb (Johns Hopkins University), an expert in immune cell function in kidney injury, during the summer of 2010. We will analyze the effects of injury and lymphocyte inhibition on renal hemodynamics and whole body blood pressure regulation. These studies will be combined with studies on the activity of isolated renal blood vessels in vitro and studies on isolated lymphocytes from injured rat kidney and assess their activation states and cytokine liberation as a function of salt diet. Finally, by utilizing adoptive transfer techniques of immune cells, we will investigate the potential that activated T-cells derived from injured animals may mediate salt sensitivity in naïve, uninjured animals.

Development of novel research interests.

It is recognized that the University expects a continued evolution of a research program to incorporate novel and developing areas of research. Although it is impractical to list all potential areas of interest, I list here 2 separate projects relating to both acute and chronic kidney disease.

Can states of resistance to kidney injury be mediated by alterations in mitochondrial function? Can engineered mitochondrial proteins be used to alleviate AKI?

I have previously published studies that demonstrate that Brown Norway rats have a profound resistance to AKI vs. other strains of rats, while other data published thus far only in abstract form suggest that this protection is present in the mitochondria, a key mediator of the injury process. We have identified a set of consomic strains of rats that convey similar protection and also demonstrated that ischemic preconditioning also conveys similar protection from I/R injury.

In collaboration with Dr. Frank Witzmann, (IUSM, Cellular & Integrative Physiology) we are attempting to identify common sets of proteins altered in protective states, particularly those with mitochondrial localization. In collaboration with Dr. Mark Payne (Pediatrics), we will generate mitochondrial targeting fusion proteins that can be used to influence activity with the long term goal of generating a compound that can be used to protect the kidney from ischemic injury. Experimental questions will determine if such protection is conveyed by reductions in the production of oxygen radicals and improvement in renal medullary blood flow. I have spearheaded an effort with these colleagues to develop an RO-1 application using the multi PI format, which is currently being revised for resubmission.

D. Recognition of the research program. I have sought to publish our work in an arena that is appreciated and acknowledged as high-quality. It is worth noting that we have published routinely

in some of the most highly-rated journals in the fields of Nephrology and Renal Physiology *(J. Am Soc Nephrol, Kidney Intl., Am J. Physiol Renal Physiol., Curr Opin Neph Hyp)*. I have received recognition a leader in the area of chronic effects of kidney injury on renal and cardiovascular function. I have been invited to submit review articles and editorials in several of these major journals. In addition, I have been invited to give lectures at special symposia at National and International meetings, including the Meeting of the American Society of Nephrology, the American Society of Pediatrics, a joint NIH sponsored meeting of Association of Specialty Professors on Chronic Kidney Disease in Aging and the World Congress of Nephrology sponsored by the International Society of Nephrology, and the Federation of Societies of Experimental Biology. These invitations attest to the increased recognition of the research program described above.

E. Summary of Grant Applications:

My main funding to date has derived from my NIH grant DK-63114 "Long term effects of acute renal failure". This grant was active when I arrived in 2005 and was refunded in 2008 after a 1 year period of "no-cost" extension. During time in rank, the funding to the IUSM of the original and renewed application will have generated a total of $1.4 M by June 2012.

I have made a concerted effort to submit at least 1-2 grants per year to the NIH or other national agencies such as the American Heart Association. Included in these were efforts to renew DK-63114, "Long-term effects of acute renal failure". In June of 2005 I submitted a grant entitled "Genetic rat model of resistance to ischemic injury- DK-074065"; this application was scored and not funded. Rather than unnecessarily hastening a resubmission, I developed significant new collaborations resulting in the submission of RO1-DK-088932 "Mitochondrial response and resistance to acute kidney injury" in September of 2009, which is currently pending. I also submitted applications as a project leader on the program project grant led by Dr. Michael Sturek"P01HL095497-01A1- Coronary and renal vascular disease in the metabolic syndrome, This application was submitted twice in June of 2008 and January of 2009 and is "pending".

Current plans for 2010 include the submission of at least 2 novel applications including an RO1 application with Dr. Hamid Rabb tentatively entitled "T-cell dependent alterations in chronic kidney function following injury". A collaboration with Dr. Mervin Yoder will generate an NIH STTR application based on the application of endothelial colony forming cells in the setting of acute kidney injury. I am a Co-Investigator on 2 active RO-1 applications by Drs. Timothy Sutton (IUSM, Nephrology) and Dr. Beth Lee (Ohio State University), and on one pending application, by Dr. Matthias Clauss.

B. Teaching.

Contribution in course work: I have participated in the teaching of *F711, Integrative Systems Physiology* from 2005-7. A new course G735 *Cardiovascular Renal and Respiratory Function in Health and Disease* functionally replaced F711 in 2008 to the present time. In both courses, my goals were to introduce students to fundamental concepts in renal physiology and body fluid homeostasis using both lecture and laboratory formats. In addition, I have participated in G717, for the newly established Integrated Biomedical Gateway (IBMG) graduate program and as a Facilitator in X604, Concepts in Health and Disease for first-year Medical Students.

I currently serve as course director for G735 as well as two additional courses which have not yet commenced. One course, G762 –*Renal Physiology* is a one credit course that is offered once every two years and is directed toward a more advanced perspective of kidney function. A second course, G833- *Concepts and Controversies in Cardiovascular Science,* a journal club

format course, was recently created for students wishing to engage themselves in up- to-date topics in cardiovascular research.

Work on the Cellular & Integrative Physiology Department Graduate Committee and development of a new minor; A significant proportion of the work of this committee is to provide continued evaluation of the progress of graduate students and the re-evaluation of the curriculum following the introduction of the IBMG program. Over the last year, in collaboration with Dr. Johnathan Tune, the most significant effort was the establishment of a new Graduate Minor in Cardiovascular Sciences. This new minor is designed to enhance the opportunities of graduate students interested in cardiovascular research across traditional departmental affiliations, attract high quality students interested in cardiovascular research, and provide a documented training program geared toward successfully competing for individual and institutional training awards. The Cardiovascular Science Minor was tentatively approved by Indiana University in January 2010.

Work as a mentor to graduate and undergraduate students; My work as a mentor has been highlighted by long-distance mentoring of a student who began studies in my laboratory at Medical College of Wisconsin and remained in that program when I transitioned to IUSM. Dr. Kimberly Spurgeon-Pechman defended her Ph.D. in June of 2007. Since arriving, I have also participated as a member of the graduate research committees of several graduate students in the Department.

I have also served as advisor to undergraduate research education; I have provided research experience for undergraduates supported through the Life Health Science Internship Program (LHSI), the summer research opportunities for minorities program, and the IUPUI Undergraduate Research opportunities program (UROP).

C Service

My University service has been highlighted by my work on the Institutional Animal Care and Use Committee (IACUC). In addition the standard duties carried out by this committee, I have worked on the implementation of an electronic Web-Based submission form which was placed into use in 2009. In addition, I am the chair of an IACUC subcommittee whose mission it is to simplify the application process, create uniformity in the review process and to reduce the time from submission to approval.

I currently also serve on Graduate Admissions committee, whose mission is to screen and recruit perspective graduate students. I have been involved in a number of local advisory committees including the Indiana Center for Biological Microscopy and the Grant Review Committee for Clinical and Translational Science Institute. At the department level, I have chaired the Departmental Seminar Committee and participated in the Graduate Committee and Job search committees. These services to the University have been rewarding since I am passionate about most of the issues surrounding these various committees and I feel grateful that I am sometimes in a position to provide a meaningful impact.

Summary: My research on the pathophysiology of acute kidney injury has allowed me to create a unique and distinguished research program that has received national and international recognition. Our work has allowed us to develop important collaborations within the IUSM and is setting the stage for a number of additional external grant applications. Moreover, I have worked diligently to impact areas of the University related to teaching and service. I hope this statement reflects my dedication to my research, teaching and service and that my accomplishments are recognized as being of significant quality and impact that is expected of the faculty at IUSM.