AWARD NUMBER: W81XWH-17-1-0201

TITLE: RAGE/Diaph1, Diabetes, and Kidney Disease: Mechanisms and Novel Therapeutic Strategies

PRINCIPAL INVESTIGATORS: Ann Marie Schmidt, MD (Initiating PI) and

CONTRACTING ORGANIZATION: New York University School of Medicine

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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13. SUPPLEMENTARY NOTES

14. ABSTRACT
In Year Two of the funded grant, we have substantial progress in the following critical areas:
First, we have established the breeding colonies for all four of the key mouse lines to test the roles of RAGE and DIAPH1 in podocytes and monocytes/macrophages in the pathogenesis of diabetes associated nephropathic changes in the kidney. As detailed in the full progress report, most of these animals (males and females) have already been rendered diabetic and are on time course. Many have already been sacrificed and the pathological analysis of tissues is underway. These samples are now with Dr. D'Agati and the investigators are naïve to the sample identification until the code is broken. Second, we have optimized podocyte isolation procedures as indicated in the grant application. This is a key step, which will enable us to probe mechanisms of RAGE and DIAPH1 biology in these cells. Third, Dr. Ramasamy identifies substantial progress in the development and validation of metabolomics and lipidomics assays here at NYU in order to understand detailed mechanisms of the role of these molecules in the diabetic kidney. Fourth, in the Aim 3 pharmacology study, our preliminary data on treated vs. untreated diabetic mice illustrates reduction in mesangial sclerosis, reduced thickening of the glomerular basement membrane and reduction in podocyte effacement in diabetic mice receiving RAGE229 medicated chow (delivering 30 mg/kg/day) vs vehicle chow. Additional mice are on study and time course at this time to complete the indicated enrollment.
Taken together, our work in Year 2 has been very productive and we await tissue and other analyses, as above, to render final conclusions.

15. SUBJECT TERMS
Diabetes; DIAPH1; Floxed Mice; Glomerulosclerosis; Glomerular basement membrane; Inflammation; Macrophage; Nephropathy; Podocyte; RAGE; Small Molecule Antagonist

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Ann Marie Schmidt, MD – Initiating PI

1. INTRODUCTION:

Our laboratory discovered the receptor for advanced glycation endproducts (RAGE) and we identified that the cytoplasmic domain (tail) of RAGE binds to the formin, DIAPH1, and that this interaction is critical for RAGE ligand-mediated signal transduction and modulation of gene expression linked to cellular perturbation. DIAPH1 mediates actin cytoskeleton functions, cellular migration and activation of the Rho GTPases. DIAPH1 is expressed by immune and vascular cells; we reported that deletion of *Diaph1* in murine macrophages protects against hypoxia-mediated upregulation of proinflammatory (*Egr1* and *Ccl2*) and prothrombotic (*Tf*) and that this protection is analogous to that observed in macrophages devoid of *Ager*. Furthermore, with Dr. Alexander Shekhtman, we have identified the precise mechanism by which the cytoplasmic domain of RAGE binds DIAPH1. Critically, we have now published that DIAPH1, like RAGE, is highly expressed in human diabetic podocytes. **The goal if this grant is to determine the specific mechanisms by which RAGE/DIAPH1 contribute to the pathogenesis of diabetes associated nephropathy and we to explore novel RAGE/DIAPH1-directed therapeutic opportunities.**

2. KEYWORDS:

Diabetes
DIAPH1
Floxed Mice
Glomerulosclerosis
Glomerular basement membrane
Inflammation
Macrophage
Nephropathy
Podocyte
RAGE
Small Molecule Antagonist

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**
  There are three specific aims of the funded grant:

  AIM 1 will test the hypothesis that RAGE and DIAPH1 mediate podocyte dysfunction in DN through disengagement of homeostatic actin cytoskeleton dynamics and upregulation of pro-inflammatory and pro-fibrotic molecules. We will generate mice in which podocyte-specific deletion of *Ager* or *Diaph1* is accomplished via breeding *Ager* or *Diaph1* floxed mice with podocin (*Nphs2*) cre recombinase mice.

  AIM 2 will test the hypothesis that RAGE and DIAPH1-expressing macrophages contribute to structural and functional derangements in DN through upregulation of

tissue-destructive and profibrotic mediators. We will generate mice in which myeloid cell deletion of *Ager* or *Diaph1* is accomplished by breeding *Ager* or *Diaph1* floxed mice with *Lysm* cre recombinase mice.

AIM 3 will determine if administration of novel small molecule antagonists of RAGE-DIAPH1 interaction in diabetic mice protects against DN.

- **What was accomplished under these goals?**

1) Major Activities
In the second year of the funded grant, we have focused on the following major activities:
1A). As noted in the project narrative, we generated four different lines of mice to directly test the hypothesis that RAGE and DIAPH1 contribute to the pathogenesis of diabetes-associated nephropathy in the podocytes and/or in myeloid cells/macrophages. All of the mouse lines are now generated and are in various stages of completion and time course – we are on full time line to complete the study.
1B). We have now characterizing each of the four mouse lines to be certain that the gene of interest is deleted under the conditions with the cre recombinase driver. In our case, this means that we are testing deletion of *Ager* or *Diaph1* in podocytes.
1C). We are isolating podocytes from the mouse models using described techniques. At this stage, we are now culturing the podocytes after the isolation and have obtained all of the needed reagents in order to secure the characterization of these cells so that in vitro experimentation might be performed.
1D). We have determined that the small molecule RAGE antagonist is best administered orally and that the RAGE antagonist survives the medicated chow pelleting, heating and irradiation. We just completed the first set of animals in which RAGE229 medicated chow 30 mg/kg/day vs. vehicle was administered.
1E). For metabolomics and lipidomics assays, Dr. Ramasamy has now set up and validated his system for all of the measurements to be performed. His laboratory will be testing the tissues/cells from the mice through the time course and he has verified all of his experimental systems for the performance of the outlined studies. This work will commence in Year 3 once we finalize the data for the 4 lines of mice testing podocyte vs. myeloid RAGE or DIAPH1.
1F). All of the colleagues and collaborators are in place, with roles and timing defined, in order to execute the outlined studies as expertly and efficiently as possible.

2) Specific objectives
Our objectives in year one were to execute the above six activities in order to be certain that the aims of the study would be completed according to the three Specific Aims outlined in Item #1 above.

3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative):
3A). Generation of Mouse Models for Study

At this time, we have generated all four of the mouse lines for study; the time course for study once diabetes (or control state) is induced is 6 months – at this time, the four lines of mice are in various stages of completion or in progress to complete the aims. Per line, we have generated the following mice and these mice are on time course (Power calculations as described in the Narrative indicated 12 final mice/condition were needed for statistical significance).
Note that the investigators handling/working with the mice are naïve to the genotype; although they know the diabetes/non diabetes status and the sex of the mice, they are not aware of the genotype until after data are entered post sacrifice.

Aim 1:

Ager flox flox *Nphs2* (+/wt) cre recombinase mice
To date we have completed:
Diabetes: Male (N=11)
Female (n=13)
Non-Diabetes Male (N=9)
Female (N=10)
Ager flox flox *Nphs2* (wt/wt) cre recombinase mice
To date we have completed:
Diabetes: Male (N=12)
Female (n=12)
Non-Diabetes Male (N=11)
Female (N=10)

Diaph1 flox flox *Nphs2* (+/wt) cre recombinase mice
To date we have completed:
Diabetes: Male (N=10)
Female (N=12)
Non-diabetes: Male (N=10)
Female (N=10)

Diaph1 flox flox *Nphs2* (wt/wt) cre recombinase mice
To date we have completed:
Diabetes: Male (N=11)
Female (N=12)
Non-diabetes: Male (N=10)
Female (N=9)
Note that at this time we are filling in the groups – breeding is well underway for all of the above lines in order to achieve final N=12 / group.
Plan is to sacrifice the mice @ 6 months diabetes or control and perform the studies indicated in the Narrative of the funded grant.

Aim 2:

Ager flox flox *lysm* (+/+) cre recombinase mice
Mice are being actively bred at this time. As born, the are being genotyped and will be placed on time course (6 months) as follows:

Diabetes: Male (N=3)
Female (N=3)
Non-diabetes: Male (N=3)
Female (N=3)
Ager flox flox *lysm* (wt/wt) cre recombinase mice
Mice have been bred, genotyped and placed on time course (6 months) as follows:
Diabetes: Male (N=3)
Female (N=3)
Non-diabetes: Male (N=3)
Female (N=3)

Diaph1 flox flox *lysm* (+/wt) cre recombinase mice
Mice have been bred, genotyped and placed on time course (6 months) as follows:
Diabetes: Male (N=12)
Female (N=12)
Non-diabetes: Male (N=8)
Female (N=9)

Diaph1 flox flox *lysm* (wt/wt) cre recombinase mice
Mice have been bred, genotyped and placed on time course (6 months) as follows:
Diabetes: Male (N=10)
Female (N=8)
Non-diabetes: Male (N=8)
Female (N=9)
Note that at this time we are filling in the groups – breeding is well underway for all of the above lines in order to achieve final N=12 / group.
Plan is to sacrifice the mice @ 6 months diabetes or control and perform the studies indicated in the Narrative of the funded grant.

Characterization of mice
We first validated antibodies for RAGE and DIAPH1 in mouse kidney and the co-localization antibodies (Synaptopodin for podocyte deletion and CD68 for macrophage deletion) as follows:
Podocyte deletion: We have sacrificed *Ager* flox flox *Nphs2* (+/wt) and (wt/wt) and our findings reveal that based on qualitative assessment of the images that the expression of podocyte RAGE is significantly lower in the CRE+ vs. CRE- mice. In the case of DIAPH1, we have prepared the animals/tissues for the validation and these experiments are pending at this time.
Macrophage deletion: We have sacrificed *Ager* flox flox *lysm* (+/+) and (wt/wt) mice and discovered that we need to use homozygote *lysm* cre for significant/sufficient deletion of *Ager*. We discovered this by assessment of the bone marrow derived macrophage expression of *Ager*. In the case of DIAPH1 we have sacrificed *Diaph1* flox flox *(+/wt*) and (wt/wt) mice and our results reveal that hemizygosity is sufficient. That is, we can use *lysm* (+/wt) in order to test the deletion of *Diaph1* in myeloid cells for these studies.

We have isolated podocytes from the above mice and just recently successfully cultured them on plastic dishes. At this time, we are performing the fluorescence microscopy and real time PCR that is needed to both show that they are podocytes and also to document their purity. This work is actively underway at this time.

Aim 3:

Below are the first highly promising data on up to 6 mice/group – at this time, we continue to add ongoing mice to groups. Note that RAGE229 is 30 mg/kg/mouse /day

MALE Mice	Mesangial Sclerosis Score	Podocyte Effacement %	GBM nm
WT NON Diabetic mouse	0.5 ± 0	5.3±0.8	159.0±9.2
Diabetic Mouse VEHICLE	3.0±0	35±6.1	218.8±16.5
Diabetic Mouse RAGE229	2.2±0.3	25.8±4	198.5±6.3

FEMALE Mice	Mesangial Sclerosis Score	Podocyte Effacement %	GBM nm
WT NON Diabetic mouse	0.5 ± 0	4.8±1.6	157.3±8.7
Diabetic Mouse VEHICLE	2.9±.2	36±5	215.1±13.2
Diabetic Mouse RAGE229	1.9±0.4	28.3±9	195.5±7.6

There are no goals unmet in this period. We have generated all of the needed mice successfully; we have characterized them; and we have assembled all of the needed collaborators to successfully execute the studies in the outlined Narrative of the funded grant.

- **What opportunities for training and professional development has the project provided?**

Dr. NARA SZOSTACZUK under Dr. Schmidt's mentorship learned a great deal during her work on this project. She learned the complexities of working with floxed/cre mice and how to understand their characterization. She learned how to induce and monitor diabetes in the animals and she learned how to keep careful monitoring records of the mice per the protocol and to prepare for their sacrifice and post-mortem studies. Dr. Szostaczuk learned how to isolate podocytes and bone marrow derived macrophages from mice and how to properly test antibodies for specificity and how to perform real time quantitative PCR with these tissues.

How were the results disseminated to communities of interest?

NOTHING TO REPORT

- **What do you plan to do during the next reporting period to accomplish the goals?**

  As we have indicated throughout the narrative above, we plan to:
  1). Add the mice to completion in the four groups of animals; sacrifice them at 6 months and continue the tissue/urine analysis.
  2). We plan to isolate podocytes and macrophages and perform the indicated analyses as outlined in the Narrative.
  3). With Dr. Ramasamy, we plan to perform metabolomics/lipidomics on the indicated cells in order to discern mechanisms of action in these models once we identify the optimal conditions from studies in Aim 1
  4). We plan to continue the pharmacological studies as outlined in the Narrative.

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?**

  Despite years of research on diabetic kidney disease, the precise cells that mediate the damage in diabetes are not fully clarified vis-à-vis RAGE and DIAPH1. We know that mice globally devoid of *Ager* or *Diaph1* (new publication 2018) are protected from diabetes associated nephropathy but we do not know the cell specific mechanism. This work as outlined in this funded grant holds great promise to uncover new insights into the mechanisms by which diabetes causes nephropathic changes in the kidney.

- **What was the impact on other disciplines?**
  NOTHING TO REPORT AT THIS TIME

- **What was the impact on technology transfer?**
  NOTHING TO REPORT AT THIS TIME

- **What was the impact on society beyond science and technology?**
  NOTHING TO REPORT AT THIS TIME

5. CHANGES/PROBLEMS

- **Changes in approach and reasons for change**
  There are no changes in approach.

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  There are no actual or anticipated problems to date that delay in any way our planned work.

- **Changes that had a significant impact on expenditures**

  Staff is hired at this time (once the grant was funded, we began the hire process) and mice are being generated and studied as outlined. Hence although there was a delay, it is not appropriate to hire staff and start mouse studies until notice of funding is achieved. We have thus adhered to this principle and all of the staffing/work is actively underway at this time.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  No human subjects
  No select agents
  No significant changes in the use of vertebrate animals – any amendments were first submitted at NYU and then submitted to DOD ACURO. These amendments had NO impact on the plans of the funded work but involved staffing and amendments to ensure that the aims were carried out as written in the funded grant.

  Approval Date of the IACUC:
  Approval Date: 04/12/2019
  Effective Date: 06/13/2019
  Annual Expiration Date: 6/13/2020
  Final Expiration Date: 6/13/2020

6. PRODUCTS

Publication:

Manigrasso MB, Friedman RA, Ramasamy R, D'Agati V, Schmidt AM. Deletion of the formin Diaph1 protects from structural and functional abnormalities in the murine diabetic kidney. Am J Physiol Renal Physiol 315:F1601-F1612, 2018

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Ann Marie Schmidt
Project Role:	PI (Initiating)
Researcher Identifier (e.g. ORCID ID):	SCHMIDTAM (eRA Commons ID)
Nearest person month worked:	1
Contribution to Project:	PI, oversight of project and all administrative work with respect to use of animals and the budgetary requirements
Funding Support:	DOD

Name:	Ravichandran Ramasamy
Project Role:	PI (Partnering)
Researcher Identifier (e.g. ORCID ID):	RAVIRAMASAMY (eRA Commons ID)
Nearest person month worked:	2
Contribution to Project:	Dr. Ramasamy developed the methods for the metabolomics and lipidomics analyses of the mice under study.
Funding Support:	DOD

Name:	Nara Jimena Szostaczuk
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	szostn01 (eRA commons ID)
Nearest person month worked:	3
Contribution to Project:	Leads studies on work in generating mice for diabetes, time course and molecular analyses
Funding Support:	DOD

Name:	Raquel Lopez-Diez
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	DIEZR01 (eRA Commons ID)
Nearest person month worked:	11
Contribution to Project:	Contributed to podocyte isolation processes and characterization with immunostaining strategies
Funding Support:	DOD

Name:	Laura Frye
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Ms. Frye assists Dr. Szostaczuk in the breeding, identifying and genotyping of mice in her study.
Funding Support:	DOD

Name:	Michael MacLean
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	mm8848 (eRA Commons ID)
Nearest person month worked:	5
Contribution to Project:	Mr. MacLean assisted in *in vitro* experiments and data analysis.
Funding Support:	DOD

Name:	Vivette D'Agati
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	VDA1234 (eRA Commons ID)
Nearest person month worked:	1
Contribution to Project:	Pathological analysis of kidney tissues

Funding Support:	DOD
Name:	Richard A. Friedman
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	FRIEDMANR (eRA Commons ID)
Nearest person month worked:	1
Contribution to Project:	Statistical and bioinformatics analyses
Funding Support:	DOD

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Schmidt, Ann Marie

ACTIVE

1R24DK103032 08/01/14-07/31/20 0.06 calendar
NIH NCE
Targeting RAGE-mDia1 in Diabetic Complications: Mechanisms & Therapeutics
Major goal of this application is to develop small molecule inhibitors of the interaction of the RAGE cytoplasmic domain with DIAPH1.
Role: PI

1R01DK109675 04/01/16-03/31/21 0.91 calendar
NIH $303,742
RAGE/mDia1, Macrophage Trafficking and Inflammation in High Fat Feeding
Major goal of this application is to understand macrophage-adipocyte interactions in high fat feeding and obesity.
Role: PI

1R01HL132516 12/09/16-11/30/20 0.46 calendar
NIH $375,255
RAGE/mDia1, Macrophage Trafficking and Inflammation in Regression of Diabetic Atherosclerosis
The major goal of this grant is to probe the mechanisms by which macrophage (Mø) RAGE impairs regression of atherosclerosis in diabetic or IR mice.
Role: Multi-PIs (Schmidt & Ramasamy-Contact-PI)

Alzheimer's Association – Zenith Award 03/01/17-02/29/20 0.24 calendar
ZEN-17-440472 $134,612
RAGE, Diaph1, Microglia and Alzheimer's disease
Major goal of this grant is to probe the hypothesis that microglial-specific Ager deletion modulates neuronal stress, accumulation of Aβ and amyloid plaques, synaptic and cognitive dysfunction in APPswe/PS1 mice.
Role: PI

1P01HL131481 (Fisher, PI) P01 05/01/17-04/30/22 2.86 calendar

NIH $1,434,208
Macrophage Dysfunction in Obesity, Diabetes and Atherosclerosis
Major goal of this application is to determine mechanisms of macrophage trafficking, metabolism and inflammation in the context of RAGE/DIAPH1 in obesity
Role: Co-I

USAMRAA Dept. of the Army 07/01/17-06/30/19 (NCE) 0.12 calendar
Receptor for AGE (RAGE) Signal Transduction in Amyotrophic Lateral Sclerosis: In Vivo Imaging and Novel Therapeutic Approaches
Major goals of this grant includes testing the hypothesis that microglia RAGE, through ligand-driven upregulation of inflammatory and pro-oxidative stress and suppression of reparative processes in the ALS spinal cord, mediates neuronal death and loss of motor function and probing the hypothesis that PBMM-specific deletion of Ager attenuates neuronal stress, accumulation of Aβ and amyloid plaques, synaptic dysfunction and cognitive impairment in APPswe/PS1 mice.
Role: PI

(THIS AWARD)
USAMRAA Dept. of the Army 09/30/17-08/31/20 0.36 calendar
W81XWH-17-1-0201/0202 $499,187
RAGE/Diaph1, Diabetes, and Kidney Disease: Mechanisms and Novel Therapeutic Strategies
Major goals for this grant involves (a) testing the hypothesis that RAGE and DIAPH1 mediate podocyte dysfunction in DN through disengagement of homeostatic actin cytoskeleton dynamics and upregulation of pro-inflammatory and pro-fibrotic molecules (b) testing the hypothesis that RAGE and DIAPH1-expressing macrophages contribute to structural and functional derangements in DN through upregulation of tissue-destructive and profibrotic mediators and (c) determining if administration of novel small molecule antagonists of RAGE-DIAPH1 interaction in diabetic mice protects against DN.
Role: PI (Ramasamy-Partnering PI)

American Heart Association 04/01/17-03/31/21 3.6 calendar
$900,663
Braking Inflammation in Obesity & Metabolic Dysfunction: Translational and Therapeutic Opportunities
The major goal of this grant is to investigate the novel hypothesis that impaired adipocyte, macrophage and other inflammatory cell signal transduction thwarts weight loss and its anti-inflammatory and metabolic benefits, at least in part through the activation of the receptor for advanced glycation endproducts, or RAGE pathway, which has been shown to regulate a unique repertoire of inflammatory and metabolic processes.
Role: Center Director, Project 1 Leader

(NEW)
1P01HL146367-01 08/01/19- 07/31/24 3.0 calendar
NHLBI $1,629,632
Macrophages, Cell-Cell Communication, Ischemic Injury in Diabetes and the RAGE/DIAPH1 Signaling Axis

The major goal of this grant application is to probe the mechanisms and identify new therapies for untoward monocyte and macrophage responses in ischemia, which, together with cellular perturbation in the microenvironment, amplify damage in myocardial infarction and peripheral arterial disease, especially in diabetes.
Role: PI

INACTIVE
(ENDED)
P01HL60901 07/15/11-11/30/18 0.12 calendar
NIH $897,537 No Cost Extension
RAGE and Mechanisms of Vascular Dysfunction
This grant focuses on the mechanisms by which diabetes accelerates atherosclerosis via RAGE.
Role: Project 1 and Core A Leader, Core C Co-Leader

(ENDED)
1R01 HL118565 06/01/13-04/30/18 0.9 calendar
NIH $248,449
RAGE, Macrophages & HDL Biology
This grant examines the molecular mechanisms by which RAGE regulates cholesterol transport.
Role: PI

(ENDED)
Harrington Discovery Institute 01/01/16-12/31/17 0.12 calendar
$50,000
Targeting RAGE/mDia1 for the Prevention and Treatment of Diabetic Complications
The goal of the Harrington Discovery Institute project is to develop LOCAL intraocular and transdermal treatments for diabetic retinopathy and diabetic wound healing, respectively.
Role: PI

OVERLAP
None

D'Agati, Vivette

ACTIVE
2 PO1 DK 56492 (Klotman) renewal 05/15/17-04/30/22 1.80 calendar
NIH NIDDK $91,325 (Core B)
Long-term Consequences of HIV Infection of the Kidney
The aim of this grant is to explore the long-term consequences of HIV infection of the kidney beyond HIVAN. The role of Core B will be to provide technical and interpretative support in light microscopy, immunohistochemistry, immunofluorescence and electron microscopy in mouse and human kidney tissues for all projects, including study of the mechanisms of ApoL1 induced kidney

injury, the kidney as long-term reservoir for HIV, the interactions between gut microbiome and HIVAN in the Tg26 model, and the effects of chronic HIV infection on progression of kidney disease through enhanced pro-inflammatory responses.
Role: Core B Leader

1R24DK103032-01 (Schmidt) 08/01/14/-07/31/20 0.12 calendar
NIH NCE
Targeting RAGE-mDia1 in Diabetic Complications: Mechanisms & Therapeutics
This application focuses on the role of the receptor for advanced glycation endproducts (RAGE) and its cytoplasmic domain binding partner, mammalian form of diaphanous1, mDia1, which is essential for RAGE signaling as a fundamental therapeutic target for diabetic complications.
Role: Co-I

1R01DK106436-01A1 (Winchester) 01/25/16-12/31/19 0.6 calendar
NIH/NIDDK $211,308
Significance of Intrarenal T Cells in SLE Nephritis
The overall goal of this study is to define the role of T cells in the development and progression of chronic lupus nephritis. It addresses the clinical problem of why nearly half of lupus nephritis cases do not adequately respond to therapy and much progress to chronic glomerulonephritis. SLE kidneys have a variable but often extensive infiltrate of predominantly clonally expanded CD4 and/or CD8 T cells with features that suggest they could drive the inflammatory process. We hypothesize that while acute glomerulitis is driven by immune complexes, chronic SLE nephritis is driven by the development of CD4 and especially CD8 T cell clonal recognition of self-peptides, resulting in glomerular and tubular cell injury. In the first aim we will delineate the extent and character of the renal CD4 or CD8 T cell infiltration in new onset nephritis and correlate these findings with short- and long-term pathological and clinical outcomes that predict poor response to therapy and progressive renal disease. In the second part of this aim we will also determine the T cell characteristics of cases with worsening renal involvement requiring repeat biopsy, comparing current and prior biopsies for T cell features that predict progression. In the second aim we will define the properties of the infiltrating T cells and their potential mechanisms to mediate renal injury, discriminating between the phenotype of clonally expanded CD4 or CD8 T cells that potentially drive renal injury and that of the polyclonal T cells secondarily recruited by inflammation.
Role: Co-I

R01 DK109544-01A1 (Lee) 04/01/17-03/31/22 0.6 calendar
NIH $225,000
Paneth cells and acute kidney injury
This proposal seeks to elucidate the mechanisms as well as therapies for remote organ dysfunction after renal ischemia and reperfusion injury.
Role: Co-I

1R01 DK115694-01 (Lee) 09/13/17–07/31/22 0.6 calendar
NIH $250,000
Peptidylarginine deiminase-4 and acute kidney injury

This project seeks to elucidate the mechanisms as well as therapies for inflammation and injury after renal ischemia and reperfusion.
Role: Co-I

1UG3DK114926-01 (Kiryluk, Barasch, Bomback) 07/01/17 - 06/30/22 0.9 calendar
NIH/NIDDK $300,000
Kidney Precision Medicine Program (KPMP): Columbia AKI Recruitment Site
The national Kidney Precision Medicine Project (KPMP) aims to reduce the significant global health burden of acute kidney injury (AKI) by elucidating mechanisms and effective therapies through precision medicine technologies.
Role: Co-I

(THIS AWARD)
USAMRAA Dept. of the Army (Schmidt, Ramasamy, MPI) 07/01/17 - 06/30/20 0.6 calendar
W81XWH-17-1-0201/0202 $499,187
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This project will test the hypothesis that RAGE and DIAPH1 mediate podocyte dysfunction in DN through disengagement of homeostatic actin cytoskeleton dynamics and upregulation of pro-inflammatory and pro-fibrotic molecules and will determine if administration of novel small molecule antagonists of RAGE-DIAPH1 interaction in diabetic mice protects against DN.
Role: Co-I

INACTIVE
(ENDED)
Dept. of the Army – USAMRAA (Gharavi) 08/15/16 – 08/14/19 0.84 calendar
Grant number: PR151419 $250,000
Multispecies, integrative GWAS for focal segmental glomerulosclerosis
Goals: The major goal of this project is to devise new targeted therapies that will impact and benefit treatment for chronic kidney disease at large in the general population as well as in the active duty personnel, veterans and their families. Aims: - Specific aim 1: A Genome-wide association study for common single nucleotide polymorphisms and rare copy number variations in 7,559 FSGS and over 50,000 controls - Specific aim 2: A GWAS for FSGS in a mouse leveraging the power of the newly developed DO strains - Specific aim 3. Cross annotation between human and mouse GWAS and identification of downstream dysregulated pathways and networks.
Role: Co-I

(ENDED)
1R01MD009223-01 (Multi-PI: Gharavi & Bomback) 07/01/14-06/30/19 0.6 calendar
NIH/NIMHD $250,000
Ancestry, Genetic Risk and Health Disparities in Immune-Mediated Nephritis
The major goal of this project is to investigate the role of shared and distinct genetic factors among Europeans, Asians, Hispanics, and African-Americans in the onset, course, and ultimate outcomes of IgA nephropathy, membranous nephropathy and lupus nephritis.
Role: Co-I

(ENDED)
1 UM1 DK100876-01 (Gharavi) 09/16/13-05/31/18 1.2 calendar
NIH/NIDDK $353,143
Advancing Clinical Research in Primary Glomerular Diseases
The major goals of this project are to develop a longitudinal observational cohort of patients with biopsy-documented forms of major glomerular diseases, including minimal change disease, focal segmental glomerulosclerosis, membranous glomerulopathy and IgA nephropathy.
Role: Co-I

OVERLAP
None

Friedman, Richard A.

ACTIVE
5 P30 CA13696-44 (Rustgi, PI) 07/01/14 - 06/30/20 1.2 calendar
NCI $2,248,065
Cancer Center Support Grant
This grant supports the leadership of Columbia University's lab, clinical & population-based cancer research programs & the shared resources serving the University's Cancer Center members. Dr. Friedman's role as a member of the Biomedical Informatics Shared Resource is to provide the bioinformatics and statistical component of cancer research projects.
Role: Staff Member of the Biomedical Informatics Shared Resource.

1R35CA210088-01 (Wang-PI) 12/09/16-11/30/23 1.2 calendar
NIH/NCI $540,000
The Role of Stem Cells and the Microenvironment in Gastrointestinal Cancer
This project seeks to investigate the role of nerves and other stromal cells in the development of digestive cancers, including stomach, esophageal, colon and pancreas. The project builds on previous work that suggests that these elements can regulate stem cells and that inhibiting stromal cells in the microenvironment, it may be possible to inhibit the development of tumors. Dr. Friedman's role is to design and analyze RNASeq and other genomic experiments and perform other statistical analyses.
Role: Co-I

1U54CA163004-06 (Wang, PI) 05/12/17-4/30/22 0.60 calendar
NIH/NCI $978,861
The Role of the Microenvironment in Barrett's Esophagus
To investigate how the tumor microenvironment leads to adenocarcinoma. Dr. Friedman's role is to design and analyze RNASeq experiments.
Role: Co-I

(NEW)
2R01DK048077-23 (Wang, PI) 09/01/16-8/31/21 0.84 calendar
NIH/NIDDK $225,000
The Function and Regulation of Histidine Decarboxylase
To investigate the role of histidine decarboxylase in digestive cancer and the immune response. Dr. Friedman's role is to analyze gene expression and other experiments.
Role: Co-I

1R01CA208711-01 Sepulveda (PI) 09/01/2016-08/31/21 1.0 calendar
NIH/NCI $228,750
Genomics and Mechanisms of Esophageal Carcinogenesis
The goal of this project is to investigate the role CDKN1A/P16 mutations the genesis of esophageal cancer; to use expression, polymorphism, and methylation data to predict progression to cancer, and to test various drugs for their ability to prevent this progression from occurring. Dr. Friedman's role is to perform statistical analyses.
Role: Co-I

1R01CA178445-04 (Su, PI) 07/01/15-06/30/20 0.6 calendar
NIH/NCI $294,426
The Role of wild-type Kras in the context of tumor progression and metastasis
To elucidate the mechanism of the role of Kras in human pancreatic ductal adenocarcinoma by means of a mouse model. Dr. Friedman's role is to design and analyze RNASeq and pcr experiments to measure gene expression.
Role: Co-I

1R01DK109675-01 (Schmidt) 04/01/16-03/31/21 0.9 calendar
NIH/NIDDK $303,742
RAGE/MDIA1, Macrophage Trafficking and Inflammation in High Fat Feeding
To characterize the mechanism by which macrophage Receptor for Advanced Glycation Endproducts regulates obesity, adiposity and metabolic dysfunction in high fat feeding, both inherently and via cross-talk with the adipocyte.
Role: Co-I

1R01HL132516-01-A1 (Ramasamy, Schmidt-MPI) 12/09/16-11/30/20 1.13 calendar
NIH/NHLBI $375,255
RAGE/mDia1, Macrophage Trafficking and Inflammation in Regression of Diabetic Atherosclerosis
The goal of this project is to characterize how RAGE and mDia1 signaling macrophages affect atherosclerotic regression. Dr. Friedman's role is to perform genomic and statistical analyses.
Role: Co-I

(THIS AWARD)
USAMRAA Dept. of the Army (Schmidt, Ramasamy, MPI) 09/30/17-09/29/20 0.6 calendar
W81XWH-17-1-0201/0202 $499,187
RAGE/Diaph1, Diabetes, and Kidney Disease: Mechanisms and Novel Therapeutic Strategies

To characterize the mechanism by which macrophage Receptor for Advanced Glycation Endproducts helps cause diabetic kidney disease and develop therapies for the treatment of this disease. Dr. Friedman's role is to analyze RNASeq and other experiments.
Role: Co-I

(NEW)
Internal Pilot (Lieberman, PI) 04/01/19-3/31/20 0.6 calendar
Identification of Precision Diagnostic and Therapeutic Targets for Advanced Prostate Cancer Patients Based on Mechanistic RNA Landscape

(NEW)
1P01 HL146367-01 (Schmidt, PI) 08/01/19-06/30/24 0.6 calendar
NIH/NHLBI
Macrophages, Cell-Cell Communication, Ischemic Injury in Diabetes and the RAGE/DIAPH1 Signaling Axis
To elucidate the mechanism of the role of the RAGE/DIAPH1 signaling pathway in ischemic injury and diabetes. Dr. Friedman's role is to design and analyze RNASeq experiments to measure gene expression.
Role: Co-I

INACTIVE
(No Longer on Grant)
1P01HL131481-01A1 (E. Fisher PI) 05/01/17-04/30/19 1.8 calendar
NIH/NHLBI $1,434,208
Macrophage Dysfunction in Obesity Diabetes and Atherosclerosis
The goal of this project is to characterize how macrophages affect atherosclerosis in patients who are diabetic, and/or obese. Dr. Friedman's role is to perform genomic and statistical analyses.
Role: Co-I

(ENDED)
W81XWH-15-1-0296 Broustas (PI) 08/31/2015-08/30/2018 0.24 calendar
DOD $108,000
Targeting MEK5 Enhances Radiosensitivity in Human Prostate Cancer
The goal of this project is to test whether inhibition of MEK5 signaling enhances the response of human prostate cancer cell lines to radiation therapy. Dr. Friedman's role is to perform statistical analyses
Role: Co-I

(ENDED)
1R01HL118565-04 (Schmidt/Friedman, PI) 06/01/13-4/30/18 1.2 calendar
NIH/NHLBI $262,295
RAGE, Macrophages, and HDL Biology
To link RAGE (Receptor for Advanced Glycation Endproducts) function to that of High Density Lipoproteins. Dr. Friedman's role is to design and analyze RNASeq experiments to discover molecular mechanisms underlying obesity.

Role: Co-I

(ENDED)
5R03CA186218-02 (Abrams, PI) 07/01/15-06/30/17 0.3 calendar
NIH/NIDCR
Randomized placebo-controlled trial of a gastrin receptor (Abrams: PI)
The goal of this project is to test the effect of a netazepide (YF476), a gastrin receptor antagonist, on biomarkers associated with progression to esophageal adenocarcinoma. Dr. Friedman's role is to design and analyze RNASeq and PCR experiments to measure gene expression.
Specific Aims:
Role: Co-I

OVERLAP
None

- **What other organizations were involved as partners?**
  Organization Name: Columbia University
  Location of Organization: New York, NY
  Collaborating Investigators: Drs. Vivette D'Agati and Richard A. Friedman
  Collaboration: Dr. D'Agati performed the pathological analysis of kidney tissues.
  Dr. Friedman performed statistical and bioinformatics analyses.

8. SPECIAL REPORTING REQUIREMENTS

Dr. Ravichandran Ramasamy, PhD (Partnering PI) progress report follows Dr. Schmidt's section.

9. APPENDICES:

Nothing to report

Ravichandran Ramasamy – Partnering PI

1. INTRODUCTION:

Our laboratory discovered the receptor for advanced glycation endproducts (RAGE) and we identified that the cytoplasmic domain (tail) of RAGE binds to the formin, DIAPH1, and that this interaction is critical for RAGE ligand-mediated signal transduction and modulation of gene expression linked to cellular perturbation. DIAPH1 mediates actin cytoskeleton functions, cellular migration and activation of the Rho GTPases. DIAPH1 is expressed by immune and vascular cells; we reported that deletion of *Diaph1* in murine macrophages protects against hypoxia-mediated upregulation of proinflammatory (*Egr1* and *Ccl2*) and prothrombotic (*Tf*) and that this protection is analogous to that observed in macrophages devoid of *Ager*. Furthermore, with Dr. Alexander Shekhtman, we have identified the precise mechanism by which the cytoplasmic domain of RAGE binds DIAPH1. Critically, Dr. D'Agati's data reveal that DIAPH1, like RAGE, is highly expressed in human diabetic podocytes. **The goal of this grant is to determine the specific mechanisms by which RAGE/DIAPH1 contribute to the pathogenesis of diabetes associated nephropathy and we to explore novel RAGE/DIAPH1-directed therapeutic opportunities. The goal of the studies by Partnering PI is to determine is to elucidate substrate metabolic mechanisms driven by RAGE/DIAPH1 in diabetic nephropathy and to explore if these specific metabolic changes can serve as RAGE/DIAPH1 target biomarkers.**

2. KEYWORDS:

Diabetes
DIAPH1
Floxed Mice
Glomerulosclerosis
Glomerular basement membrane
Inflammation
Macrophage
Nephropathy
Podocyte
RAGE
Small Molecule Antagonist

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**
  There are three specific aims of the funded grant:

  AIM 1 will test the hypothesis that RAGE and DIAPH1 mediate podocyte dysfunction in DN through disengagement of homeostatic actin cytoskeleton dynamics and upregulation

of pro-inflammatory and pro-fibrotic molecules. We will generate mice in which podocyte-specific deletion of *Ager* or *Diaph1* is accomplished via breeding *Ager* or *Diaph1* floxed mice with podocin (*Nphs2*) cre recombinase mice.
AIM 2 will test the hypothesis that RAGE and DIAPH1-expressing macrophages contribute to structural and functional derangements in DN through upregulation of tissue-destructive and profibrotic mediators. We will generate mice in which myeloid cell deletion of *Ager* or *Diaph1* is accomplished by breeding *Ager* or *Diaph1* floxed mice with *Lysm* cre recombinase mice.

AIM 3 will determine if administration of novel small molecule antagonists of RAGE-DIAPH1 interaction in diabetic mice protects against DN.

- **What was accomplished under these goals?**

1) Major Activities
In the second year of the funded grant, along with Dr. Schmidt we have focused on the following major activities:
PI, Dr. Schmidt and her team are generating four different lines of mice to directly test the hypothesis that RAGE and DIAPH1 contribute to the pathogenesis of diabetes-associated nephropathy in the podocytes and/or in myeloid cells/macrophages. They are characterizing each of the four mouse lines in order to be certain that the gene of interest is deleted under the conditions to be expected with the cre recombinase driver.

Dr. Schmidt and her team are isolating podocytes from the mouse models using described techniques. At this stage, they are culturing the podocytes after the isolation and have obtained all of the needed reagents in order to secure the characterization of these cells. Once they characterize these cells, we will obtain these cells from them for metabolite measurements to determine RAGE/DIAPH1 specific metabolism changes in podocytes.
For metabolomics and lipidomics assays, we have set up the validation for all of the measurements to be performed. We will be testing the tissues/cells from the mice through the time course of lipid and intermediary metabolite changes. We have optimized and fine-tuned the metabolite measurements using spectroscopic approaches as in the outlined studies.

All of the colleagues and collaborators are in place, with roles and timing defined, in order to execute the outlined studies as expertly and efficiently as possible.

2) Specific objectives
Our objectives in year one were to execute the above metabolism studies in order to be certain that the aims of the study would be completed according to the three Specific Aims outlined in Item #1 above.

3) Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative):

3A). Generation of Mouse Models for Study
At this time, Dr. Schmidt and her team have made extensive progress in three of the four mouse lines for study; the time course for study once diabetes (or control state) is induced is 6 months – at this time, the first cohort of mice (7 animals) have already completed the time course and tissues are being processed as outlined in the Narrative of the funded grant. Per line, they have generated the following mice and these mice are on time course (Power calculations as described in the Narrative indicated 12 final mice/condition were needed for statistical significance).

Note that the investigators handling/working with the mice are naïve to the genotype; although they know the diabetes/non diabetes status and the sex of the mice, they are not aware of the genotype until after data are entered post sacrifice.

See the Section in Dr. Schmidt's progress report for the current status of the mouse numbers.

We will obtain tissue and cells from these mice for the outlined metabolism experiments.

3B). Characterization of mice

3Bi). Dr. Schmidt and her team validated antibodies for RAGE and DIAPH1 in mouse kidney and the co-localization antibodies (Synaptopodin for podocyte deletion and CD68 for macrophage deletion) as follows:

In the case of podocytes, RAGE is validated; DIAPH1 is underway.
In the case of macrophages, RAGE and DIAPH1 are validated.

3Bii). They have isolated podocytes from the above mice and just recently successfully cultured them on plastic dishes. At this time, they are performing the fluorescence microscopy and real time PCR that is needed to both show that they are podocytes and also to document their purity.

After characterization of these mice and podocytes by Dr. Schmidt, we will obtain tissue and cells from her for the metabolism studies outlined.

4) Optimization of metabolite measurements:
4A). We have used ^{13}C heavy isotope (non-radioactive) and known lipid and intermediary metabolite standards to establish calibration and sensitivity limits for metabolomics/lipidomics measurements using mass spectrometry.

4B). We have used primary macrophages and HEK cells to optimize lipids and intermediary metabolite extraction efficiency. Using ^{13}C heavy isotope labeled lipid standards and ^{13}C heavy isotope labeled TCA cycle metabolites we have been able to get a consistent extraction efficiency in the 75-80% ranges for lipids and 88-90% range for intermediary metabolites. Intra sample and inter sample variation of less than 5% for detection of known standards have also been established.

5) Other achievements.
There are no other achievements to report at this time. There are no goals unmet in this period. We have generated all of the needed mice successfully; we have characterized them; we have established the spectroscopic methodology for metabolites/lipid measurements; and we have assembled all of the needed collaborators to successfully execute the studies in the outlined Narrative of the funded grant.

- **What opportunities for training and professional development has the project provided?**

NOTHING TO REPORT

- **How were the results disseminated to communities of interest?**

NOTHING TO REPORT

- **What do you plan to do during the next reporting period to accomplish the goals?**

As we have indicated throughout the narrative above, we plan to work with Dr. Schmidt to obtain indicted cells from various mice to perform metabolomics/lipidomics to discern mechanisms of action in these models.

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
  Despite years of research on diabetic kidney disease, the precise cells that mediate the damage in diabetes are not fully clarified vis-à-vis RAGE and DIAPH1. We know that mice globally devoid of *Ager* or *Diaph1* (DIAPH1 manuscript, publication 2018) are protected from diabetes associated nephropathy but we do not know the cell specific mechanism. This work as outlined in this funded grant holds great promise to uncover new insights into the mechanisms by which diabetes causes nephropathic changes in the kidney.

- **What was the impact on other disciplines?**
  NOTHING TO REPORT AT THIS TIME

- **What was the impact on technology transfer?**
  NOTHING TO REPORT AT THIS TIME

- **What was the impact on society beyond science and technology?**
  NOTHING TO REPORT AT THIS TIME

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

There are no changes in approach.

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  There are no actual or anticipated problems to date that delay in any way our planned work.

- **Changes that had a significant impact on expenditures**
  Staff is hired at this time (once the grant was funded, we began the hire process) and mice are being generated and studied as outlined.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  No human subjects
  No select agents
  No significant changes in the use of vertebrate animals – any amendments were first submitted at NYU and then submitted to DOD ACURO. These amendments had NO impact on the plans of the funded work but involved staffing and amendments to ensure that the aims were carried out as written in the funded grant.

Approval Date of the IACUC:
Approval Date: 04/12/2019
Effective Date: 06/13/2019
Annual Expiration Date: 6/13/2020
Final Expiration Date: 6/13/2020

Cells and tissue from mice will be obtained from Dr. Schmidt's team for metabolite analysis. As in outlined studies for the partnering portion of the grant, no independent studies will be conducted on mice.

6. PRODUCTS:

Publication:

Manigrasso MB, Friedman RA, Ramasamy R, D'Agati V, Schmidt AM. Deletion of the formin Diaph1 protects from structural and functional abnormalities in the murine diabetic kidney. Am J Physiol Renal Physiol 315:F1601-F1612, 2018

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

- **What individuals have worked on the project?**

Name:	Ravichandran Ramasamy
Project Role:	PI (Partnering)
Researcher Identifier (e.g. ORCID ID):	RAVIRAMASAMY (eRA Commons ID)
Nearest person month worked:	2

Contribution to Project:	Dr. Ramasamy developed the methods for the metabolomics and lipidomics analyses of the mice under study
Funding Support:	DOD

Name:	Ann Marie Schmidt
Project Role:	PI (Initiating)
Researcher Identifier (e.g. ORCID ID):	SCHMIDTAM (eRA Commons ID)
Nearest person month worked:	1
Contribution to Project:	PI, oversight of project and all administrative work with respect to use of animals and the budgetary requirements
Funding Support:	DOD

Name:	Nara Jimena Szostaczuk
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	szostn01 (eRA commons ID)
Nearest person month worked:	3
Contribution to Project:	Led studies on work in generating mice for diabetes, time course and molecular analyses
Funding Support:	DOD

Name:	Charlotte Detremmerie
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	9
Contribution to Project:	Performed *in vitro* experiments, data interpretation and analysis
Funding Support:	DOD

Name:	Qing Li
Project Role:	Associate Research Scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	11
Contribution to Project:	Worked on optimizing assay conditions for metabolite extraction in cells from diabetic mice.
Funding Support:	DOD

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Ramasamy, Ravichandran

ACTIVE

1R24DK103032 (Schmidt) 08/01/14-07/31/20 0.60 calendar
NIH NCE
Targeting RAGE-mDia1 in Diabetic Complications: Mechanisms & Therapeutics
Major goal of this application is to develop small molecule inhibitors of the interaction of the RAGE cytoplasmic domain with DIAPH1.
Role: Co-I

1R01DK109675 04/01/16-03/31/21 1.80 calendar
NIH $303,742
RAGE/mDia1, Macrophage Trafficking and Inflammation in High Fat Feeding
Major goal of this application is to understand macrophage-adipocyte interactions in high fat feeding and obesity.
Role: Multiple PIs (Schmidt (Contact PI) & Ramasamy)

1R01 HL132516-01 (Al) 12/09/16-11/30/20 2.40 calendar
NIH $375,255
RAGE/mDia1, Macrophage Trafficking and Inflammation in Regression of Diabetic Atherosclerosis
The major goal of this grant is to probe the mechanisms by which macrophage (MΦ) RAGE impairs regression of atherosclerosis in diabetic or IR mice.
Role: Multi-PIs (Schmidt & Ramasamy-(Contact-PI))

1P01 HL131481-A1 (Fisher, PI) 05/01/17-04/30/22 2.40 calendar
NIH $1,434,208
Macrophage Dysfunction in Obesity, Diabetes and Atherosclerosis
Major goal of this application is to determine mechanisms of macrophage trafficking, metabolism and inflammation in the context of RAGE/DIAPH1 in obesity
Role: Co-I

1 R01 HL135987-01A1 05/01/17 – 04/30/21 1.81 calendar
NIH $356,158
Fatty Acids: Ischemic Protection and Repair
The goal of this project is to assess how lipid metabolism in cardiomyocytes and white blood cells affects heart injury and repair after ischemia/reperfusion.
Role: Multi-PIs (Goldberg (contact PI) & Ramasamy)

THIS AWARD
USAMRAA Dept. of the Army 09/30/17-08/31/20 0.96 calendar
W81XWH-17-1-0201/0202 $499,187
RAGE/Diaph1, Diabetes, and Kidney Disease: Mechanisms and Novel Therapeutic Strategies
Major goals for this grant involves (a) testing the hypothesis that RAGE and DIAPH1 mediate podocyte dysfunction in DN through disengagement of homeostatic actin cytoskeleton dynamics and upregulation of pro-inflammatory and pro-fibrotic molecules (b) testing the hypothesis that

RAGE and DIAPH1-expressing macrophages contribute to structural and functional derangements in DN through upregulation of tissue-destructive and profibrotic mediators and (c) determining if administration of novel small molecule antagonists of RAGE-DIAPH1 interaction in diabetic mice protects against DN.
Role: Partnering PI (Schmidt, Contact PI)

(NEW)
2R01HL073029-12A1 (Goldberg) 08/10/18-07/31/22 0.91 calendar
$319,764
Mechanisms of Fatty Acid Uptake by Cardiac Muscle
The goal of this project is to understand how lipids are obtained by the heart and their roles in normal physiology and in pathological conditions.
Role: Co-I

(NEW)
1P01HL146367-01 08/01/19- 07/31/24 3.00 calendar
NHLBI $1,629,632
Macrophages, Cell-Cell Communication, Ischemic Injury in Diabetes and the RAGE/DIAPH1 Signaling Axis
The major goal of this grant application is to probe the mechanisms and identify new therapies for untoward monocyte and macrophage responses in ischemia, which, together with cellular perturbation in the microenvironment, amplify damage in myocardial infarction and peripheral arterial disease, especially in diabetes.
Role: Project 1 and Scientific Core Leader

INACTIVE
(ENDED)
P01HL60901 (Schmidt) 07/15/11-11/30/18 0.12 calendar
NIH No Cost Extension
RAGE and Mechanisms of Vascular Dysfunction
This grant focuses on the mechanisms by which diabetes accelerates atherosclerosis via RAGE.
Role: Project 2, 3, Core B Leader, Core C Co-Leader

(ENDED)
1R25DK092170-01A1 07/2012-06/2017 0.2 calendar
NIH $472,130
A New Era of Targeted Drug Discovery and the Path of Development from Molecular
This grant funds a graduate level course focused on challenges in drug discovery for the treatment of diabetic complications and obesity.
Role: Multiple PIs (Ramasamy & Gold-von Simson)

8. SPECIAL REPORTING REQUIREMENTS:

- **COLLABORATIVE AWARDS:**
  A copy of this report will be submitted to *https://ebrap.org/eBRAP/public/index.htm.*

9. APPENDICES:

Nothing to report