My research focus:

- Immunology of kidney disease
  - autoimmune injury
  - alloimmune injury
  - inflammatory injury
- Transition between innate and adaptive immunity
- Factors promoting a functional vs. pathological immune response

Objectives

- To introduce experimental mouse models of human glomerulonephritis
- To examine the role of RIP2 in experimental crescentic glomerulonephritis
- To provide “lessons learned” from my experience as a new investigator

Autoimmune disease of the kidney: glomerulonephritis

- “Inflammation” of the glomerulus
- May involve one or many cell types within the glomerulus
- Numerous types and etiologies
- Diagnosis: clinical syndrome + pathologic classification
**Origins of autoimmunity:**

Genetic predisposition → T cell activation → Antigen presentation → Adaptive immune response → Antigen-specific tissue injury

- HLA polymorphisms
- GWAS studies
- Thymic T cell maturation
- Regulatory T cells
- Inflammation

**Are PRRs important in autoimmune disease?**

- Non-specific inflammation perpetuates tissue injury
- Antigen presentation in the context of "inflammation" is necessary for activation of auto-reactive T cells

**Influencing adaptive immunity:**

- APC-T cell interaction
- Activation of APCs by microbes, innate immune response

**Influencing the innate immune response:**

1. Strength of antigen-MHC-TCR interaction
2. Local environment of APC activation and antigen presentation
3. Costimulation between APC and T cell
4. Signaling cytokines from APC to T cell

**T cell activation and differentiation:**

- Repertoire of T helper cell responses
- Th1 response: IL-2, IFN-γ, IL-12
- Th2 response: IL-4, IL-5, IL-13
- Peripheral Treg response: TGF-β, IL-10
- Th17 response: IL-17, IL-22

**Toll-Like Receptor 2 Agonist Elicits Moderate Accelerated Nephrotic Nephritis**

Toll-like receptor 2 agonist elicits moderate accelerated nephrotic nephritis. This study investigated the effect of N-palmitoyl-S-(2,3-bis(palmitoylhydroxy)palmitoyl)glycine (Pam3CysSK4) on renal injury. It was hypothesized that the TLR2 ligand Pam3CysSK4 can increase renal inflammation and acute inflammation by recruiting CD4-positive T cells. Therefore, the more severe disease that was seen in the group that was administered the inhibitor 7 days after disease initiation decreased glomerular leukocyte recruitment and serum creatinine, which was attenuated by comparison with wild-type and TLR4-deficient mice. These findings suggest that renal cell TLR4 stimulation increases disease severity, and differences were mirrored by changes in serum creatinine, which was attenuated by comparison with wild-type and TLR4-deficient mice. These findings suggest that renal cell TLR4 stimulation increases disease severity, and differences were mirrored by changes in serum creatinine, which was attenuated by comparison with wild-type and TLR4-deficient mice. The role of TLR4 in experimental crescentic glomerulonephritis is confirmed, with antibody to glomerular basement membrane. The resulting glomerular injury is characterized by glomerular hypercellularity, thrombosis, and immune complex deposition. The role of TLR4 in experimental crescentic glomerulonephritis is confirmed, with antibody to glomerular basement membrane. The resulting glomerular injury is characterized by glomerular hypercellularity, thrombosis, and immune complex deposition. The role of TLR4 in experimental crescentic glomerulonephritis is confirmed, with antibody to glomerular basement membrane. The resulting glomerular injury is characterized by glomerular hypercellularity, thrombosis, and immune complex deposition.
Animal models of human autoimmune disease:

Experimental mouse models of human glomerulonephritis:

- Most models of human disease are studied in rodents (mice > rats)
- In general: 3 types of models:
  - Induced
  - Spontaneous
  - Genetically manipulated (transgenics, knockouts)

In general, IL-23/IL-17 pathway significantly contributes to renal injury in autoimmune disease: experimental rodent models of glomerulonephritis. Here, we identified renal IL-17-producing T cells in the T cell-mediated model of glomerulonephritis. Recently identified interleukin-17 (IL-17)-producing T cells (Th17 cells), are incompletely understood in proliferative and crescentic glomerulonephritis. These results demonstrate that the IL-23/IL-17 pathway significantly contributes to renal injury in these experimental rodent models of glomerulonephritis. In vitro, IL-17 enhanced the production of the proinflammatory chemokine thymus and activation-regulated chemokine (TARC) in mouse peritoneal macrophages.

The IL-23/Th17 Axis Contributes to Renal Injury in Goodpasture Antigen.

The role of Th17 in glomerulonephritis: STAT-3

BASIC RESEARCH


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Genetic deletion of Th1 cytokines such as IL-12 p404 and IL-23 p19 results in hyporeactivity including but extending beyond a deficient Th17 responses in the absence of IL-23 were not due to increased regulatory T cells; IL-12p40-deficient mice are protected from experimental autoimmune anti–glomerular basement membrane (anti-GBM) glomerulonephritis, seemingly defining a role for IL-12 in this disease; however, the recent identification of IL-23, a heterodimer composed of IL-12p35 and IL-23p19 as an essential Th17 activator, is a promising therapeutic strategy for the treatment of proliferative and crescentic glomerulonephritis.

IL-12, not IL-23, Directs Autoimmunity to the Goodpasture Antigen

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Infiltrating T cells can induce tissue injury, either directly or indirectly by activating macrophages. In experimental rodent models of glomerulonephritis, T cells infiltrate the kidney in both human and experimental glomerulonephritis, and several lines of evidence indicate that T cell-mediated tissue damage plays an important role in the immunopathogenesis of glomerulonephritis. Here, we identified renal IL-17-producing T cells in the T cell-mediated model of glomerulonephritis.3 We induced nephrotoxic nephritis in IL-23 p19-deficient mice, which have reduced numbers of Th17 cells, and in IL-12p40-deficient mice, which have reduced numbers of Th17 cells. In comparison with nephritic wild-type mice, IL-23 p19-deficient mice, which have reduced numbers of Th17 cells, and IL-12p40-deficient mice, which have reduced numbers of Th17 cells, show significantly less tissue damage in crescentic and proliferative forms of glomerulonephritis.2 Substantial evidence exists for the involvement of glomerular crescent formation. These results demonstrate that the IL-23/IL-17 pathway significantly contributes to renal injury in these experimental rodent models of glomerulonephritis.

The recruitment of T cells into the kidney is a hallmark of chronic kidney disease. T cell infiltration is a feature of both human and experimental glomerulonephritis. IL-23, a heterodimer composed of IL-12p35 and IL-23p19, induces the differentiation of monocytes to dendritic cells (DC) and promotes T helper 17 (Th17) cell differentiation. Th17 cells produce IL-17, which is required for the development of effector and memory CD4 T cells and is involved in the activation and expansion of B cells and the production of antibodies. IL-17 is a key cytokine for the development of autoimmune disease.

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Spectrum of glomerular disease:

- Nephrotic syndrome
- Nephritic syndrome
  - Hematuria
  - Proteinuria
    - "Less inflammatory"
    - "More inflammatory"

- Minimal change disease
- Membranous glomerulopathy
- Focal segmental glomerulosclerosis
- Membranoproliferative glomerulonephritis
- Diffuse proliferative glomerulonephritis
- Crescentic glomerulonephritis
- Necrotizing glomerulonephritis

Anti-GBM (Goodpasture’s) Disease:

- Severe form of glomerulonephritis
- Characterized by rapidly progressive kidney failure
- Antibodies against NC1 domain of α3 type 4 collagen (a component of the glomerular basement membrane)
- T cells required for disease pathogenesis
- Etiology of disease unknown

Nephrotoxic nephritis: a model of anti-GBM disease

- NT serum: immunization of sheep with mouse glomerular extract
- Immunologic priming: Sheep IgG antibody + CFA s.c. injection
- Disease induction: I.V. injection of nephrotoxic serum
- Heterologous injury
- Autologous injury
- Animal sacrifice

Pathogenesis of ANCA vasculitis:
A model of ANCA vasculitis:

**Step 1:** Development of immunity against MPO

- Creation of an MPO-/- transgenic mouse
- Immunization with MPO protein in CFA
- Development of immunity against MPO
- Collection of:
  - (a) splenocytes (anti-MPO T & B cells)
  - (b) anti-MPO antibodies

**Step 2:** Transfer of MPO antibodies to susceptible host

- Susceptible mouse (MPO expressing)
  - (a) wild-type
  - (b) RAG-/-
- Transfer of anti-MPO immunity
  - (i) anti-MPO antibodies
  - (ii) anti-MPO splenocytes
- Development of ANCA vasculitis

Experimental mouse models of lupus:

- **Spontaneous development of disease:**
  - New Zealand White (NZW) x New Zealand Black (NZB) F1 hybrid (NZB/W)
  - MNL Fox
  - BXSB mice

- **Transgenic mice:**
  - >20 unique genes manipulated to cause disease
  - Common Bcl-2 transgene, ApoE-/-

**Categories of immune defects:**
- Promote presentation of response to apoptotic debris
- Alter lymphocyte signaling
- Promote survival of autoreactive

Animal models of human autoimmune disease:

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlled variability between subjects</td>
<td>Inbred/congenic</td>
</tr>
<tr>
<td>Ease of genetic manipulation</td>
<td>Multiple susceptibility genes</td>
</tr>
<tr>
<td>Inbred</td>
<td>Variable susceptibility to renal injury</td>
</tr>
<tr>
<td>Targeted gene knockout</td>
<td>Mixed background</td>
</tr>
<tr>
<td>Inducible</td>
<td>Not spontaneous</td>
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</tbody>
</table>

Tip #1:
Choose the most appropriate model in which to test your hypothesis and be prepared to defend your choice
The role of RIP2 in autoimmune glomerulonephritis

The unknown role of RIP2 in autoimmunity:

EAE is exacerbated in RIP2−/− mice:

RIP2−/− T function in vitro:
There is a greater ratio of IL-17-producing to IFN-γ-producing T cells in lymph nodes and CNS in RIP2−/− mice:

Nephrotoxic nephritis: experimental design

C57BL/6 RIP2−/−
C57BL/6 RIP2+/+

Day 0 7 17
Immunologic priming: Sheep IgG antibody + CFA s.c. injection
Disease induction: I.V. injection of nephrotoxic serum

Tip #2:
Choose a research topic based on your previous work

Hypothesis:

We predict that RIP2−/− mice will have an an exacerbated (?) course of crescentic glomerulonephritis due to an increased Th17 response
Survival data: (mortality as an endpoint)

Survival data: (induction of experimental glomerulonephritis)

Crescentic glomerulonephritis: renal function

Crescentic glomerulonephritis: proteinuria day 2
Crescentic glomerulonephritis: proteinuria day 10

Crescentic glomerulonephritis: glomerular pathology

Crescentic glomerulonephritis: cytokine response

Tip #3:
Choose/develop an efficient and reliable method/assay in which to test your hypothesis and collect results
Conclusions I:

• Loss of RIP2 does not attenuate experimental glomerulonephritis
• NOD2 not required for renal injury?
• RIP2 deficiency increases mortality after induction of experimental glomerulonephritis
• Dependent vs. independent of renal injury?
• No difference in immune reactivity between wild-type and RIP2-deficient auto-reactive T cells

Further questions:

• Is there a defect in activation of RIP2−/− antigen-presenting cells?
• Is there an effect of Complete Freund’s adjuvant?
• Large amount of NOD2 ligands present in Tuberculosis
• Does RIP2 play an intrinsic role in T cell activation?

RIP2−/− macrophage activation by TLR/NLR stimulation

The role of CFA: Delayed-type hypersensitivity reaction
The role of CFA: Delayed-type hypersensitivity reaction

T cell cytokine production after CFA/sheep serum inoculation

Does loss of RIP2 (NOD2) in dendritic cells affect T cell activation?

Conclusions 2:

- NOD2 signaling doesn’t appear to be necessary for initiation of autoimmunity
- CFA doesn’t appear to be the cause of exacerbated autoimmune responses in RIP2-/- mice

Further research directions:

- Examination of the intrinsic role of RIP2 in T cells
- Do T cells have a functional NOD2 PRR?
- The role of RIP2 in NF-kB signaling
Objectives revisited:

• To introduce experimental mouse models of human glomerulonephritis
• To examine the role of RIP2 in experimental crescentic glomerulonephritis
• To provide “lessons learned” from my experience as a new investigator

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