Outline of Introduction to the Immune System and Potential Sources of Dysregulation by Thomas A. Fleisher, M.D.

I. General concept of innate and adaptive immunity

II. Specific T cell subsets are defined by cytokine production patterns (Th1, Th2, Th9, Th17)

III. IgG4 is the product of a Th2 response
   A. Requires T cell – B cell interaction via CD40L - CD40 binding
   B. IL-4/IL-13 dependent

IV. IgG4 is an unusual antibody
   A. Does not activate complement
   B. Does not bind Fcγ receptors (non-opsonic)
   C. Is generate after prolonged exposure to antigen
   D. Seen following successful allergen immunotherapy and in the setting of helminthic parasite infection

V. Antigen receptor generation
   A. Process of somatic mutation
   B. Generates self-reactive cells that need to be eliminated or inactivated

VI. Self-tolerance
   A. Central: thymus (T cells), bone marrow (B cells)
   B. Peripheral
     1. Native Tregs
     2. Inducible Tregs
     3. Anergy
     4. Apoptosis
     5. Alternative immunoregulatory cells (CD8 T cells, NK cells, myeloid cells)

VII. Monogenic autoimmune disorders
   A. APECED/APS1
   B. IPEX
   C. ALPS
   D. Early component complement deficiencies

References:

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Introduction to the Immune System and Potential Sources of Dysregulation

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Two Major Classes of Immune Response

- Innate immune system: older system of host defense that is "hard wired"
- Specific cells: phagocytic cells (monocyte, macrophage, dendritic cell, PMN, eosinophil); cytotoxic cells (NK cell, monocyte)
- Pattern recognition system: toll like receptor (TLR), lectin-like receptor (eg dectin)
- Systemic mediators: complement & cytokines,
- Local mediators: defensins & cathelicidins
- Provides a first line of host protection
- Interacts with the adaptive immune system

Two Major Classes of Immune Response

- Adaptive immune system: involves a process that depends on antigen specific recognition and response
- Specific immunologic memory central to the adaptive response (eg protection following vaccination)
- T cells and B cells central cellular players
- Cytokines are critical effectors of the adaptive immune response and are pleomorphic

Current Concepts of Adaptive Immunity

- T cells determine the immune response
  - Th1: IFN gamma, IL-12; response to intracellular pathogens (eg. TB)
  - Th2: IL-4, IL-5, IL-13: IgE and eosinophils; response to parasites and allergens
  - Th9: IL-9, IL-10, IL-17, IL-4, IFN-γ; mast cells
  - Th17: IL-17, IL-22: neutrophilic inflammation, host response to surface pathogens (eg candida)
  - T reg: immune down regulation; direct cell-cell interaction; cytokine mediated (IL-10, TGF-β)
- T cells and cytokines determine Ig class (eg IL-4 = IgE & IgG4; TGF-β = IgA)

Th2 Response

- Naive T cells stimulated by agn with co-stimulatory signal in the presence of IL-4 (basophils, mast cells, eosinophils, NK T cells, other Th2 cells)
- Involves STAT6 & transcription factor GATA3
- Response to parasite (eg helminth), allergen
- Th2 cells produce IL-4, 5, 10, 13
- IL-5 - eosinophil differentiation and activation
- IL-4/13 together with agn and CD40 (B cell)-CD40L (T cell) interaction = IgE and IgG4
IgG4 Is an Unusual Antibody

Features of IgG4

- IgG subclass with lowest concentration (<5%)
- Does not activate the classical complement pathway or bind to Fcγ receptors (non-opsonic)
- Is heterobivalent – it can bind to more than one antigenic determinant based on half molecule (one heavy and light chain) exchange
- Binds IgG on solid surface (i.e. mimics RF) but this involves the Fc (not Fab) portion of IgG

Features of IgG4 (cont)

- Production is Th2 dependent (IL-4, 5, 13)
- "Anti-inflammatory" cytokines (e.g. IL-10) drive the production of IgG4 producing plasma cells
- Generated after prolonged parasite exposure
- Seen following successful allergen sIT and in beekeepers (multiple stings), ? protective role in modulating IgE mediated disease
- Seen in association with pemphigoid diseases, sclerosing autoimmunity, membranous GN
- ? IgG4 levels useful in monitoring therapy

Immunologic Response Driving Autoimmunity

Antigen Specificity in the Adaptive Immune Response

- The generation of T cell (TcR) and B cell (Ig) antigen receptors depends on a process of somatic recombination between various genetic elements (VDJ)
- The recombination is random and thus generates cells expressing self-reactive receptors that need to be eliminated or inactivated to prevent autoimmunity

Immunologic Mechanisms Driving Autoimmunity

- Self-reactive helper T cells produce pro-inflammatory cytokines initiating inflammation
- Self-reactive helper T cells provide help for self-reactive B cells which leads to the production of autoantibodies
- Autoantibodies contribute further to the inflammatory process and tissue damage
Mechanisms of Immune Tolerance

Central (primarily through elimination)

Peripheral (through multiple mechanisms)

Mechanisms Inducing Tolerance

- Central tolerance: deletion of self-reactive cells in the thymus (T cells) and bone marrow (B cells)
- Peripheral tolerance
  - Regulatory T cell (Tregs): natural (thymic) & inducible
  - Cytokines: inducible Tregs - IL-10 (Tr1), TGF-β (Th3)
  - Anergy: lack of costimulatory signal associated with TcR stimulation yields an specific unresponsiveness
  - Peripheral elimination of autoreactive cells (via extrinsic apoptosis – e.g., Fas mediated)
  - Other immunoregulatory cells: CD8 T cells, NK cells, myeloid suppressor cells

Congenital Defects Associated with Immune Dysregulation

Lessons from genetically defined disorders of tolerance that lead to autoimmunity

- Autoimmune Polyendocrinopathy, Candidiasis, Ectodermal Dystrophy (APECED)/Autoimmune Polyendocrinopathy Syndrome Type 1 (APS1)
  - Hypoparathyroidism 85%
  - Adrenal failure 72%
  - Ovarian failure 60%
  - Type 1 DM 18%
  - Testicular atrophy 14%
  - Pancreatic cell atrophy 13%
  - Hypothyroidism 6%
  - Alopecia 27%
  - Hepatitis 13%
  - Enamel hypoplasia 77%
  - Nail dystrophy 52%
  - TM calcification 33%
  - Mucocutaneous candidiasis (100%)
  - Vitiligo (13%)

APECED

- Found primarily in selected populations: Finns, Sardinians, Iranian Jews
- Genetics: autosomal recessive defect in AIRE (Autoimmune REgulator protein)
- AIRE: a transcription factor involved in self antigen expression in the thymus for T cell negative selection (central tolerance)
- Evaluation focuses on the clinical findings and the presence of autoabys

APECED: AIRE Is Critical for the Elimination of Some Self-reactivity

- Defined genetic defect alters central tolerance with specific autoimmune processes typically with autoantibodies
- Primary targets are endocrine system with some involvement of skin and GI
- Mucocutaneous candidiasis as a result of autoantibodies to IL-17/IL-22
Immune dysregulation Polyendocrinopathy Enteropathy X-linked (IPEX)

Clinical syndrome described by Powell et al (1982)

IPEX – Clinical Findings

- Early onset enteropathy (watery diarrhea/villous atrophy; ~100%)
- Eczema/rash (~95%)
- Early onset type I DM (~75%)
- Thyroiditis (~40%)
- Cytopenias (~50%)
- Lethal without BMT/SCT

FOXP3 Expression is Absent in IPEX

Normal IPEX

XL defect in gene encoding FOXP3 results in absence of Tregs

Lessons from IPEX

- IPEX clarifies the critical nature of Tregs in maintenance of self-tolerance
- Primary targets are β cells, Gl tract, skin, blood cells and thyroid gland
- Very early onset of autoimmunity
- Owing to the severity of initial disease, the constellation of early clinical symptoms may not fully define role of Tregs in maintaining tolerance

Autoimmune Lymphoproliferative Syndrome - ALPS

Lymphadenopathy

Adult Control alpha-beta TCR DNT = 0.2% (5/mm$^3$)  
ALPS Patient alpha-beta TCR DNT = 33.7% (1071/mm$^3$)

Autoimmune Lymphoproliferative Syndrome (ALPS)

- Diagnostic criteria (requires 3 of 4)
  - Non-malignant lymphadenopathy
  - Increased level of α/β DNT cells
  - Defined genetic defect (Fas, Fasl, caspase 10)
  - Defective in vitro lymphocyte apoptosis
- Additional features supporting diagnosis
  - Autoimmune disease usually involves blood cells
  - Increased levels of IL-10, Fasl, vit B12
  - Typical histological findings in lymphatic tissue
  - Family members with similar clinical findings
  - History of lymphoma in the family
Lesons from the Autoimmune Lymphoproliferative Syndrome

- Demonstrates role of Fas mediated apoptosis:
  - Lymphocyte homeostasis - lymphoproliferation
  - Eliminating certain self-reactive cells - autoimmunity
  - Eliminating lymphocytes with potential for malignant transformation - increased risk for lymphoma
- Additional factor(s) contribute to development of autoimmunity since not all patients with Fas mutations in the same family are affected - these include genetic and likely other factors

Autoimmunity: It Is Not All About Defective Tolerance

- C1q deficiency: ~90% incidence of SLE; very rare (<50 cases); impaired apoptotic cell + immune complex (IC) clearance
- C4A/B deficiency: ~75% incidence of SLE; very rare (<30 cases), impaired IC clearance
- C2 deficiency: ~40% incidence of SLE; most common complete C defect; impaired IC clearance; also increased bacterial infections (e.g. S. pneumo, H. flu) during childhood

Summary

- Our understanding of the immune response involves various subtypes of T cells based on their pattern of cytokine production
- Control of self-reactivity requires both multiple central and peripheral mechanisms
- Single gene defects with specific patterns of autoimmunity identify the complexity of these control mechanisms as well as the potential role of other genetic and/or environmental factors in the disease evolution
Autoimmune Pancreatitis and Retroperitoneal Fibrosis

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Summary

Retroperitoneal fibrosis complicated by ureteral obstruction and hydronephrosis can be a manifestation of IgG4-related sclerosing disease. Affected patients almost always have similar fibroinflammatory lesions at other sites, with the pancreas being the best-known. This review will detail the histologic features of autoimmune pancreatitis (with a digression on subtypes of the condition) and summarize the extra-pancreatic manifestations of IgG4-related disease, with emphasis on retroperitoneal fibrosis.

Autoimmune pancreatitis

Autoimmune pancreatitis (AIP) is a fibroinflammatory condition of the pancreas that can mimic malignancy but usually responds to steroids. Most patients have elevated levels of serum IgG4 and most patients have increased numbers of IgG4-positive plasma cells in the inflamed pancreas. Similar IgG4-rich inflammatory lesions can occur in almost any organ; all such conditions are currently (tentatively!) grouped under the rubric IgG4-related sclerosing disease or IgG4-related systemic disease (IgG4-RSD).
IgG4-RSD produces a characteristic but not specific set of histologic changes, modified by the microanatomy of the particular site involved. Cheuk and Chan have provided a superb recent review of the topic. [1] Lymphoplasmacytic inflammation, fibrosis, phlebitis and increased numbers of IgG4-positive plasma cells make up the characteristic mix, and the resulting pattern is usually sufficient to suggest the correct diagnosis. While the original work on the pathology of AIP was based on resection specimens, the fact that this is a non-neoplastic, steroid-responsive condition means that we are now being asked to contribute to the diagnosis based on findings in needle biopsies.

The literature on the pathology of autoimmune pancreatitis is complicated by the fact that the term encompasses two probably separate conditions. Notohara et al termed them “lymphoplasmacytic sclerosing pancreatitis” and “idiopathic duct-centric pancreatitis” ([2], while Despande et al chose the terms lobulocentric AIP and duct–centric AIP. [3] The two patterns have come to be called type 1 and type 2 AIP [4, 5]

**Pathology of AIP, type 1**
Type 1 AIP is the pancreatic manifestation of IgG4-related sclerosing disease. Affected patients are generally males (M:F ratio is 4-5:1) in their 7th decade. The usual presentation is painless jaundice, but some patients complain of abdominal pain and rare patients present with pancreatic insufficiency. [5]

Type 1 AIP usually involves the entire pancreas, but can be localized enough to mimic pancreatic tumor. Histologically, focal involvement is not rare; Chandan et al found at least focal sparing in 32/39 resected examples of AIP. [6] When focal involvement does occur, the uninvolved areas are histologically normal and IgG4-positive plasma cells are not increased. Biopsies taken from uninvolved areas will necessarily be non-diagnostic.

Pancreatic tissue involved by AIP has lymphoplasmacytic inflammation. There is almost always a cuff of lymphocytes and plasma cells surrounding branches of the pancreatic duct. While the inflammatory cuff can be quite dense, the duct epithelium remains intact. Neutrophils are not seen in or around the epithelium; in fact, the presence of intraepithelial neutrophils favors type 2 AIP over type 1.
Lymphoplasmacytic inflammation involves the pancreatic lobule as well. At least a few eosinophils are often present, and may be quite numerous at times. [7] As in the periductal infiltrate, the presence of more than a few neutrophils should raise doubt about the diagnosis of IgG4-RSD. Lymphoepithelial lesions (infiltration of glands by lymphocytes) are not a feature of this condition. Instead, the infiltrate is concentrated between acini. At first, the dense infiltrate contrasts with interlobular fibrosis to accentuate the lobular architecture, but further damage ultimately obliterates the acini, leaving only islets and small duct branches. The lymphoid infiltrate is dominated by T-lymphocytes, specifically CD8-positive T-suppressor cells. No clonality has been demonstrated in T or B cell subsets. [8] Lymphoid aggregates are sparse in the pancreas itself, but are prominent in peripancreatic soft tissue. [2] Perineural inflammation is a common feature of type 1 AIP.

The atrophic pancreatic lobule is replaced by a mix of lymphoplasmacytic inflammation and fibrosis, often producing a storiform pattern. This pattern tends not to occur in other forms of chronic pancreatitis (including type 1 AIP) or in the pancreatitis adjacent to neoplasm, making it a very helpful diagnostic feature, particularly in needle biopsies. The distinctive
appearance may partly be due to the type of collagen that makes up the fibrotic component: Song et al have demonstrated that AIP tends to have much less type III collagen than alcoholic chronic pancreatitis, and more type IV collagen. [9] They speculate that the collagen profile accounts for the reversibility of fibrosis in some cases of AIP, since type IV collagen is an element of the normal pancreatic stroma rather than a manifestation of scarring. One might further speculate that “burned out” AIP has more collagen III and is therefore irreversible. [10]

Phlebitis is invariably seen in resected examples of type 1 AIP. It is easily located by finding large muscular arteries, then locating the adjacent vein. The vein lumen is narrowed (sometimes completely obliterated) by a dense infiltrate of lymphocytes and plasma cells. Neutrophils are not a part of this process, nor is there fibrinoid necrosis or nuclear dust that might suggest a vasculitic process. Needle biopsies generally don’t contain large veins, but small artery-vein pairs can occasionally be seen. A histochemical stain for elastic is said to be helpful in identifying vein remnants. [11]

Immunohistochemical staining for IgG4
Antibodies against IgG4 perform very well in formalin-fixed, paraffin-embedded tissue, and provide a useful adjunct to the diagnosis of type 1 AIP. Plasma cells producing IgG4 are decorated with a very dense cytoplasmic stain, leaving the nucleus unstained and visible. An IgG4-positive infiltrate alone is not specific for a diagnosis of type 1 AIP (or any particular manifestation of IgG4-RSD), but in the appropriate clinicopathologic setting, positive staining for IgG4 can clinch the diagnosis. The first attempt to quantify the IgG4 infiltrate in this setting used a semiquantitative scale with irregular breakpoints and considered a density of 10 IgG4-positive plasma cells/high power field (hpf) as a positive result. [12] We used this scoring system in a study of pancreatic resections, and found that 21/29 AIP cases had a positive score, compared to 1/9 examples of alcoholic chronic pancreatitis and 3/25 pancreatic cancers. [13] Deshpande et al used a slightly different scale, but found similar results. [3] A recent study from Memorial Sloan-Kettering found that a cut-off value of 50 IgG4-positive cells/hpf gave a sensitivity for AIP of 84% and a specificity of 100%. [14] The authors also emphasized that the IgG4-positive infiltrates tend be diffuse in AIP and patchy in peritumoral infiltrates.
Diagnosing type 1 AIP in small biopsies

Because this condition generally responds to medical management, the goal is to avoid surgical resection of the pancreas. The tissue-based component of the clinical work-up has come to depend on needle biopsy; most of the documented experience in this area has been with trucut biopsies obtained under endoscopic ultrasound guidance. [15, 16] Naturally, this makes the pathologist’s task more difficult, but assuming that the biopsy shows chronic pancreatitis and no evidence of malignancy, one can usually point the diagnosis in favor of or against type 1 AIP. Even small duct branches, if present, will often show a dense periductal lymphoplasmacytic infiltrate. If vein branches are present, phlebitis is a characteristic finding. In practice, neither ducts nor veins are commonly seen in needle biopsy; in that case the cell-rich storiform fibrosis becomes the critical finding. Immunohistochemistry for IgG4 is invaluable in this setting. Increased numbers of IgG4-positive plasma cells (more than 10/hpf), particularly if they are diffuse, strongly support the diagnosis of type 1 AIP.

Some studies have shown that fine needle aspiration of the pancreas can provide sufficient diagnostic material in this clinical setting. [17] This
diagnosis depends on recognizing stromal fragments on cytology preparations. Carcinoma can also have stromal fragments, but AIP lacks atypical epithelial cells. The inflammation typical of type 1 AIP makes the stromal fragments more cellular in AIP than in carcinoma.

**Type 2 AIP**

A subset of patients originally grouped under the heading of autoimmune pancreatitis seems to be different on both clinical and histologic grounds. ([2, 3] Table 1 highlights the observed differences between type 1 and type 2 AIP. Briefly, patients with type 2 AIP tend to be younger than those with type 1, and have an equal sex distribution. The disease is confined to the pancreas. Some patients have inflammatory bowel disease, but systemic manifestations of IgG4-RSD are absent. A very recent review by Despande emphasized the importance of making this distinction, and highlighted the fact that peripancreatic lymph nodes almost always show a distinctive IgG4-rich hyperplasia in AIP1, but are not abnormal in AIP2. [18]
Table 1: Clinical and Pathologic comparison of Type 1 and Type 2 AIP

<table>
<thead>
<tr>
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<th>Type 1</th>
<th>Type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>60-70</td>
<td>50-60</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>4-5M:1F</td>
<td>M=F</td>
</tr>
<tr>
<td><strong>Jaundice</strong></td>
<td>common</td>
<td>&lt;20%</td>
</tr>
<tr>
<td><strong>Abdominal pain</strong></td>
<td>rare</td>
<td>usual</td>
</tr>
<tr>
<td><strong>IgG4-RSD</strong></td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td><strong>Periductal inflammation</strong></td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td><strong>GELs</strong></td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td><strong>Phlebitis</strong></td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td><strong>Storiform pattern</strong></td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td><strong>IgG4 IHC</strong></td>
<td>+++</td>
<td>0 to +</td>
</tr>
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In resected specimens, the diagnosis is usually easy – the ducts are made prominent by a dense cuff of lymphoplasmacytic inflammation and the epithelium contains neutrophils. The epithelium may be eroded. The neutrophils often form microabscesses, producing the very characteristic
“granulocytic epithelial lesion.” [19] Features of type 1 AIP (storiform fibrosis, phlebitis, lymphoid aggregates in peripancreatic parenchyma) are either poorly developed or absent. IgG4-positive cells are either absent or sparse. I often struggle with the diagnosis in needle biopsies; unless the needle happens to catch a duct with neutrophils, the biopsy can look very non-specific. Often, the best one can do is to say that there are no malignant cells and no features of type 1 AIP.

**Retroperitoneal manifestations of IgG4-related sclerosing disease**

The idea that IgG4 might serve as a marker for a systemic inflammatory condition is relatively recent. [12] Now, the list of sites/organs involved by IgG4-RSD seems to grow by the day. It is not yet clear whether all of these conditions represent a single disease or simply a stereotypic response to various stimuli, but there is absolutely no doubt that individual patients can have multisystem involvement, either synchronously or metachronously. At many sites, the term IgG4-RSD encompasses or “explains” a subset of a previously named condition. In the salivary gland, for example, some examples of Mikulicz’s disease and some examples of Kuttner’s tumor are IgG4-rich and have histology characteristic of IgG4-RSD. The same can be
said for some examples of Riedel’s thyroiditis, orbital pseudotumor and inflammatory pseudotumors in various locations. IgG4 disease also seems to account for a substantial subset of retroperitoneal fibrosis.

Retroperitoneal fibrosis is an idiopathic chronic inflammatory fibrosing condition, probably with multiple causes. Affected patients can have similar fibrosing lesions at other sites; this condition has been referred to as multifocal fibrosclerosis. [20] Even before the IgG4 story developed, pancreatic disease was noted as a sometime association with multifocal fibrosclerosis. [21] Now, it appears that IgG4 disease accounts for many examples of multifocal fibrosclerosis. [22, 23] Zen et al recently described 17 patients with retroperitoneal fibrosis, 10 of whom were thought to be IgG4-related. [24] In general, there were no histologic features specific for IgG4-related fibrosis: both set of patients had variable degrees of inflammation, with or without lymphoid aggregates. Multinucleated giant cells were present in about 20% of both groups. Phlebitis, however, was much more common in the IgG4-related group (6/10 vs 1/7). IgG4 immunohistochemistry showed significant differences, both in terms of IgG4-positive plasma cells (68/hpf vs 3/hpf) and IgG4/IgG ratios (62% vs
4%). Strikingly, all 10 patients with IgG4-related disease were male, while 6 of the other 7 were female.

The male predominance noted by Zen et al has also been a feature of other cases described in the literature, mostly in the form of case reports. In fact, all 14 cases collected in a recent review of the literature were male. [25] Among those 14 patients, 11 also had pancreatitis, two had salivary gland involvement and one had mediastinitis. All 11 patients for whom treatment was described were said to have a positive response to corticosteroid treatment.

**Conclusion**

The combination of clinical features and histology can usually establish a diagnosis of type 1 AIP without the need for pancreatic resection. Affected patients will often have other manifestations of IgG4-related sclerosing disease. There is no one histologic feature specific for IgG4-RSD, but a mix of lymphoplasmacytic inflammation, storiform fibrosis and phlebitis is characteristic. Immunohistochemistry for IgG4 is also nonspecific, but large numbers of IgG4-positive cells are a useful adjunct to the diagnosis.
Retroperitoneal fibrosis (and other conditions linked to multifocal fibrosclerosis) often feature IgG4-rich infiltrates and are associated with other manifestations of IgG4-related sclerosing disease. Thus far, there is a striking male predominance in this subset of IgG4-RSD (a condition in which males already predominate). The etiology remains unknown.

Renal manifestations of IgG4-related systemic disease

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While autoimmune pancreatitis (AIP) has been recognized since the first description by Sarles et al in 1961, its involvement of other organs has been recognized more recently. Now, some form of IgG4-related systemic disease (IgG4-RSD) has been described in nearly every organ system, including the liver, gallbladder, other gastrointestinal sites, kidney, salivary and lacrimal glands, orbit, breast, lung, retroperitoneum, aorta, lymph nodes, skin, pituitary gland, and prostate. Involvement usually consists of inflammatory masses but may also manifest as a skin rash or as inflammatory bowel disease.

In the kidney, IgG4-RSD can also present as an inflammatory mass or masses. Biopsy of these masses reveals a histologic pattern of tubulointerstitial nephritis (TIN); glomerular disease has been reported in a subset of cases. Besides radiographic findings of a mass or masses, renal involvement by IgG4-RSD may also present clinically as acute or chronic renal failure, proteinuria with associated glomerular disease, or obstruction related to retroperitoneal fibrosis.

**Tubulointerstitial nephritis**

In the kidney, we attempt to classify TIN according to its cause. In general, TIN/nephropathy can be divided into broad categories of drug-related, autoimmune, hereditary/toxic/metabolic, infectious (direct infection or reactive to a distant infection), and idiopathic/other. Some overlap exists between the different categories.

The cause of TIN in a particular case can be determined by biopsy features by light microscopy, immunofluorescence (IF), and electron microscopy (EM) in conjunction with clinical history and clinical laboratory results, and correlation with radiographic studies in some entities. By light microscopy, the renal pathologist recognizes the pattern of inflammation and types of cells in infiltrate; by IF, the presence or absence of immune deposits and anatomic and immunophenotypic pattern of deposition; and by EM, the absence or presence of immune deposits, pattern of deposition, and presence of any substructure to the deposits.

Autoimmune TIN may be may be associated with systemic autoimmune disease. Examples of systemic autoimmune disease with associated TIN are interstitial inflammation as part of systemic lupus erythematosus (usually accompanied by glomerular disease), sarcoidosis, and Sjögren syndrome. Although not always present, some types of autoimmune TIN show tubular basement membrane (TBM) immune complex deposits, which can suggest an autoimmune etiology.

**IgG4-related tubulointerstitial nephritis**

TIN as part of IgG4-RSD is an autoimmune TIN, which we term IgG4-related TIN. TIN is the most common renal manifestation of IgG4-RSD. Indeed, 30% of patients with AIP
show evidence of renal parenchymal involvement by TIN based on the distinctive radiographic appearance. A history suggestive of IgG4-RSD is helpful in making the diagnosis, but this history is not always present in patients at the time of renal biopsy, or particular aspects of the history are not recognized by the clinician as relevant. Certain laboratory results, along with radiographic findings, can also help make the diagnosis of IgG4-related TIN.

Common clinical, radiographic, and clinical laboratory features in IgG4-related TIN include radiographic abnormalities (present in 85% of cases), elevated serum IgG4 or total IgG levels or hypergammaglobulinemia on serum protein electrophoresis (present in 81%), eosinophilia (present in 28%), and a history of other organ involvement by associated inflammatory conditions including inflammatory masses (present in 84%). Patients may show some but not all of these clinical, radiographic, and laboratory features together. Seventy-five percent of patients with renal specimens had renal failure, either acute or chronic and progressive, as the main indication for renal biopsy, while ~25% of patients with renal specimens had a renal mass or masses as the indication for biopsy or nephrectomy.

Pathologic features of IgG4-related TIN

By light microscopy, the biopsy may show diffuse, multifocal, or focal involvement of the renal cortex by TIN. The infiltrate is composed of mononuclear cells, many plasma cells, and sometimes numerous eosinophils. Often, cases show a distinctive expansion of the interstitium by a fibroinflammatory process with prominent myofibroblasts, resulting in the residual tubules being pushed apart. Tubules are atrophic, sometimes with thickened basement membranes containing immune deposits. Residual fragments of basement membranes of destroyed tubules may be seen focally. Mononuclear cell (sometimes plasma cell) tubulitis and tubular injury are present. Individual cases show a range of appearances, ranging from a very cellular, less fibrotic lesion to a very sclerotic lesion with fewer inflammatory cells. The inflammatory involvement of the kidney resembles that of other organs involved by IgG4-RSD.

Approximately 84% of cases show TBM immune complex deposits. By immunofluorescence, there is granular TBM staining for IgG, C3, and kappa and lambda light chains, sometimes with accompanying interstitial granular staining. Some cases show lesser TBM staining for IgM or C1q. Corresponding amorphous electron dense deposits can be seen by electron microscopy. Immune deposits may be focal, and are present in areas of interstitial inflammation. In some cases, TBM deposits may be seen on an immunohistochemical stain for IgG4.

Value of IgG4 immunohistochemical staining

The pancreatic literature categorizes IgG4+ infiltrates according to the number of IgG4+ plasma cells per 40x field (hpf) in the most concentrated area(s): 0-5 IgG4+ cells, no increase; 5-10, mild increase; 11-30, moderate increase; and >30, marked increase. In the pancreas, increased IgG4+ plasma cells distinguish inflammatory infiltrates of AIP from those associated with other conditions. In a study by Zhang et al (Mod Pathol 2007), 72% of AIP cases showed moderate to marked increase in IgG4+ plasma cells, compared to 11 and 12% of cases of chronic alcoholic pancreatitis and pancreatic adenocarcinoma.
A series of 32 IgG4-related TIN cases from Mayo Clinic, Nephropath, and Massachusetts General Hospital (Raissian USCAP 2011 abstract and Cornell 2007) used as one criterion for IgG4-related TIN diagnosis the presence of increased IgG4+ plasma cells; consequently, 100% of these cases showed moderate to marked increased IgG4+ plasma cells. In comparison, only 10 of 114 (9%) renal biopsies with plasma cell-rich interstitial inflammation due to other causes showed at least moderate increase in IgG4+ plasma cells (excluding cases of pauci-immune necrotizing and crescentic glomerulonephritis; see below). These results provide a sensitivity of 100%, a specificity of 91%, and a positive predictive value of 76% for IgG4-related TIN with at least moderately increased IgG4+ plasma cells.

**Differential diagnosis and pitfalls of IgG4 staining**

Some other causes of interstitial inflammation with increased plasma cells show increased IgG4+ plasma cells, usually with a moderate increase (11-30/hpf focally). Most notably, 25% of cases of pauci-immune necrotizing and crescentic glomerulonephritis show at least moderate increase in IgG4+ plasma cells, in both the kidney and in extra-renal ANCA-associated disease. This disease can be mass-forming and may resemble IgG4-RSD. Rare cases of TIN in other categories may also show increased IgG4+ plasma cells, including chronic pyelonephritis, Sjögren syndrome (<10% of cases), and drug reactions. These entities should not show the other clinical or radiographic features of IgG4-RSD, however.

One entity similar to IgG4-related TIN is “idiopathic hypocomplementemic tubulointerstitial nephritis with extensive tubulointerstitial deposits” (Kambham, *Am J Kid Dis* 2001), some cases of which may represent IgG4-RSD.

**Proposed diagnostic criteria for IgG4-related TIN**

| Histology | Plasma cell-rich tubulointerstitial nephritis with >10 IgG4+ plasma cells/hpf field in the most concentrated field*  
Tubular basement membrane immune complex deposits by immunofluorescence and/or electron microscopy** |
|---|---|
| Imaging | Small peripheral cortical nodules, round or wedge-shaped lesions, or diffuse patchy involvement  
Diffuse enlargement of kidneys |
| Laboratory results | Elevated serum IgG4 or IgG level  
Hypergammaglobulinemia  
Eosinophilia |
| Other organ involvement | Includes autoimmune pancreatitis, sclerosing cholangitis, inflammatory masses in any organ, salivary/lacrimal gland involvement, inflammatory aortic aneurysm, lung involvement, retroperitoneal fibrosis |

*Mandatory criterion  
**Present in ~84% of cases
To make the diagnosis of IgG4-related TIN, a typical histologic appearance with >10 IgG4+ plasma cells/hpf in the most concentrated area must be present, along with at least one other feature in the categories of Imaging, Laboratory, or Other organ involvement. Other organ involvement need not be concurrent with renal manifestations. Supporting features are the presence of TBM immune complex deposits, as well as marked improvement with steroid therapy, particularly with respect to masses or other radiographic abnormalities.

**Treatment of IgG4-related TIN**

The standard therapy for AIP with pancreatic involvement is steroid therapy. Patients typically show a marked response to steroids, but there is a high relapse rate. A small subset of patients does not respond to steroids; in these patients, rituximab has been used, with a good response. Some AIP patients have been reported to respond to azathioprine, mycophenolate mofetil, or cyclophosphamide, sometimes in combination with steroids.

Less is known about the response to treatment in IgG4-related TIN. In a Mayo Clinic IgG4-related TIN biopsy series (Raissian USCAP 2011 abstract), 11 of 12 patients with elevated serum creatinine showed a response to prednisone therapy (one responder also received mycophenolate mofetil), while 2 untreated patients showed continued elevated creatinine. One patient with a mass lesion was treated with prednisone and mycophenolate mofetil. Similarly in the Sacki series, 10 of 11 patients with elevated creatinine at diagnosis showed decreased creatinine 1 month after prednisolone therapy, while another patient presented with a high creatinine and remained on hemodialysis even after treatment.

**Other renal involvement**

*Glomerular disease*

Glomerular diseases have also been seen in patients with IgG4-RSD. Membranous glomerulonephritis (MGN) is most commonly observed, present in 9% of IgG4-RSD patients in the largest published series of renal parenchymal involvement (Sacki, *Kidney Int* Nov 2010). MGN has also been noted in association with AIP in various case reports. MGN in patients with IgG4-RSD may or may not have concurrent interstitial nephritis. Of interest, MGN is also an IgG4-dominant disease.

In the series of Sacki et al of 23 patients with IgG4-related TIN, 6 (26%) patients had concurrent glomerular disease: 2 with membranous glomerulonephritis, one with IgA nephropathy, and 3 with mesangial immune deposits with mesangial or endocapillary proliferative GN without a more definitive glomerular diagnosis.

A few individual cases have been reported of membranoproliferative glomerulonephritis, IgA nephropathy, and an endocapillary proliferative GN.

*Obstruction related to retroperitoneal fibrosis or ureteral mass(es)*

Extra-renal manifestations may give rise to hydronephrosis, with or without accompanying renal parenchymal involvement.
Pathogenesis of IgG4-RSD

IgG4 is an unusual molecule, with some unusual physical characteristics. Compared to IgG1, IgG4 has weaker inter-chain bonds, resulting in a high rate of dissociation of immunoglobulin half-molecules. In this way, the IgG4 molecule cannot fix complement and cannot form large immune complexes. IgG4 may block antigen from the more pathogenic IgG1 or IgE.

Despite being thought of as an “anti-inflammatory” immunoglobulin, IgG4 nevertheless is found in high levels in IgG4-related autoimmune disease. IgG4 class switching depends on IL-4 and/or IL-13 mainly secreted by T-helper 2 cells. IL-10 has an effect on IgG4 versus IgE class switching and may be required for IgG4 class-switched B cells to differentiate into IgG4-secreting plasma cells. One may speculate that, in IgG4-related systemic disease, an initial insult and process involving production of anti-inflammatory cytokines, including IL-10 and tumor necrosis factor-alpha, along with fibrogenic IL-13, drives increased fibrosis, induction of IgG4 class-switched B cells, and production and massive expansion of IgG4-secreting plasma cells.

Selected references

Anti-PLA2R IgG4: Is it more than a marker of primary membranous nephropathy?

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Membranous nephropathy (MN) is an organ-specific autoimmune disease characterized by the presence of subepithelial immune deposits containing IgG and complement. Work on the Heymann nephritis model of MN in rats established that the immune deposits form in situ, and that the membrane attack complex of complement mediates podocyte injury. The target antigen gp330/megalin was subsequently identified and shown to reside on rat podocytes; however megalin is not expressed on human podocytes. More recent observations in an alloimmune form of neonatal membranous nephropathy in babies born to mothers with homozygous deficiency of neutral endopeptidase (NEP) validated the in situ paradigm in human MN.

With this background we embarked on a search for the target antigen in idiopathic MN using patient sera and proteins extracted from glomeruli isolated from normal human donor kidneys that were unsuitable for transplantation. On western blot analysis we have found that sera from 75-80% of patients with primary (idiopathic) MN, but not secondary (lupus and hepatitis B) MN, other proteinuric diseases, or normal controls have circulating antibodies to a reduction-sensitive glycoprotein that is expressed on normal human podocytes. Proteomic analysis of the target antigen showed it to be the phospholipase A2 receptor (PLA2R), a member of the mannose receptor family.

Another interesting finding is that most of the reactivity to PLA2R resides in the IgG4 subclass. This is significant because most of the immunoglobulin that is deposited in the glomeruli of patients with primary MN is IgG4, which does not fix C1q and activate complement via the classical pathway. Although, the serum of several patients also has lesser amounts of anti-PLA2R IgG1 and IgG3, which do activate the classical pathway of complement, C1q is typically absent from the glomerular deposits in primary MN. This suggests the possibility that anti-PLA2R IgG4 might activate complement via the alternative or lectin pathways. Preliminary data supporting such a role will be presented. Moreover, recent studies have shown that the presence of anti-PLA2R IgG4 antibodies correlates with disease activity in all ethnic groups. Thus, these findings have implications for the diagnosis of primary MN and for monitoring the response to treatment and may implicate a pathogenic role for anti-PLA2R IgG4.