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Inflammatory cells in renal allografts

Volker Nickeleit[1], Kenneth Andreoni[2]

The University of North Carolina Medical School, Department of Pathology and Laboratory Medicine, Nephropathology Unit[1], and Division of Abdominal Transplant Surgery[2], Chapel Hill, North Carolina 27599-7525, USA

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1. ABSTRACT

Renal transplants are injured by a variety of diseases and pathways. One important cause for acute and chronic graft failure is rejection. Since the advent of kidney transplantation, it has become apparent that rejection is a cellular and/or antibody mediated inflammatory process with different histologic phenotypes, and clinical degrees of severity. In recent years, the immunohistochemical detection of the complement degradation product C4d has further helped to unravel mechanisms of graft injury. Our brief review of 'renal allograft inflammation' focuses on basic morphologic aspects of rejection. Our goal is to foster the close correlation between 'histologic variants of rejection/inflammation' and molecular signalling cascades including chemokine induced effects.

2. INTRODUCTION

This special issue of *Frontiers in Bioscience* focuses on chemokine mediated effects in the kidneys and underscores the tremendous advances made over the past years to define basic biologic pathways controlling "inflammation". Our view of inflammatory cells in renal allografts is constantly changing as new technological advances allow us to better characterize allograft ischemia-reperfusion injury and repair, cellular and humoral rejection, accommodation, tolerance, or complications such as infections or glomerulonephritides. Cell activation and signalling cascades have occupied center stage and 'subcellular' molecular key events including chemokine induced effects

have been described.. However, while pushing the molecular frontiers of our biologic understanding it becomes increasingly apparent that all signalling events have to be interpreted in the proper cellular microenvironment and appropriate histologic context. In a renal allograft, for example, why does *one* activated lymphocyte under the endothelium of an artery have a different clinical and prognostic significance, i.e. rejection mediated arteritis, than *one* lymphocyte in the interstitial compartment, i.e. non-specific change? If five lymphocytes are observed in five glomeruli the finding is interpreted as non-specific, whereas five lymphocytes in one dilated glomerular capillary trigger the diagnosis of rejection mediated glomerulitis. Thus, much remains to be studied about inflammatory events and pathways occurring in specific renal tissue compartments. The most rewarding and informative analyses, although technically challenging and expensive, will be careful laser microdissection and gene expression profiling studies targeting specific anatomic structures: "chemokines meet anatomy".

Here we will briefly focus on the (diagnostic) significance of inflammatory events found in different compartments of kidney allografts (for a detailed review see (1)). We hope that our introduction fosters an in-depth understanding of the complexity of inflammatory events seen in renal allografts and helps to put subsequent articles from this issue into a broader 'histologic' and 'clinical' context.

3. INFLAMMATORY CELLS

In renal transplants, different inflammatory cell types are involved in a wide spectrum of effects ranging from ischemia-reperfusion injury, cellular and antibody mediated rejection, the defense against infectious agents, allergic reactions, to graft accommodation and tolerance.

3.1. Classic inflammatory cells

3.1.1. Neutrophilic leukocytes

Neutrophils (2, 3) are most commonly associated with the host defense against bacterial infections, such as seen in cases of pyelonephritis. The inflammatory response is promoted by chemotactic factors and proinflammatory cytokines (e.g. chemokines, bioactive lipids, neuroendocrine hormones, histamine or adenosine) that attract and activate circulating neutrophils. The neutrophils bind to adhesion molecules on the surface of activated endothelial cells, predominately in the peritubular capillaries and vasa recta spuriae/verae,, pass through the endothelial cell layer, and travel towards the chemoattractants. Neutrophils not only respond to, but also secrete a number of cytokines, which may act in an autocrine or paracrine manner, such as interleukin-8, interferon gamma, and the complement factor C5a. Cytokines may also aid in the resolution of the inflammatory response through macrophage inflammatory protein-1 alpha (MIP-1alpha). (2). In some renal allografts neutrophils can be associated with pure, T-cell poor antibody mediated rejection (4, 5).

3.1.2. Lymphocytes

T (thymus derived) and B (bursa or bone marrow derived) lymphocytes are active in adaptive immunity, whereas natural killer cells are part of the innate immune system (6). Lymphocytes can be differentiated from each other by cell surface proteins known as 'cluster of differentiation' markers (CD).

T lymphocytes are the traditional mononuclear cells described in acute cellular rejection. They can kill parenchymal cells through various means, including direct cell contact and active secretalogues. The tissue distribution and the number of T lymphocytes defines the severity of the rejection episode and will be discussed below. The cells carry the "T cell receptor" complex, and express CD3; additionally T helper cells express CD4 and cytotoxic T cells CD8. T helper lymphocytes are activated by professional antigen presenting cells, such as macrophages. The T-cell receptor and closely associated CD3 complex of a CD4 positive cell bind to a foreign protein presented by the major histocompatibility (MHC) complex of an antigen presenting cell. As this occurs, the CD4 complex also binds to the MHC complex allowing for "signal 1" to be sent through activation of intracellular kinases. This "signal 1" must be followed by costimulation via a "second signal", usually through binding of CD80 or CD86 on the antigen presenting cell to CD28 on the T cell (7, 8). Signals 1 and 2 promote signal 3, i.e. the expression of the interleukin-2 receptor complex (CD25) (9). T helper cells have no direct phagocytic or cytotoxic activity but activate CD8 positive cytotoxic T cells, B lymphocytes, and macrophages.

CD8 positive T effector or cytotoxic lymphocytes are responsible for the "classic" host cellular attack against foreign antigens expressed in renal allografts. The CD8 complex binds along with the T cell receptor/CD3 complex to MHC class I molecules. Similar to CD4+ T helper cells, cytotoxic T cells are activated by antigen presenting cells and undergo signal transduction. In contrast to CD4 positive cells, however, CD8+ cytotoxic lymphocytes can destroy and lyse other cells through granzyme and perforin release or possibly FAS ligand induced apoptosis (10, 11).

More recently T regulatory cells CD4 (+), CD25 (+), Foxp3 (+) have been characterized that may potentially prevent host reactivity toward donor antigens (12-14). The direct biologic significance of T regulatory cells in renal allografts is, however, at the present time undetermined (15)

B lymphocytes are CD19, CD20, and CD21 positive. They express MHC class II antigens on their surface. B lymphocytes generally mature into antibody producing plasma cells and can participate in "inflammatory diseases" such as cellular rejection episodes (see below). The B cell receptor is a membrane bound immunoglobulin responsible for B cell activation. Upon binding of the receptor and (co)stimulation from a T helper cell, a B cell can differentiate into a plasma cell or memory cell (16). During activation, the B cell internalizes foreign protein and subsequently expresses it with the MHC class II complex on the cell surface where it can be recognized

by T helper cells (17). This interaction results in the secretion of cytokines (by T helper cells) which further stimulate B cell activation, differentiation, and antibody production (18). Mature plasma cells are CD138, CD38 positive and synthesize immunoglobulins, but lose expression of MHC class II, CD19, CD20, CD22, CD44 and CD 45 (19). CD20 positive B lymphocytes and plasma cells have also been implicated in graft rejection (20-23). The process through which plasma cells actually injure an allograft are not well defined; most plasma cell rich rejection episodes are C4d negative, thus, appear to be purely cell mediated (see below). Plasma cells also appear to be able to present antigens in certain circumstances (24, 25).

Natural killer cells commonly express CD16, CD28, and CD56 without the TCR complex or surface immunoglobulins (18). They play a vital role in innate immunity protecting the host from viruses and tumor cells. They kill by release of perforin and granzyme causing apoptosis in the target cells. Their role in renal allograft dysfunction is not fully determined (26-28).

3.1.3. Mast cells

Mast cells are resident cells in various tissues and contain intracytoplasmic histamine and heparin granules. They are involved in allergy, anaphylaxis, wound healing and host defense against invading pathogens. Mast cells can release vasoactive granules by direct injury, IgE receptor binding, or complement stimulation. Active compounds of mast cell granules in addition to histamine and heparin include: serine proteases, prostaglandin D2, leukotriene C4, and cytokines. Mast cells and their circulating counterpart, the basophil, can activate T and B lymphocytes and dendritic cells. Mast cells have been postulated to act directly within renal allografts and/or to induce indirect effects via inflammatory mediator release into the circulation (such as from regional lymph nodes) (29).

3.1.4. Monocytes/Macrophages/Histiocytes

Circulating monocytes are attracted to an area of injury via chemotaxis. They settle into tissues as macrophages/histiocytes, can survive for months, and show phagocytotic activity. Tissue macrophages (CD 68+) also serve as professional antigen presenting cells for cell mediated immunity by stimulating T helper and B cells through foreign antigen presentation on MHC class II complexes (30). .Activated histiocytes secrete a wide variety of monokines, complement factors, interleukins, growth factors, and even early matrix proteins such as fibronectin. The plasticity and diversity of (circulating) monocytoid cell elements appears to be much broader than traditionally believed. Histiocytes are major components of rejection (31-36).

3.2. Non-classic inflammatory cells: dendritic cells, endothelial cells, tubular cells, platelets and myofibroblasts

Non-inflammatory cells can contribute to and (co)stimulate inflammatory reactions including rejection by various mechanisms. One very important “non-classic” inflammatory cell element is the dendritic cell. Dendritic cells are professional antigen presenting cells of the monocyte lineage (36) that are active in innate immunity and can express CD68 (37). They are found in tissues in contact with the environment, such as the skin, lungs, mucosal surfaces as well as the renal parenchyma (37, 38). Once activated, dendritic cells upregulate the chemokine receptor 7 (CCR7), migrate out of tissues and into the circulation. They travel to lymphatic organs, present foreign antigens to T helper cells, cytotoxic T cells, and B cells, and provide important co-stimulatory activation signals. Dendritic cells can produce IL-12 to activate naïve CD4 T cells (39, 40). Co-stimulatory lymphocyte activation signals can also be provided by other cell types, such as tubular epithelial cells, by upregulation of MHC-class II, CD80, and CD86 (41-46)..

Another non-classical inflammatory cell of great importance is the myofibroblast. Myofibroblasts are of undetermined origin. They play a pivotal, currently largely underestimated role in the development of fibrosis during active inflammation and rejection (31, 47).

3.3. Others

The effects of extra-renal donor and/or recipient inflammatory cells or circulating progenitor cells on allograft rejection, remodelling, and graft acceptance are profound; a detailed description is far beyond the scope of this short review (29, 36, 47-50).

4. INFLAMMATION IN THE KIDNEY (TRANSPLANT)

The renal parenchyma can be subdivided into different anatomic compartments: tubulo-interstitial, glomerular, and arterial/arteriolar. Veins and lymphatics that are only fully formed at the cortico-medullary junction play important functional roles for inflammatory cell trafficking, but they are of only limited significance for classifying inflammation. Inflammatory changes can affect the different anatomic compartments alone or in combination. Depending on the time of diagnosis, the natural disease course, and the response to therapy, inflammation can be “acute” and active or protracted and smoldering, also known as chronic active inflammation. Inflammation, in particular in the acute phase, can heal with complete functional and morphologic restitution (i.e. restitutio ad integrum) or lead to varying degrees of fibrosis and tubular atrophy with “inactive scar” formation. The development of fibrosis and tissue remodelling are of particular importance in smoldering, chronic active inflammation that shows not only “classical” inflammatory cell elements but also myofibroblasts which synthesize matrix proteins including collagens types I and III (Figure 1). Smoldering inflammation in glomeruli and peritubular capillaries can result in the activation of other “non-classical inflammatory cells”, i.e. endothelial cells, with synthesis of basement membrane components including laminins and collagen type IV. If the right window of opportunity is provided, inflammation can reoccur and flare at any time involving normal parenchyma or areas of fibrosis. In renal allografts,

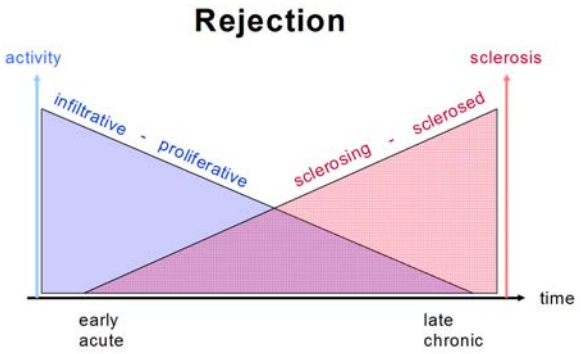

Figure 1. Acute/active and chronic/sclerosing rejection phenomena represent a continuum rather than two sharply separated disease entities. Myofibroblasts are crucial cell elements that are recruited during acute/active rejection. They promote sclerosis by synthesizing collagens.

reoccurring "active" rejection episodes are of particular clinical significance.

Besides cellular and antibody mediated rejection, the most significant types of allograft inflammation, practically all other inflammatory diseases found in native kidneys can also affect transplants. These include de-novo or recurrent glomerulonephritides, infections, ANCA (anti-neutrophilic cytoplasmic antibody) associated vasculitides, or allergic interstitial nephritides. In order to render a specific diagnosis, an (invasive) renal biopsy is often crucial for proper therapeutic intervention and patient management. It is considered to be the diagnostic "gold standard". The microscopic examination of a biopsy, typically amended by immunohistochemical, immunofluorescent and electron microscopic analyses, provides powerful information on disease activity, chronicity, and reversibility of primary and potential secondary disease processes. During the histologic decision making process renal transplant pathologists are guided by several important aspects: the inflammatory cell composition and compartmental tissue involvement, immunohistochemical/electron microscopic findings, the time of disease occurrence post grafting, and functional/clinical data including information on the urine sediment. Listed below are some examples of inflammatory "look-alikes" that require careful analysis during the diagnostic work-up.

Look-alikes in the tubulo-interstitial compartment. Tubules including collecting ducts can be the primary sites of severe, active and destructive inflammatory conditions caused by ascending E. coli infections, i.e. pyelonephritides. The inflammation is rich in polymorphonuclear leukocytes with characteristic, dense intra tubular inflammatory cell casts and focal "melt-down" of nephrons. The interstitium typically shows edema and polymorphonuclear cell infiltrates; other tissue compartments such as arteries or glomeruli are, however, usually spared. Patients generally require intensive therapy with antibiotics commonly resulting in full morphologic recovery.

If polymorphonuclear leukocytes predominate in the interstitium, mainly in peri-tubular capillaries, they can indicate an antibody mediated rejection episode that is typically associated with pronounced complement factor C4d deposits along peri-tubular capillaries (noted by immunohistochemistry or immunofluorescence microscopy; see below). Antibody mediated rejection requires intensive and specific immunosuppressive therapy.

Early after transplantation tubulo-interstitial inflammation including scattered intra tubular polymorphonuclear leukocytes can be seen in self-limiting ischemia-reperfusion injury that is caused by ischemic tubular and endothelial cell injury induced during the transplantation process. Ischemia –reperfusion injury is typically found in organs of cadaveric origin and is mainly promoted by cold ischemia time encountered during organ storage and transport.

Look-alikes in the glomerular compartment. Significant inflammation in glomeruli is typically either caused by rejection or recurrent/de-novo glomerulonephritides. Both diseases show similar intra glomerular inflammatory cell compositions, i.e. varying numbers of lymphocytes, mono-/histiocytes, polymorphonuclear leukocytes, and activated endothelial cells. A definitive diagnosis is generally rendered based on additional findings such as rejection induced changes in other tissue compartments (favoring rejection) or the detection of intra glomerular immune complex deposits and an abnormal urine sediment (favoring a glomerulonephritis). Scattered, rare inflammatory cells, mainly lymphocytes and polymorphonuclear leukocytes can also be seen in glomerular capillaries as non diagnostic changes; they often represent circulating cells en route to the inflamed interstitium.

Look-alikes in the arterial compartment. Inflammation rich in lymphocytes and histiocytes is generally caused by rejection. Similar inflammatory conditions, often transmural and associated with vascular wall necroses, can be seen in vasculitides, such as ANCA disease.

5. THE MANY MORPHOLOGIC FACES OF REJECTION

Rejection – cellular and/or antibody mediated – represents a specific type of inflammation targeting donor specific antigens. Thus, perfectly MHC matched organs, best exemplified by transplantation between identical twins, lack significant rejection episodes and can survive for decades with little or no immunosuppressive therapy. All other transplants, however, despite maintenance immunosuppressive treatment, are at risk for rejection that might occur at any time post grafting. Rejection associated inflammation follows the same basic principles outlined above, i.e. different cell compositions, compartmental tissue involvement, degrees of activity and chronicity. Rejection episodes can be purely cellular, mixed cellular and antibody, or purely antibody

Table 1. The "Banff '97-update 2005" classification scheme of renal allograft rejection. The system is based on morphologic changes in different anatomic compartments with due regard to clinical parameters and outcome (51)

1. Normal
2. Antibody-mediated rejection
Due to documented anti-donor antibody ('suspicious for" if antibody not demonstrated); (may coincide with categories 3-6)
Acute antibody-mediated rejection
Type (grade)
I. ATN-like—C4d+, minimal inflammation
II. Capillary-margination and/or thromboses, C4d+
III. Arterial—v3, C4d+
Chronis active antibody-mediated rejection
Glomerular double contours and/or peritubular basement membrane multilayer and/or interstitial fibrosis/tubular atrophy and/or fibrous intimal thickening in arteries, C4d+
3. Borderline changes: 'suspicious' for acute T-cell-mediated rejection This category is used when no intimal arteritis is present, but there are foci of tubulitis (t1, t2, or t3 with i0 or i1) although the i2 t2 threshold for rejection is not met (may coincide with categories 2, 5 and 6)
4. T-cell-mediated rejection may coincide with categories 2, 5 and 6
Acute T-cell-mediated rejection
Type (grade)
IA. Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of moderate tubulitis (t2)
IB. Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of severe tubulitis (t3)
IIA. Cases with mild to moderate intimal arteritis (v1)
IIB. Cases with severe intimal arteritis comprising >25% of the luminal area (v2)
III. Cases with 'transmural' arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation (v3)
Chronic active T-cell-mediated rejection
'Chronic allograft arteriopathy' (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima)
5. Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology
Grade
I. Mild interstitial fibrosis and tubular atrophy (<25% of cortical areas)
II. Moderate interstitial fibrosis and tubular atrophy (26-50% of cortical area)
III. Severe interstitial fibrosis and tubular atrophy/loss (>50% of cortical area)
(may include non-specific vascular and glomerular sclerosis, but severity graded by tubulointerstitial features)
6. Other: Changes not considered to be due to rejection-acute and/or chronic; may coincide with categories 2-5

mediated with varying degrees of fibrosis and remodeling (Figures 1 and 2) (4, 31, 51-53). The non-specific and confusing term "chronic allograft nephropathy" has been abandoned (51).

Table 1 illustrates the "Banff" classification scheme of renal allograft rejection (51). "Banff" represents the most widely accepted "histology based" attempt to classify rejection related inflammatory and fibrosing lesions.. "Banff" is continuously updated and recently amended by the incorporation of immunohistochemical markers, i.e. C4d staining results. Likely, in the future, "molecular data", "transcriptomics", or "chemokine-profiling" will help to fine tune the "Banff" system (54, 55) - a seemingly easy but surprisingly challenging task. For example, in the past, "gene chip" based studies suggested an inferior graft survival of CD20 positive, B-cell rich, active rejection episodes (56). This observation could not be confirmed in subsequent reports (23, 57, 58). More recently animal studies demonstrated that T regulatory lymphocytes, i.e. Foxp3 expressing cells, were associated with graft "accommodation" and "functional tolerance" (59, 60). Histologic examination of human renal biopsy material could, however, so far not support this hypothesis (15). Although complement factor C4d deposits were shown to be associated with inferior graft survival and elevated antibody titers in the 1990's (61, 62), it took many years to incorporate C4d staining results into the Banff classification system (63). Thus, much detailed work and patience are required before "molecular markers" will amend "histologic signs" of rejection in the future. At present, the careful morphologic analysis and classification of rejection remains the diagnostic gold standard against which all other tests and findings have to be measured. Described below are key morphologic changes found in the major types of kidney transplant rejection.

5.1. Tubulo-interstitial rejection

Tubulo-interstitial or acute cellular rejection (Banff category 4, type 1; Table 1) is characterized by mononuclear cell infiltrates in the interstitial compartment of the cortex. Edema and tubulitis are typically seen. The mononuclear cells are predominately composed of activated and mitotically active lymphocytes (CD3, CD4, CD8 positive) and histiocytes / monocytes (CD68 positive). Often, varying numbers of eosinophils, CD20 positive B-cells and/or CD 138 positive plasma cells are found. Polymorphonuclear leukocytes are characteristically scant and, if present, normally detected adjacent to severely injured tubules (differential diagnosis: antibody mediated rejection, pyelonephritis). In some tubulo-interstitial rejection episodes CD20 positive lymphocytes and plasma cells are abundant. Mononuclear cells, i.e. lymphocytes and histiocytes, but not plasma cells and eosinophilic leukocytes, pass through the tubular basement membranes and infiltrate under and in between tubular epithelial cells, i.e. tubulitis. Injured tubules with tubulitis occasionally contain scattered intra tubular polymorphonuclear leukocytes. Tubulitis is only considered to be "diagnostic" of rejection if non-atrophic tubules are involved. There is, however, some evidence that also inflammation and tubulitis in atrophic tubules located in fibrosed regions may contribute to graft demise and constitute "cellular rejection" (64, 65). Tubulitis is a focal phenomenon, which predominately affects distal tubules. It is commonly not associated with severe necrosis or tubular destruction. Tubulo-interstitial rejection only involves the renal medulla in severe cases.

Tubulo-interstitial cellular rejection can be "pure", i.e. limited to the tubulo-interstitial compartment, or it can additionally show glomerular involvement, i.e. transplant glomerulitis, and/or arterial rejection, i.e. transplant endarteritis (in approximately 30% to 40% of cases).

In typical cases of tubulo-interstitial cellular rejection immunohistochemical incubations to search for immunoglobulins or complement factors are unrevealing. The immunohistochemical detection of HLA-DR and ICAM-1 in tubular epithelial cells as signs of "activation" may be diagnostically helpful to confirm the diagnosis of rejection.

Approximately 20%-30% of cases demonstrate the accumulation of the complement degradation product C4d along peritubular capillaries; these cases represent combined cellular and antibody mediated rejection episodes (combined Banff category 4, type 1 and category 2 rejection).

Pure tubulo-interstitial cellular rejection episodes usually respond well to bolus steroid treatment, and renal function typically returns to baseline levels (66). The extent of tubulo-interstitial inflammation, defined by the degree of parenchymal inflammation and the number of tubules with tubulitis does not correlate with one year graft function/failure. This observation is of particular importance, since it challenges the common clinical practice of simply grading the severity of rejection episodes based on the extent of cortical inflammation (66). Tubulo-interstitial cellular rejection rich in plasma cells seems to fare less favorably (20).

5.2. Arterial rejection

Rejection episodes in arteries can be divided into four subtypes: thrombosing hyperacute rejection, necrotizing transplant arteriopathy, transplant endarteritis, and sclerosing/sclerosed transplant arteriopathy. They can be seen as isolated events (less than 10% of all "arterial" rejection episodes) or more frequently in combination with tubulo-interstitial and/or glomerular rejection.

a) In hyperactue rejection or transplant arteriopathy with massive intravascular coagulation/thrombosis and vascular immunoglobulin deposition (Banff category 2 rejection, not further specified; Table 1) endothelial cell damage and occlusive thrombi in large and small arteries and capillaries are found. This phenomenon can be grossly observed following the restoration of blood flow during surgery. The kidney grafts develop a reddish mottled aspect, increase in size and rapidly turn reddish-blue. Nephrectomy specimens are usually markedly swollen, hemorrhagic and infarcted. Impaired blood flow causes acute ischemic type injury of tubular epithelial cells (acute tubular injury, ATI) and in severe cases parenchymal infarction with hemorrhage. Significant lympho-histiocytic infiltrates, i.e. signs of cellular rejection, are generally lacking.

During the early phases of hyperacute rejection linear IgG, IgM, and complement factor deposits are generally present along the vascular endothelium of arteries, glomeruli, and peritubular capillaries (by immunofluorescence microscopy). In nephrectomy specimens with marked ischemic injury and necrosis, however, such deposits may be scant or even lacking. Transplant arteriopathies with massive intravascular coagulation are generally C4d positive.

Hyperacute rejection is caused by circulating, preformed alloantibodies that bind to the endothelium of arteries and lead to rapid thrombotic occlusion of all caliber vessels. It is the "prototype" for an antibody mediated rejection phenomenon. Since the introduction of crossmatch testing prior to transplantation, rejection episodes with widespread thrombus formation have become exceptionally rare events (less than 0.1. % of all rejection episodes).

b) Necrotizing transplant arteriopathy (Banff category 4, type 3 or Banff category 2, type 3 acute rejection; Table 1) demonstrates vascular necrosis and varying degrees of inflammation in all caliber arteries. The kidneys are grossly enlarged and show hemorrhagic and anemic infarcts ("mottled appearance"). Typically only short arterial segments are necrotic with either transmural (from the intimal to the adventitial layer) or intramural (inner medial smooth muscle layer) vascular wall destruction. Surprisingly, endothelial cells are often viable and occlusive thrombus formation is uncommon. In many cases, fibrinoid arterial necrosis is associated with intimal and transmural inflammation (i.e. lymphocytes and histiocytes). During the healing phase necrotic arteries undergo scarring with occasional aneurysma formation.

The tubulo-interstitial compartment often shows signs of "cellular" rejection (see above) and in most severe cases hemorrhage and infarction. Different changes are found in glomeruli: i) non-specific ischemic glomerular collapse, ii) massive glomerular capillary dilatation with blood stasis, iii) fibrin thrombi, or iv) transplant glomerulitis (see below).

By immunohistochemistry or immunofluorescence microscopy typically fibrin can be found in necrotic vessel walls and in the interstitium. However, IgG or IgM deposits along the endothelium of arteries or capillaries are generally lacking. Approximately 50% of cases with necrotizing transplant arteriopathy show C4d deposits along peritubular capillaries.

The specific etiology of fibrinoid arterial wall necrosis is undetermined. Antibodies (de-novo ?) likely cause injury in C4d positive cases. Arteries may also show signs of cell mediated rejection with intramural inflammation and endothelialitis. Necrotizing transplant arteriopathy is typically seen within the first weeks after grafting, and it is associated with poor transplant survival. It is an uncommon form of rejection (seen in less than 5% of early rejection episodes) (66). According to the updated Banff '97 classification scheme (see Table 1), C4d positive cases are classified as "category 2 (type 3)" and C4d negative cases as category 4 (type 3) acute rejection. Of note: necrotizing transplant arteriopathy can also be seen many years after transplantation in non-functioning grafts/ patients off immunosuppression; it is then typically accompanied by transplant endarteritis and sclerosing transplant vasculopathy (see below).

c) Transplant endarteritis (Banff category 4, type 2 acute rejection; Table 1) typically affects "large caliber" vessels, i.e. peripelvic, interlobar, arcuate type arteries, and large interlobular branches; arterioles are less frequently involved. Per definition, one or more mononuclear inflammatory cells are always present in

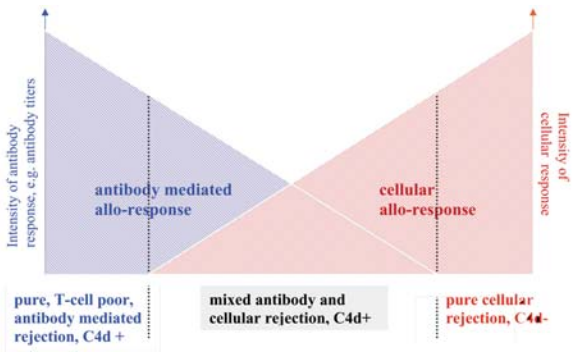

Figure 2. Active rejection episodes can either be 'pure' antibody or 'pure' cellular mediated events – or – represent mixed rejection with varying degrees of humoral and cellular components (4).

the intimal layer under activated and enlarged endothelial cells. Endothelial cell necrosis is generally inconspicuous, although it can occasionally be noted in association with small intimal fibrin deposits. Occlusive thrombi, however, are not a feature of transplant endarteritis. The inflammatory cells in the intima are mainly CD68 positive monocytes / histiocytes and CD8/CD4 positive T lymphocytes. During the course of transplant endarteritis, i.e. in days or few weeks, the histiocytes in the intima can transform into foam cells. In addition varying numbers of alpha-smooth muscle actin positive myofibroblasts are found that become progressively abundant during persistent intimal inflammation (31). Proliferation markers, such as KI-67, reveal a high proliferative activity. While the number of myofibroblasts increases over time, usually the number of lymphocytes and histiocytes decreases; foam cells persist. The accumulation of myofibroblasts is associated with the deposition of "early" extracellular matrix proteins, in particular fibronectins and collagen type IV. Of note: "scar" collagens I and III are absent (their deposition marks the transformation of transplant endarteritis into "chronic" sclerosing transplant vasculopathy, see below; Figure 1) (31). Transplant endarteritis hardly ever shows eosinophilic leukocytes or CD-20 positive B cells; CD138 positive plasma cells are absent. Since inflammation is limited to the intima, both the lamina elastica interna and the media remain unchanged.

Transplant endarteritis may be accompanied by transplant glomerulitis and/or by transplant glomerulopathy (see below). Tubulo-interstitial cellular rejection with tubulitis is common (approximately 95% of rejection episodes with transplant endarteritis also show tubulo-interstitial cellular rejection; 5% are pure events limited to the arterial tree). Transplant endarteritis is a cell mediated type of injury, mainly driven by T-cells and macrophages. C4d is found in approximately 40%-50% of cases indicating a concurrent antibody mediated rejection component (mixed Banff category 4, type 2 and category 2 rejection; Figure 2).

Transplant endarteritis is a common phenomenon, seen in approximately 30% of rejection episodes during the first year. We have diagnosed it as early as 6 days and as late as 14 years post transplantation. The diagnosis of transplant endarteritis carries great prognostic and therapeutic significance (66). Patients usually do not respond to conventional bolus steroid therapy, but rather require potent treatment with anti-lymphocyte preparations. Treatment is most efficient during early phases of transplant endarteritis when myofibroblasts are scant. Myofibroblasts are of utmost importance since they do not respond to conventional anti-rejection therapy and serve as machineries for intimal scar formation and the development of chronic, sclerosing transplant arteriopathy (see below).

d) Sclerosing/sclerosed transplant arteriopathy, or chronic vascular rejection, (Banff category 4 "chronic rejection", Table 1) is rejection induced arterial intimal thickening due to de-novo deposition of collagens types I and III.. It imperceptively evolves from transplant endarteritis and can show varying degrees of activity, i.e. mononuclear inflammatory cell infiltrates, ranging from marked to absent in the final burnt-out and sclerosed stage (Figure 1).

Nephrectomy specimens are generally fibrosed and shrunken; occasionally hemorrhagic infarcts are seen indicating an "active" rejection component. Arteries are stenosed by concentric intimal fibrosis, which displays distinct features: 1) Elastic lamellae are lacking, i.e. an absence of marked intimal elastosis. 2) The fibrotic intima contains scattered, irregularly arranged myofibroblasts with enlarged "activated" nuclei. 3) The fibrotic intima may contain mononuclear inflammatory cell elements, i.e. lymphocytes, histiocytes, foam cells. 4) During intimal remodeling, myofibroblasts may occasionally form a new rudimentary neo-media. 5) Endothelial cells are activated and enlarged.

Sclerosing transplant arteriopathy is often associated with transplant glomerulitis and glomerulopathy, i.e. signs of glomerular rejection (in our experience in approximately 40% of cases). In cases with significant ongoing activity, i.e. intimal inflammation, tubulo-interstitial cellular rejection is common. Arterial stenosis results in parenchymal ischemia, tubular atrophy and interstitial fibrosis.

Sclerosing transplant arteriopathy, i.e. chronic vascular rejection, is the fibrosing stage of rejection episodes involving the arterial tree. It is most frequently found in association with or subsequent to transplant endarteritis and can develop within few weeks. Intimal inflammation including the influx of macrophages promotes the proliferation of myofibroblasts that synthesize extracellular matrix proteins, in particular scar collagens I and III. Matrix synthesis is promoted by various cytokines and growth factors including platelet derived growth factor (PDGF), transforming growth factor beta (TGF-beta), basic fibroblast growth factor (bFGF) and others. Thus, sclerosing transplant vasculopathy is an immune mediated type of injury. Likely, also circulating donor specific antibodies play a currently undefined role in the

pathogenesis, as suggested by the detection of C4d along capillaries in some cases (in our experience approximately 30%).

Sclerosing and especially sclerosed/scarred transplant arteriopathies do not respond well to anti rejection treatment. Overall prognosis is poor with graft loss frequently occurring within months.

5.3. Glomerular rejection

Rejection phenomena in glomeruli can be divided into two overlapping subtypes: transplant glomerulitis and transplant glomerulopathy. They nearly always concur with arterial and/or tubulo-interstitial rejection.

a) Transplant glomerulitis or acute transplant glomerulopathy, shows endocapillary hypercellularity with mononuclear, polymorphonuclear, and activated endothelial cells occluding dilated capillary loops. Occasionally, minute fibrin thrombi are found, and in severe cases, segmental mesangiolysis.

The most common antigens detected in the glomeruli are C4d, IgM and complement factor C3 followed by fibrin and other immunoglobulins. Additionally, approximately 60% of cases show C4d deposits along peritubular capillaries. These latter cases are characterized by abundant. intraglomerular monocyte and/or polymorphonuclear leukocyte accumulations (67, 68).

Transplant glomerulitis is an infrequent finding, encountered in only approximately 5% to 10% of biopsies with acute/active rejection. Glomerulitis is closely associated with transplant endarteritis or fibrinoid vascular necrosis (in 50%-60%). This close association seems logical since the primary target of mononuclear cells in both types of rejection is the endothelial cell layer.

b) Transplant glomerulopathy is characterized by thickening and duplication of peripheral glomerular capillary walls lacking significant “mesangial” cell interpositions. The glomerular remodeling resembles changes seen in “thrombotic microangiopathies/hemolytic uremic syndromes”. Mesangial matrix expansion is inconspicuous; it only becomes apparent in advanced disease stages. Frequently, glomeruli show features of glomerulopathy and concurrent activity, i.e. transplant glomerulitis. In about half the cases, glomerulopathy is associated with vascular rejection, most often sclerosing transplant arteriopathy. Tubulo-interstitial rejection is mainly limited to those biopsies also displaying transplant glomerulitis.

The most common antigens found in the glomeruli are C4d, IgM and complement factor C3, followed by scanty deposits of IgG and fibrin. C4d can be found in a linear distribution pattern along the GBM (an observation of undertermined significance). Approximately 50% of biopsies with transplant glomerulopathy (in particular those with concurrent transplant glomerulitis) reveal C4d deposits along peritubular capillaries.

Transplant glomerulopathy is a distinct, rejection induced lesion that is frequently preceded or accompanied by (active) transplant glomerulitis. The remodeling phenomena are not limited to the glomerular capillaries but can also be noted along peritubular capillaries (i.e. multilayering of basement membranes) and arteries (i.e. sclerosing transplant arteriopathy). Approximately 50% of cases are antibody mediated with C4d deposits along peritubular capillaries; the remaining C4d negative cases are presumably cell mediated rejection episodes. In order to diagnose both transplant glomerulitis and glomerulopathy other glomerular diseases, such as glomerulonephritides or thrombotic microangiopathies, have to be excluded.

5.4. Capillary rejection and complement factor C4d deposits

Capillary rejection with C4d deposits represents antibody mediated allograft injury (Figure 2) (4, 5), either alone, i.e. pure antibody mediated rejection, or in combination with a cellular response.

C4d is the degradation product of the activated complement factor C4, a component of the classical complement cascade which is typically initiated by binding of antibodies to specific target molecules. Following activation and the degradation of the C4 molecule, thio-esther groups are exposed which allow for transient, covalent binding of the split product C4d to endothelial surfaces and adjacent matrix components of vascular basement membranes. The immunohistochemical detection of C4d along peritubular capillaries in renal transplant biopsies is generally regarded as an indirect marker, a “footprint”, of an antibody mediated alloresponse that remains often unapparent based on standard light microscopic analysis. During the post transplantation period, C4d is detected in approximately 30% of all diagnostic graft biopsies. Since C4d generally indicates an independent, de-novo antibody response, it is not surprising to detect it in association with various histologic changes, most often with signs of cellular and glomerular rejection (Figure 2). Based on the detection of C4d, “acute cellular” and “antibody” mediated rejection can concur in approximately 20%-30% of tubulo-interstitial cellular rejection episodes (Banff category 4, type 1), approximately 40%-50% of cases with transplant endarteritis (Banff category 4, type 2), and approximately 50% of biopsies with fibrinoid arterial necrosis (Banff category 2 or 4, types 3) (53, 69). The tightest associations are found between C4d deposits and injury to glomerular and peritubular capillaries.

Dominant capillary rejection with C4d deposits typically lacks a conspicuous cell mediated component and is frequently mediated by anti class I or class II antibodies (Figure 2). It is characterized by diffuse capillary injury with microthrombi, intra-capillary polymorphonuclear leukocytes and mononuclear cell elements.. Concurrent transplant glomerulitis is common. Capillary occlusion, ischemic injury, and vascular leakage result in hemorrhage, interstitial edema, and ischemic injury. The edematous interstitium can contain scattered inflammatory cells, i.e.,

lymphocytes, histiocytes and polymorphonuclear leukocytes, although they are never abundant and tubulitis remains inconspicuous (differential diagnosis: mixed cellular and concurrent antibody mediated rejection; see above; Figure 2).

C4d positive dominant capillary rejection (i.e. pure antibody mediated rejection) is a newly recognized rejection type, ,and much remains to be learned. It is typically diagnosed within the first weeks post transplantation and seems to be mediated by antibodies with various specificities. In contrast to transplant vasculopathy with massive intravascular thrombosis and vascular immunoglobulin deposition, the donor specific antibodies are often formed "de novo" post transplantation.

Therapeutic attempts are made with high dose IVIG treatment and/or plasmapheresis or immunoabsorption. Graft survival varies. It is poor in cases with marked capillary thrombosis and parenchymal necrosis. Dominant capillary rejection episodes are more common in kidney transplants originating from ABO incompatible donors.

6. CONCLUSION

Inflammatory changes in renal allografts are complex events. Neither morphology nor molecular biology alone can fully explain all pathobiologic events in a satisfying manner. Rather, it will take a multidisciplinary approach to unravel the entire mystery of inflammatory events, to optimally guide patient management, and to design new and effective treatment strategies. For more than 100 years pathology and the morphologic characterization of tissue remodeling have proven to provide detailed and significant insights into the pathogenesis of many diseases. Consequently, future research efforts should focus on the design of a gene and chemokine expression map explaining and reflecting morphologic changes in specific anatomic compartments of the kidney. It is time to do it.

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Abbreviations: Banff = the name of a small Canadian resort town, commonly used to refer to a classification scheme of renal allograft rejection, C4d = complement degradation product C4d, a split component of the activated complement factor C4 molecule, IVIG treatment = intravenous immunoglobulin (treatment)

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Send correspondence to: Volker Nickeleit, The University of North Carolina Medical School, Department of Pathology and Laboratory Medicine, Nephropathology Unit, CB#7525, Chapel Hill, NC 27599-7525, USA, Tel: 919-966-2421, Fax: 919-966-4542, E-mail: volker_nickeleit@med.unc.edu