

# Symposium 11

## Relevant topics in pathology of transplantation

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### The Banff 97 classification of renal allograft pathology

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#### Introduction

The original Banff classification was developed at a meeting held in Banff, Canada in 1991, organized principally by Professor Kim Solez. Its stated aim was to facilitate international standardization of nomenclature and evaluation of renal transplant biopsies "to guide therapy in transplant patients and to help establish an objective rejection endpoint in clinical trials" (1). This schema has become widely accepted. It has been used in numerous clinical trials and has become incorporated into routine clinical practice in many transplant centers. However, it was always viewed as a "working classification", with the intention that further changes and improvements would be incorporated as time went on. Inevitably, evidence of problems emerged and suggestions for improvements were put forward. This process was facilitated by the subsequent series of meetings at Banff (2) and by the ground-breaking use of the Internet as a discussion tool in the development of histopathological consensus. Some relatively major changes were agreed upon at the fourth Banff conference in 1997. At the time of writing these have not yet become available in conventional published form (3) but in the interim a "pre-print" is available on the Internet (4). The purpose of this paper is to present the main features of the new classification and to discuss some of the problems which have arisen.

#### Changes in the classification

The most important changes are in the classification of acute rejection changes. The original classification (Banff 93) fused "cellular" and "vascular" rejection, and Grade 2 rejection included cases with severe tubulitis (2a) and mild intimal arteritis (2b). The meeting accepted evidence from several large studies that this fusion was probably a mistake, as vascular changes indicate a poorer prognosis and response to therapy (5, 6). Tubulitis in atrophic tubules is now specifically excluded as a criterion for rejection. Other minor changes were incorporated, but arguments for inclusion of new features, such as infiltration by eosinophils, neutrophils or plasma cells were not accepted; such features may be recorded, and noted with an asterisk on the "I" score, but do not form part of the classification.

The scoring of lesions most relevant to acute rejection is now as follows:

Tubulitis ("t") score (applied only to tubules which are no more than mildly atrophic)

- t0 No mononuclear cells in tubules;
- ti Foci with 1 to 4 mononuclear cells per tubular cross section or 10 tubular epithelial cells;
- t2 Foci with 5 to 10 mononuclear cells per tubular cross section or 10 tubular epithelial cells;
- t3 Foci with 1 to 4 mononuclear cells per tubular cross section or 10 tubular epithelial cells "disappearing tubules" in at least 2 places and t2 elsewhere

Intimal arteritis ("v" score)

- v0 No arteritis;
- vi Mild to moderate intimal arteritis in at least one cross section. (Lymphocytes must be beneath endothelium in arteries. Venulitis is not included, nor is mononuclear adherence to endothelium);
- v2 Severe intimal arteritis with at least 25% luminal area lost in at least one cross section;
- v3 Arteritis with fibrinoid change and/or transmural arteritis with smooth muscle necrosis;

These changes make it necessary to be clear whether one is using Banff 93 or Banff 97. Partly to emphasize this, and partly to emphasize the recognition that arterial changes are qualitatively different, "borderline" has been replaced with "suspicious for acute rejection" and "grades" of acute rejection have been replaced with "types".

#### Severity of acute rejection

No acute rejection.

Suspicious for acute rejection: ti present, v0.

Acute/active rejection:

- Type IA Mild tubulitis with interstitial infiltration (t2, at least i1, v0)
- Type 1B Severe tubulitis (t3, at least i1, v0)
- Type 2A Mild to moderate intimal arteritis (vi)
- Type 2B Severe intimal arteritis (v2)
- Type 3 Arterial fibrinoid/smooth muscle necrosis (v3)

Other changes in definitions of specimen adequacy, chronic allograft nephropathy grading etc. are documented in detail elsewhere (3, 4, 7).

These changes have been viewed by some as an inconvenience, but they should be seen as evidence of strength in a system which is capable of adaptation and development in order to avoid obsolescence. It is recognized that in some circumstances, such as ongoing clinical trials, it may be necessary to continue to use the Banff 93 formulation until the trial is complete.

#### Drawbacks of the classification

The Banff classification is widely perceived as a success in the context of clinical trials, in harmonizing approaches and in facilitating meaningful communication. There is evidence that it has improved reproducibility of assessment, and it is particularly popu-

lar with pathologists who are relatively inexperienced in renal transplant pathology, as it provides a clear framework for evaluation. However, evidence of improvement in the management of individual patients has been harder to find. In the author's personal opinion, this stems largely from an inappropriate rigidity in the application of the schema to individual patients.

Firstly, as a simple example: the scheme provides a clear definition of specimen adequacy. Adequate for what? A definition is clearly necessary in the context of clinical trials, but when dealing with an individual patient, flexibility is needed. If a specimen does not fulfill the criteria for adequacy, this should be clearly stated, but the pathologist should not refuse to make any assessment. Even a sample which contains only medulla may be adequate for patient management if there is clear evidence of acute rejection, though it remains inadequate for grading of the severity of the rejection. Medulla alone obviously cannot be adequate to exclude rejection.

The basis of the decision to treat acute rejection has been the subject of much controversy, largely because of over-rigid implementation. The Banif classification attempts to grade the severity of changes within the needle biopsy; but we know that biopsies sometimes fail to sample evidence of rejection, and conversely histological evidence of "rejection" may be present in protocol biopsies from completely stable grafts (8). Attempts to define a clear point in the schema at which treatment is justified should not be made in the absence of clinical data. The original Banif paper stated: "Clearly individual centers will develop their own clinical strategies for dealing with various biopsy findings" (1), and this needs to be reemphasized. Studies in this area are fraught with hidden dangers from differences in practices and populations in different centers, which may not be immediately apparent. For example, we recently developed a computer-based system which integrated 12 different histological variables to produce a highly reliable system for the diagnosis of very early acute rejection (9). When the pathologist whose observations had been used to "train" the system moved from the UK to Pakistan, he found that it no longer worked; it required retraining with data from his new institution. The inference is that although a system to grade acute rejection is valuable, attempts to impose a single "action point" for treatment are likely to fail unless the characteristics of the local institution are taken into account.

### Recommendations

The appropriate course of action, when reporting renal transplant biopsies, is first to give a description of the histological changes which includes the Banff grading system. This aspect of the report is most important in clinical trials. However, the pathologist is also a physician, so the conclusion should include a recommendation for appropriate management which is based not only on the Banff classification, but also on the clinical features and experience of local conditions and practices. It may also be appropriate to consider "minor" histological features of acute rejection, which although less reliable than tubulitis and intimal arteritis may be of assistance in difficult cases (9).

At the time of writing the 1999 Banff conference has not been held. It is anticipated that a brief report of this meeting, to be held in June 1999, will be incorporated into the presentation in Barcelona in September 1999.

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## A simplified classification system for acute renal allograft rejection (CCTT)

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### Introduction

A simplified, standardized scoring system for acute renal allograft rejection was sought which would avoid some of the perceived limitations of the Banff system. This description is excerpted and updated from that report (1) and a recently published review (2).

A panel of renal pathologists participating in the National Institutes of Health supported Cooperative Clinical Trials in Transplantation program (CCTT) defined and tested the clinical utility of the three following categories of acute rejection (1):

Type I: At least 5% of the cortex must have interstitial mononuclear infiltration with at least two of the three following features present: edema, tubular degeneration/injury or reactive lymphoblasts. Tubulitis must be present, with at least three tubules affected in 10 serial high power fields (40x) from the areas with the most infiltrate.

Type II: Arterial mononuclear endothelial inflammation (endarteritis or endothelialitis) is present (with or without features of type I).

Type III: Arterial fibrinoid necrosis or transmural inflammation is present and may be accompanied by thrombosis, parenchymal necrosis/recent infarction or hemorrhage.

In contrast to Banff (3), CCTT adds criteria that reflect ongoing parenchymal injury (edema, tubular injury) or immunological activity (activated lymphocytes) to help separate active rejection from inactive infiltrates. CCTT excludes the subcapsular area for scoring (which often shows mild inflammation and fibrosis) and does not score tubulitis in areas of tubular atrophy (where it is often seen nonspecifically). CCTT regards the presence or absence of endarteritis as potentially more fundamental and it therefore is the basis of separating Type I from Type II.

Interobserver reproducibility of the CCTT classification showed 91% agreement on the presence or absence of rejection (0.80 kappa score) (1). The agreement was almost as good for the type

of rejection ( $\kappa$  0.72) and the presence or absence of endarteritis ( $\kappa$  0.65). The design of this study did not intrinsically favor agreement, in that the scoring was done over several years by almost a dozen pathologists. Thus, the CCTT classification system appears to be statistically robust. The original Banif system compares unfavorably with CCTT in  $\kappa$  values, it is obviously difficult to compare  $\kappa$  values between these two studies, however, it is notable that the agreement rate for endarteritis was equivalent in the two studies. Since this feature is defined similarly by the two groups, this result suggests that the other marked differences in agreement are intrinsic to the classification schemes rather than the design of the studies or the skills of the pathologists.

The pathologists in this study found that the CCTT classification of the biopsies takes no more time than the usual diagnostic examination of a transplant biopsy. In contrast to Banff, little has to be quantitated: the estimated percentage of cortex involved with the infiltrate has to exceed 5% and the tubulitis occasionally has to be counted to be certain that at least three tubules are affected. Nothing else is graded: there are no "mild", "moderate" or "severe" degrees of any lesions. As a measure of the efficiency, the review panels typically took about 5 min per biopsy.

The CCTT criteria have a sensitivity of 90% for detection of rejection in one core and a calculated sensitivity of 99% for two cores, which is quite satisfactory for clinical management. The specificity of the pathological criteria is difficult, if not impossible, to determine since the biopsy is widely regarded as the "gold standard". When rejection was defined solely by clinical criteria and the biopsy was interpreted without any clinical information, the CCTT classification performed acceptably, with a sensitivity of 86% and a specificity of 72%. When judged by the clinical course, a significant fraction of patients who lack clinical evidence of rejection (28-38%) have a biopsy that meets the pathological criteria for rejection (4). It must be considered possible, if not likely, that these discrepancies are not due to a lack of specificity of the biopsy criteria, but rather that rejection is subclinical. Past published data do not support prognostic significance of the extent of the infiltrate or tubulitis, even if it could be accurately graded (1, 2). When the diagnostic criteria for the number of tubules with tubulitis and the percent infiltrate were varied, the greatest agreement with a clinical course consistent with rejection were using the original criteria, validating the thresholds set. Banif has a higher threshold of infiltrate for Grade I rejection (25% vs. 5%). Our results indicate that the infiltrate involves less than 25% of the cortex in 31% of the cases of Type I rejection. Thus, CCTT would classify many of the Banif borderlines as a Type I rejection. The addition of the requirements for edema, activated lymphocytes or tubular injury made no difference in diagnostic accuracy. However, 90% of these biopsies were for graft dysfunction, and the criteria may be useful for protocol biopsies.

The CCTT types of rejection correlate with clinical severity. Type I rejection is more often completely steroid responsive (5). Type II rejection was six times more likely to be clinically severe than Type I (1), confirming several previous studies noted above that have suggested that endarteritis has adverse prognostic significance. Type III rejection has a well-known adverse prognostic significance in all series. Hemorrhage and glomerulitis also are adverse prognostic features. Thus, the CCTT system has certain objective and major advantages, notably simplicity and reproducibility, while retaining sensitivity, specificity and clinically relevant prognostic implications.

The revised Banff classification recognizes the validity of the CCTT system and uses the same major categories ("borderline"

has become "suspicious"). Major remaining issues for the future are to define more markers for active rejection versus harmless (?beneficial) infiltration in an accepted graft and to incorporate acute humoral rejection and glomerular lesions into the system.

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## Polyomavirus infection of renal allografts

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A morphologically manifest *Polyomavirus* infection of renal allografts with the BK-virus strain is a new and highly unusual complication carrying an unfavorable prognosis. *Polyomavirus*, a subgroup of the papovavirus family, is a double-stranded nonencapsulated DNA virus. After a mostly asymptomatic primary infection early in life, *Polyomavirus* frequently resides in a dormant state in the kidneys and ureters of healthy individuals. In immunocompetent hosts, it does not cause symptomatic disease. On the other hand, immunocompromised patients are at risk of a clinically manifest infection. Human disease can be caused by two *Polyomavirus* strains: JC and BK. JC-virus is the causative agent of progressive multifocal leukoencephalopathy. BK-virus is associated with changes in the kidney and the urothelium, i.e., "viral nephritis" and, proposed by some, hemorrhagic cystitis. However, a clinically symptomatic *Polyomavirus* infection is exceptional, even under immunosuppression. The kidney, a common site of dormant viruses, is hardly ever affected. In Basel we did not encounter a single case of a manifest renal allograft infection with *Polyomavirus* before 1996, whereas nine cases were diagnosed in the following 3 years, which points to new risk factors (1).

## Polyomavirus disease

*Polyomavirus* disease defines a histologically manifest renal allograft infection with viral inclusion bodies, associated with rather varying degrees of interstitial inflammation and deterioration of graft function (i.e., an increase in serum creatinine). The initial diagnosis is made months after transplantation (range: 4-25 months) (2, 3). In man, the disease is caused by the BK-virus strain (2).

The diagnosis of *Polyomavirus* disease is only made histologically in a graft biopsy (2). The morphological hallmark is the detec-

tion of intranuclear viral inclusions, which are exclusively found in epithelial cells (with the exception of podocytes). Intranuclear inclusion bodies can be seen in four different types with similar frequencies along the entire nephron: i) an amorphous ground-glass variant; ii) a central, eosinophilic, granular type surrounded by a (mostly incomplete) halo; iii) a homogenous, finely granular form lacking a halo; and, iv) a vesicular type with enlarged nuclei and clumped, irregular chromatin. The latter (type 4) inclusion body is the least characteristic and often only distinguished after immunohistochemical incubations searching for *Polyomavirus*. Type 4 inclusions are primarily seen in grafts with longstanding disease. Cells with viral changes are often (but not always) enlarged and can have polymorphic nuclei, especially with type 4 inclusions. Frequently, tubular cells are rounded-up, necrotic and extruded from the epithelial cell layer into tubular lumens causing marked denudation of basement membranes. Intratubular "cellular" casts and denuded basement membranes are sometimes the first diagnostic clue on low power microscopic examination drawing the attention to an underlying viral infection. Although cytopathic signs are seen along the entire nephron, they are often abundant in distal tubular segments and collecting ducts. Sporadically, infected cells are noted in the parietal epithelium lining Bowman's capsule, occasionally even forming small crescents. In the renal pelvis and ureters, viral inclusion bodies are mainly seen in superficial transitional cells, rarely in the basal cell layer. Although the morphological changes are typical of an infection with polyomavirus, they are not pathognomonic. Herpes simplex virus, adenovirus and possibly even cytomegalovirus (CMV) have to be considered in the differential diagnosis. The latter viruses can easily be excluded by immunohistochemistry (employing an antibody to detect SV 40 large T antigen, i.e., "pan" *Polyoma virus* antigen) or electronmicroscopy (2). In our cases of *Polyomavirus* disease, we did not encounter a coinfection with CMV, Epstein-Barr virus, herpes simplex virus, varicella or adenovirus (1, 2).

If inclusion bearing cells are sloughed into the urine, they can easily be detected in cytological preparations as "decoy cells". Decoy cells are a characteristic and constant finding in *Polyomavirus* disease. They were excreted in all of our patients during the course of disease and preceded the histological diagnosis by months. However, the detection of decoy cells in a renal allograft recipient does not necessarily indicate *Polyomavirus* disease with renal parenchymal involvement. In general, decoy cells only indicate an asymptomatic activation of *Polyomavirus* which is normally fully reversible. Polymerase chain reaction (PCR) is an inadequate tool for screening urine samples since the technique is by far too sensitive.

In our patients, BK-virus was constantly detectable in the serum during the course of *Polyomavirus* disease by PCR (4). Similar to the excretion of decoy cells, also viremia could precede a histologically manifest disease by months. Viremia was asymptomatic and not associated with hematogenous spread to organs outside the kidney/urothelium.

In *Polyomavirus* disease, graft function is impaired and graft survival decreased (45% graft loss) (1). Two factors contribute to functional impairment: i) tubular necrosis induced by *Polyomavirus* infection, and ii) interstitial and intimal fibrosis. *Polyomavirus* infection causes frank tubular necrosis and, thus, alters renal function significantly. Sloughed necrotic epithelial cells form intraluminal casts leaving behind denuded tubular basement membranes. Denuded areas of basement membranes permit leaking of fluid into the interstitial compartment which is associated with functional impairment. In addition, tubular casts may cause an obstructive coin-

ponent. Such pathways are not unique to *Polyomavirus* disease but are well described in other forms of acute tubular necrosis. Since *Polyomavirus* in our experience never cleared from the kidneys (even months after the initial diagnosis), tubular injury did not heal, and, therefore, functional impairment persisted. In addition, overtime recurrent rejection episodes with transplant endarteritis led to interstitial and intimal fibrosis (i.e., "chronic allograft rejection") (2).

Controversy exists whether the interstitial inflammatory cell infiltrate in *Polyomavirus* disease represents virally induced interstitial nephritis or cellular rejection (1-3). Our data strongly suggest that *Polyomavirus* does not stimulate a marked inflammatory response, since a manifest infection with intranuclear inclusion bodies is often associated with an inconsistent, randomly distributed and occasionally even scant inflammatory reaction which is mostly due to tubular injury/necrosis (1, 2). Tubules with viral inclusions do not upregulate major histocompatibility complex (MHC)-class II, and intercellular adhesion molecule-1 (ICAM-1) is only sporadically and weakly expressed (1). Cellular rejection should be considered if abundant cortical mononuclear cell infiltrates and typical tubulitis are found, randomly affecting tubules with and without cytopathic signs. This interpretation is supported by the observation that tubulitis is seen in areas lacking virally infected cells. If typical signs of interstitial cellular rejection are present, tubules characteristically express MHC-class II and ICAM-1 (1) – phenomena well known to occur during rejection.

### What triggers *Polyomavirus* disease

*Polyomavirus* disease is a new complication in renal allograft recipients. In a previous study (5) we stressed that new immunosuppressive drugs are major risk factors in stimulating disease, in particular protocols containing high dose tacrolimus. In a recent publication from Pittsburgh 20/22 patients (90%) with *Polyoma virus* disease were on tacrolimus (3). All of our patients suffered from biopsy-proven rejection episodes in the months preceding disease (2). All patients had BK viremia and excreted decoy cells in the urine (4, 5). Decoy cells and viremia preceded disease (1, 2, 4). Thus, we propose the following risk profile: i) activation of *Polyomavirus* with significant decoy cell excretion in the urine (asymptomatic, fully reversible); ii) long-lasting tubular injury rendering epithelial cells susceptible to the virus (such as rejection or ischemia); iii) high dose immunosuppression, frequently tacrolimus based; and, iv) heinogenous viral spread. Several risk factors have to concur.

### Conclusion

*Polyomavirus* disease of renal allografts caused by BK virus is a new complication. The diagnosis is only made in a graft biopsy. The detection of decoy cells in the urine and BK viremia are useful adjunct tools to screen high risk patients and to monitor the course of this disease. *Polyomavirus* disease causes impaired graft function, mainly due to protracted severe tubular injury/necrosis. At present, there is no specific antiviral therapy available. The only current therapeutic option is a decrease of maintenance immunosuppression, perhaps cyclosporine based, to promote clearance of the virus (1, 3, 6). This approach is granted, since *Polyomavirus* does not trigger marked inflammation or rejection (1). If rejection coincides with *Polyomavirus* disease, it can be diagnosed in a graft biopsy.

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## Capillary C4d: A tool for the diagnosis of transplants at risk

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The occurrence of delayed graft function (DGF) together with early acute rejection, and the development of chronic rejection account for the majority of *graft* losses in recipients. Clinical and experimental observations suggest that acute transplant rejections, depending on their severity and type, have a strong impact on the long-term survival of renal allografts as they may be related to the development of chronic rejection. It is generally assumed that rejection episodes are dominated by T-cell mediated reactions since infiltrating lymphocytes within the interstitium and in vessel walls are easily detectable upon histopathological examination of graft biopsies. In contrast, the role of humoral immunity in transplantation remains ill-defined because humoral immune reactants are usually not detectable in graft biopsies and the manifestations of humoral antigraft reactions are elusive.

It is puzzling, however, that high levels of preformed alloantibodies before transplantation portend a poor graft outcome in recipients. Likewise, rejections in the presence of circulating anti-donor antibodies carry a worse prognosis than rejections in the absence of such antibodies. It appears that studies of humoral alloreactivity are hampered by the lack of indicating histological markers in biopsy specimens.

We have developed a diagnostic technique that allows the comprehensive assessment of humoral alloreactions in graft biopsies. Our technique takes into account several important aspects as can be seen below.

Endothelial cells within organ grafts form the primary targets for immunological attacks but will remove deposited antibodies and most complement components very rapidly from their surface. Conventional immunohistochemical staining techniques therefore fail to detect transiently bound humoral immune reactants in graft capillaries. Transient deposition of antibodies can be visualized, however, by the assessment of complement fragment C4d, a stable remainder of classical complement activation within capillaries *in vivo*. Assessment of capillary C4d using an indirect immunoperoxidase staining technique can thus reveal otherwise undetectable humoral antigraft reactions in biopsies. Deposition of complement

C4d in interstitial capillaries is a unique finding in renal allografts and is not observed in other immunological renal diseases such as glomerulonephritis or vasculitis.

Using this method, we analyzed biopsies from grafts with delayed function (n=93). Capillary C4d was present in half of the biopsies from transplants with DOF and was encountered predominantly in vascular rejections, but also in the majority of grafts that showed preservation injury or combined pathological findings. Importantly, capillary C4d was associated with subsequent early graft loss (18 vs. 4 losses; p=0.0027).

In a second series, we investigated the capillary deposition of C4d in biopsies derived from 218 cadaveric renal grafts.

Capillary C4d was present in 46% of biopsies from first grafts and 72% of regrafts. Grafts with capillary C4d had a markedly shorter survival than grafts without C4d (50% graft survival: 4 vs. 8 years; p=0.0001). Among several risk factors, capillary C4d is the strongest predictor of subsequent graft loss in a multivariate analysis. Furthermore, humoral alloreactivity that is detectable within 6 months after transplantation has a much stronger impact on graft survival than alloreactivity beyond this period.

Using a sensitive cytofluorometric method, we could further demonstrate that capillary C4d is indeed related to the presence of either preformed, or *de novo* formed circulating alloantibodies in recipients.

In summary, humoral alloreactivity, manifested by the capillary deposition of complement C4d in graft biopsies, exerts a strong impact on graft survival when it operates within 6 months after transplantation.

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## Post-transplant lymphoproliferative disease

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Epstein-Barr virus associated post-transplant lymphoproliferative disease (PTLD) affects approximately 1% of renal transplant recipients, and allograft involvement is reported in 36-100% of cases (1-4). PTLD is the result of excessive immunosuppression leading to uncontrolled proliferation of Epstein-Barr virus (EBV) transformed B-cells. The clinical presentation varies from a mild febrile syn-

drome with pharyngitis and lymphadenopathy to aggressive B-cell lymphoma. There is a need to report these lesions using standardized histopathological criteria, since currently used nomenclature varies somewhat from one medical center to another (4). Some workers classify lesions as i) Epstein-Barr virus positive lymphadenitis resembling infectious mononucleosis, ii) polymorphic PTLD, or ii) monomorphic PTLD (1), while others have suggested a three tier system comprised of i) plasmacytic hyperplasia, ii) polymorphic B-cell hyperplasia/polymorphic B-cell lymphoma, and ii) immunoblastic lymphoma or multiple myeloma (3). More elaborate classification systems have also been formulated (4). Unfortunately, morphological appearances cannot reliably predict clinical prognosis in individual cases, although monomorphic lesions tend to have a less favorable prognosis.

The separation of renal PTLD from severe acute rejection at biopsy is important, since the appropriate treatment is reduction of immunosuppression for PTLD, and aggressive anti-T-cell therapy for severe acute cellular rejection. PTLD may show expansile or nodular mononuclear infiltrates with irregular foci of serpiginous necrosis. These nodular infiltrates should be distinguished from the follicular lymphoid hyperplasia, which occurs in rejection as a result of intense allogeneic stimulation. PTLD lesions can be focal or diffuse, and the latter may result in extensive involvement of the perilymphatic adipose tissue and nerves. The infiltrates in polymorphic PTLD typically show the entire range of lymphocyte differentiation, including immunoblasts, plasma cells, large cleaved/noncleaved cells, and small round lymphocytes. The presence of cells with marked nuclear atypia helps in the differential diagnosis from acute cellular rejection. A predominance of transformed cells characterizes monomorphic PTLD, and these lesions are easier to distinguish from rejection, since they have a monotonous appearance resembling conventional large cell or small cell lymphomas. Although, not as readily demonstrable as in rejection, PTLD lesions may result in tubulitis. Likewise, some small intraparenchymal arteries entrapped within PTLD lesions may show lesions resembling arteritis, which if taken out of context, may be confused with rejection. When allograft nephrectomies are evaluated, infiltration of the hilar soft tissues should not be used to favor a diagnosis of PTLD over acute cellular rejection.

While the quality of the cellular infiltrate, its expansile nature, and the presence of serpiginous necrosis are helpful criteria in the separation of PTLD and acute cellular rejection on routine light microscopy, difficulties can be encountered with limited biopsy material. In the latter circumstance, the final diagnosis must await the results of immunophenotyping, and EBV *in situ* hybridization. Although there are occasional exceptions, PTLD lesions are B-cell preponderant and EBV positive, while rejection is associated with a primarily T-cell infiltrate which is EBV negative. The most sensitive method for detecting EBV in routinely processed tissue is *in situ* hybridization for Epstein-Barr virus encoded small RNA (EBER R A). CD20 (B-cell marker) and CD3 (T-cell marker) immunohistochemistry is a reliable technique for phenotypic analysis of infiltrates in formalin fixed material. In lesions with a substantial component of plasma cells, staining for kappa and lambda light chains offers a convenient way to identify lesions which are clearly clonal. If sufficient fresh tissue is available, immunoglobulin gene rearrangement and oncogene studies should also be performed, since molecular findings have some bearing on the ultimate prognosis (3, 5). A final point to remember is that PTLD and acute cellular rejection are not always mutually exclusive diagnoses. Since PTLD frequent-

ly arises in the setting of acute cellular rejection treated by OKT3, evidence of both processes can be found in some specimens.

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## What is borderline renal allograft rejection?

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We think a transplant recipient either suffers from rejection, and should thus be treated promptly, or, the patient is free of rejection. According to our experience as a major referral center for renal allograft biopsies, the creation of a "borderline" or "suspicious" category is misleading. "Borderline rejection" does not reflect the pathobiological situation. The term is often simply abused to disguise diagnostic uncertainty.

## Background

It is common practice in most transplant centers worldwide to perform "diagnostic" renal allograft biopsies if sudden graft dysfunction occurs, *i.e.*, if the serum creatinine rises by more than 10–20% above baseline levels. This is a "soft" clinical criterion of "rejection". The significance of minimal mononuclear cell infiltrates in the tubulointerstitial compartment in these "diagnostic" graft biopsies is undetermined. In the two major classification schemes of renal allograft rejection [Cooperative Clinical Trials in Transplantation (CCTT) protocol and the Banff classification scheme] (1, 2) "threshold levels" to establish the diagnosis of interstitial cellular rejection (ICR) are arbitrarily set and quite different from each other. Banff tries to incorporate histological changes found in protocol biopsies. This results in high cut-off points for making a definitive diagnosis of ICR and the creation of a soft intermediate "borderline category" (see P.N. Furness, page 334). The CCTT criteria were primarily based on diagnostic graft biopsies, resulting in lower cut-off levels (see RB. Colvin, page 335). The initiators of the CCTT scheme noted that further lowering of "their" thresholds of ICR increased the agreement rates between clinical and pathological definitions of rejection, however, also seemed to decrease the specificity (1). Our interest is focused on the evaluation of diagnostic graft biopsies with minimal tubulointerstitial changes. How can we make more definitive diagnoses in equivocal cases?

## Acute rejection

Renal allograft rejection of abrupt onset (also termed "acute rejection") can be divided into the following three major subgroups according to the affected anatomical structures: vascular rejection (*i.e.*, transplant endarteritis) and/or glomerular rejection (*i.e.*, transplant glomerulitis) and/or interstitial rejection (3). Vascular rejection is found in 34-54% of acute rejection episodes, depending on the time post-transplantation. Vascular rejection has to be further subdivided into necrotizing and infiltrative variants in order to provide crucial therapeutic and prognostic information (4). Rejection involving the glomeruli, *i.e.*, transplant glomerulitis, is infrequently seen (22% in our experience). It is important to diagnose, since transplant glomerulitis is tightly associated with vascular rejection ( $\chi^2$ ,  $p < 0.0001$ ). Histological criteria for making the diagnosis of vascular rejection and transplant glomerulitis are well defined and do not cause diagnostic problems (3, 5).

Most frequently renal pathologists are confronted with ICR, seen in its pure and pathognomonic form, *i.e.*, not associated by vascular rejection or transplant glomerulitis, in approximately 59% of cases (personal observation). Typically, ICR shows a marked mononuclear cell infiltrate in the interstitium and widespread tubulitis. The diagnosis is no challenge. "Pure" ICR should respond well to bolus steroid therapy regardless of the extent of tubulointerstitial involvement (4), provided that vascular rejection or transplant glomerulitis are absent. Thus, a "grading" of the extent of tubulitis and interstitial inflammation into mild, moderate and severe is not useful for clinical purposes (1, 4, 5).

## Borderline tubulointerstitial changes

Although in typical cases ICR is easily diagnosed, great uncertainty exists as to what to do with patchy mononuclear cell infiltrates and scant tubulitis, *i.e.*, "borderline" or "suspicious" changes. Since the extent of tubulointerstitial involvement does not correlate with the clinical severity of rejection, even minor lesions might represent full-blown ICR requiring prompt bolus steroid therapy. Minimal tubulointerstitial changes (according to our definition: mononuclear cells in the interstitium and either no tubulitis, or tubulitis involving less than three tubular cross sections) are frequent findings in renal allograft biopsies. At our center in Basel such minimal lesions are seen in 46% of graft biopsies during the first 6 months and 74% after 6 months. Note that these changes are well below the threshold levels for ICR defined in the Banff and CCTT protocols.

In order to evaluate the biological significance of minimal tubulointerstitial changes, standard light microscopy alone may be insufficient. The addition of interstitial edema, activated lymphocytes or tubular injury to the histological criteria of ICR, thus, signs of ongoing parenchymal injury, did not improve the diagnostic accuracy (see RB. Colvin, page 335). In this diagnostic gray zone, the immunohistochemical detection of up-regulated tubular "activation" markers might be helpful to establish the diagnosis of rejection. We concentrated on the tubular upregulation of major histocompatibility complex (MHC)-class II [human lymphocyte antigens (HLA)-DR] and intercellular adhesion molecule-1 (ICAM-1); both molecules, which are normally not expressed in tubular cells. However, HLA-DR and ICAM-1 can be upregulated by interferon- $\gamma$ , a cytokine released by activated T-cells. We based our investigation on the hypothesis that rejection would be associated with expression of tubular HLA-DR or ICAM-1; accordingly, upregulation should not be found with "irrelevant" tubulointerstitial changes (6, 7).

## Material and methods

A total of 418 renal allograft biopsies (271 patients; 5-7,165 days post-transplantation) were analyzed. All biopsies were performed due to unexplained deterioration of allograft function. Seventeen distinct histological changes (ranging from cyclosporine toxicity, "recurrent" glomerulonephritis to different degrees of interstitial and vascular rejection) were scored. These features were correlated with tubular MHC-class II and ICAM-1 upregulation judged by immunohistochemistry. Special emphasis was placed on the "borderline category" with minimal tubulointerstitial changes (definition see above). In this group of 231 biopsies, MHC-class II and ICAM-1 expression were correlated with response to bolus steroid therapy and serum-creatinine levels.

## Results

From 17 scored histological features, the extent of tubulitis correlated most tightly with tubular ICAM-1 or HLA-DR expression by univariate and multivariate analyses ( $p < 0.0001$ ). Cases with interstitial infiltrates and tubulitis in three tubules ( $n=184$ ) revealed upregulation of HLA-DR in 99% and ICAM in 89%. Biopsies with minimal tubulointerstitial changes showed HLA-DR in 47.6% and ICAM in 48.6%; 58% of ICAM positive cases coexpressed HLA-DR. "Borderline cases" with HLA-DR and/or ICAM up-regulation responded to bolus steroid therapy by a marked decrease in 5-creatinine  $\uparrow$  and 3 months postbiopsy ( $p < 0.05$  for HLA-DR up-regulation).

## Conclusion

Both the Banff '97 and the CCTT classification schemes define minimal threshold levels for diagnosing interstitial cellular rejection solely based on light-microscopical features. These thresholds appear too high if in addition to standard light microscopy tubular activation markers are analyzed, *i.e.*, MHC-class II and ICAM-1. Based on our data, renal allograft biopsies with minimal tubulointerstitial changes and with expression of tubular "activation markers" should be diagnosed and treated as ICR. If tubular activation markers are not expressed, discrete interstitial infiltrates likely do not reflect graft rejection. Thus, at the lower end of the histological spectrum, adjunct immunohistochemical analyses help to make a definitive diagnosis, rendering the term "borderline/suspicious for rejection" obsolete. This also answers the question raised in the title: we do not think borderline renal allograft rejection really exists. Future prospective studies, however, are required to shed more light on renal allograft biopsies with minimal tubulointerstitial changes.

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