Meeting Report

Banff '05 Meeting Report: Differential Diagnosis of Chronic Allograft Injury and Elimination of Chronic Allograft Nephropathy ('CAN')

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The 8th Banff Conference on Allograft Pathology was held in Edmonton, Canada, 15–21 July 2005. Major outcomes included the elimination of the non-specific term 'chronic allograft nephropathy' (CAN) from the Banff classification for kidney allograft pathology, and the recognition of the entity of chronic antibody-mediated rejection. Participation of B cells in allograft rejection and genomics markers of rejection were also major subjects addressed by the conference.

The 8th Banff Conference on Allograft Pathology was held in Edmonton, Canada from 15 to 21 July 2005. A large group of clinicians, pathologists, and researchers met in plenary and specialty sessions and participated in several active consensus discussions. A summary of major topics and results of consensus discussions are provided in this manuscript.

Allograft Fibrosis and Atrophy Revisited

A major topic discussed at the 8th Banff Conference was the elimination of the term 'chronic allograft nephropathy' or CAN from the Banff schema for diagnosis and grading of renal allograft rejection (1,2). Originally coined fifteen years ago in 1991 as a more generic alternative to the then popular and misleading term 'chronic rejection,' acceptance of 'CAN' did succeed in reversing the misconception that all late scarring of the graft was due to alloimmune injury/rejection. However, there are now over 550 PubMed citations using the term, many fostering the misconception that 'CAN' is a specific disease rather than just another term for non-specific parenchymal scarring. In this consensus report are outlined targeted alterations in the Banff schema replacing 'CAN' as a diagnostic term. The rationale for this update of the Banff schema is the misusage of 'CAN' as a generic term for all causes of chronic renal allograft dysfunction with fibrosis that inhibits the accurate diagnosis and appropriate therapy. In order to treat something, first you would need a definitive diagnosis, which is not artificial but rather specifies the underlying disease process(es). Thus there is an emerging need for an appropriate classification of chronic allograft injury. On the other hand, with the burgeoning recent literature, the role of alloantibody in chronic renal allograft deterioration and the corresponding morphological changes are increasingly recognized, making the identification of an antibody-mediated

component of chronic rejection reaction possible. The second part of the revisions on the Banff schema reflects the outlined pathological criteria for chronic antibody-mediated rejection (AMR) in kidney allografts which emerged from a consensus process after in-depth discussions at the 2005 Banff meeting.

Chronic Alloimmune Injury/Rejection versus Non-Immune Injury

Use of the non-specific term 'CAN' has tended to undermine recognition of morphological features enabling diagnosis of specific causes of chronic graft dysfunction. For example, many allograft recipients are hypertensive, which can lead to chronic allograft injury with fibrosis; pathological changes recognizable in the allograft include arterial fibrointimal thickening with duplication of internal elastica (fibroelastosis), arteriolar and small artery hyalinosis, glomerulosclerosis, interstitial fibrosis and tubular atrophy (IF/TA) (3). Chronic calcineurin inhibitor toxicity produces hyaline arteriolar changes, sometimes with peripheral hyaline nodules, and IF/TA either in 'striped' ischemic or diffuse form (4–6). Co-incident thrombotic microangiopathy and/or isometric vacuolization of tubular cells suggests ongoing toxic injury (7,8). Chronic obstruction in or extrinsic to the ureter can lead to IF/TA with relative glomerular sparing: dilated tubules, atubular glomeruli and intratubular Tamm–Horsfall protein casts with extravasation into the interstitium are pathological features suggestive of obstruction, which can be recognized in the allograft (9). Chronic polyomavirus infection can lead to IF/TA with chronic inflammation— intranuclear viral inclusions, highlighted on immunostaining for the SV40 large T antigen, are diagnostic of infection, though they may be sparse or even absent in very late fibrotic stages of polyoma virus nephropathy (10). Many recurrent and de novo glomerular or vascular diseases can also lead to glomerulosclerosis and IF/TA, both early and late post-transplant. In addition, de novo diabetic changes are becoming more common in allografts. All of these specific causes of IF/TA can and should be recognized by the pathologist (Table 1).

In addition, chronic alloimmune injury is an important cause of IF/TA in the graft. The Banff schema already mandates recognition and notation of morphological features of 'true' chronic rejection. Arterial and capillary changes have been emphasized as discriminating features (1). Recent data on alloantibodies and C4d in chronically failing renal allografts indicates a pathogenic role of humoral immunity in a subset of patients with chronic allograft dysfunction. There is strong evidence that anti-HLA antibodies participate in chronic rejection and previous studies have associated circulating anti-HLA antibodies with chronic vascular damage and late graft failure (11–13). In a large prospective trial, HLA antibodies were detected in 20.9% of 2278 renal allograft recipients, and graft failure at 1 year occurred more frequently in patients who developed de novo alloantibod-

Table 1: Morphology of specific chronic diseases

Causes of IF/TA (non-rejection)	
Etiology	Morphology
Chronic hypertension	Arterial/fibrointimal thickening with reduplication of elastica, usually with small artery and arteriolar hyaline changes.
CNI[1] toxicity	Arteriolar hyalinosis with peripheral hyaline nodules and/or progressive increase in the absence of hypertension or diabetes. Tubular cell injury with isometric vacuolization.
Chronic obstruction	Marked tubular dilation. Large Tamm–Horsfall protein casts with extravasation into interstitium, and/or lymphatics.
Bacterial pyelonephritis	Intratubular and peritubular neutrophils, lymphoid follicle formation.
Viral infection	Viral inclusions on histology and immunohistology and/or electron microscopy.

[1]CNI, calcineurin inhibitor toxicity.

ies than in those who did not (8.6% vs. 3%) (14). De novo production of donor HLA-specific antibodies was shown in 51% of 112 renal transplant recipients with graft failure compared with 2% of 123 stable controls and the presence of alloantibodies predicted the subsequent development of chronic allograft rejection and graft loss (13). However, the majority of patients with anti-donor HLA antibodies do not demonstrate a progressive loss of transplant function within the follow-up periods. It is possible that the accumulation of antibody-mediated injury takes a longer time, or that only certain classes of anti-donor antibodies can mediate chronic injury or that cellular regulatory mechanisms are in play that counteract the injury mechanisms. Alternatively, the presence of anti-donor antibodies may not be sufficient to mediate the full spectrum of allograft injury without the concomitant activity of cell-mediated allograft immunity.

Recent reports have described morphologic features of chronic rejection in association with capillary-endothelial C4d deposits and concomitant circulating anti-donor antibodies (15–20). Mauiyyedi et al. (15) demonstrated deposition of C4d in peritubular capillaries (PTC) in 61% of 38 chronic rejection cases with chronic transplant glomerulopathy (TG) and/or 'chronic allograft arteriopathy' (arterial intimal fibrosis with intimal mononuclear inflammatory cell and/or foam cell infiltration) and most of the C4d positive chronic rejection cases had antidonor HLA antibody (88%). Regele et al. (16) detected C4d deposits in PTC in 34% of 213 renal allograft recipients with chronic allograft dysfunction. PTC C4d deposition was strongly associated with TG (53% of positive vs. 14% of negative biopsies) and severe PTC basement membrane multilayering (PTCBMML) (15 of 21 in positive vs. 3 of 22 in negative cases). Furthermore, C4d deposits in PTC preceded the development of

TG in follow-up biopsies. Vongwiwatana et al. (18) reported C4d deposition in PTC in 25% of 24 patients with TG but none with recurrent IgA nephropathy. PTCBMML was significantly increased in TG. Thus, the authors suggested that the association of TG with PTCBMML and C4d in PTC indicates a generalized disorder of the graft microcirculation and its basement membrane due to AMR in at least some cases. Sijpkens et al. (19) identified TG in 18 (1.6%) of 1111 kidney transplants with at least 6 months of graft function, and found C4d deposits in the glomerular capillary walls in 10/11 biopsies with TG. PTC C4d deposits were demonstrated in 4 and anti-HLA antibodies in 3 of the 10 biopsies with glomerular C4d deposits, suggesting that some of the glomerular staining was non-specific. Smavatkul et al. (21) reported increased graft loss over a 2-year period in patients with biopsy-proven graft fibrosis that were C4d positive (60%) compared to those that were negative (30%), and found TG and macrophage infiltrates as predictors of graft failure in grafts that were C4d positive.

The Diagnostic Triad of Late or Chronic Antibody-Mediated Rejection

Based on this accumulated literature, at the 2005 Banff meeting criteria for identification of late or chronic AMR were discussed and defined. The diagnostic criteria of late/chronic AMR include the following: (1) *morphological features* including TG (duplication or 'double contours' in glomerular basement membranes, Banff score cg1–3, see Figure 1) and/or PTCBMML (see Figure 2) and/or IF/TA with or without PTC loss, and/or fibrous intimal thickening in arteries without duplication of the internal elastica; (2) diffuse *C4d* deposition in PTC and (3) the presence of *donor specific antibody* (DSA) (Table 2). Diffuse C4d positivity has
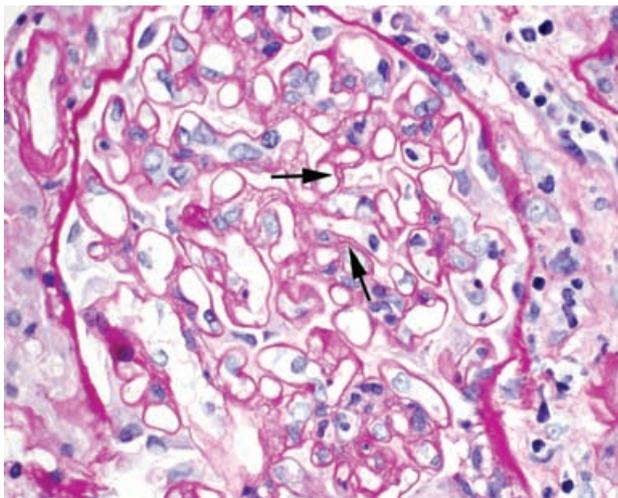

Figure 1: Chronic transplant glomerulopathy with numerous double contours (arrows) in glomerular basement membranes (PAS, original magnification ×600).
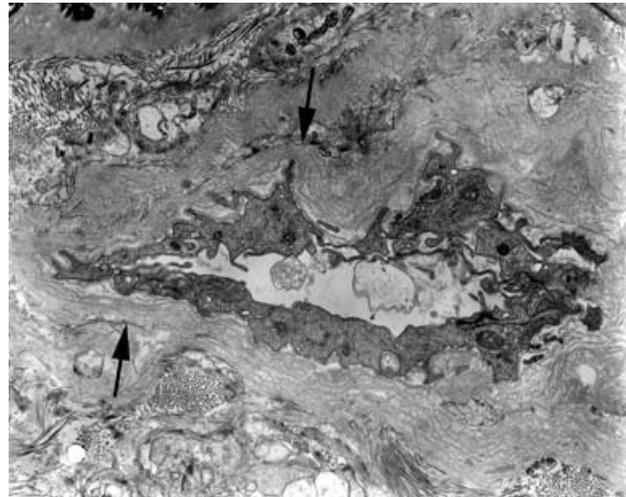
Figure 2: Marked splitting and multilayering in peritubular capillary basement membranes (arrows) in a renal allograft biopsy (uranyl acetate-lead citrate, original magnification ×5000).

been defined as bright linear staining along PTC involving over half of sampled capillaries (2). The term 'late or chronic' means a slow but active process extending over some time (22). Indeed, the presence of C4d itself provides the best in situ evidence for an active humoral immunologic process (22,23). Other morphologic features that may accompany late AMR are aggregation of mononuclear inflammatory cells in PTC (16) (see Figure 3), transplant glomerulitis (19) (see Figure 4), and a plasma cell infiltrate in the interstitium (24). As with acute AMR, if only C4d deposits (with no DSA) or DSA (with no C4d) is present, with documented morphologic capillary changes, a diagnosis of 'suggestive of chronic AMR' can be made, although activity is more difficult to assess in the absence of C4d.

Endothelial cells are thought to be the predominant target of antibody mediated injury (22,23). It has been suggested that the binding of complement-fixing alloantibody to endothelium induces tissue injury and acute rejection through the lysis of endothelial cells, coagulation (endothelial cell activation), complement activation and subsequent recruitment of macrophages and neutrophils. Recently, late/chronic AMR has been proposed as a partial accommodation (resistance of a graft to alloantibody-mediated injury) state which might be sufficient to prevent cell lysis through incomplete inhibition of complement but insufficient to prevent smoldering endothelial cell injury and activation (23). Dr. Jeffrey Platt emphasized accommodation as a possible contributor to chronic rejection in his presentation at the Banff meeting. Indeed, it has been shown that nucleated cells exposed to sublytic doses of the complement membrane attack complex become resistant to lytic complement doses (25). Dr. Platt suggested that accommodation may allow the allograft to survive long enough to acquire chronic rejection. Further studies are needed to determine whether true accommodation occurs, or whether

Table 2: Banff 97 diagnostic categories for renal allograft biopsies—Banff'05 update

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+

Chronic active antibody-mediated rejection[1]

Glomerular double contours and/or peritubular capillary basement membrane multilayering 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 diagnosis is not met (may coincide with categories 2, 5 and 6)
4. T-cell-mediated rejection[1] (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[1]

'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[1]

Grade

   I. Mild interstitial fibrosis and tubular atrophy (<25% of cortical area)

   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 (the diagnoses given in Table I); may coincide with
   categories 2–5

[1]Indicates changes in the updated Banff'05 schema.

the presence of alloantibody and complement in the absence of classical histological changes simply reflects subtle allograft injury over a long time frame (26).

TG and PTCBMML tend to occur concomitantly, and both lesions show basement membrane thickening and multilayering, which are regarded as markers of past or recent
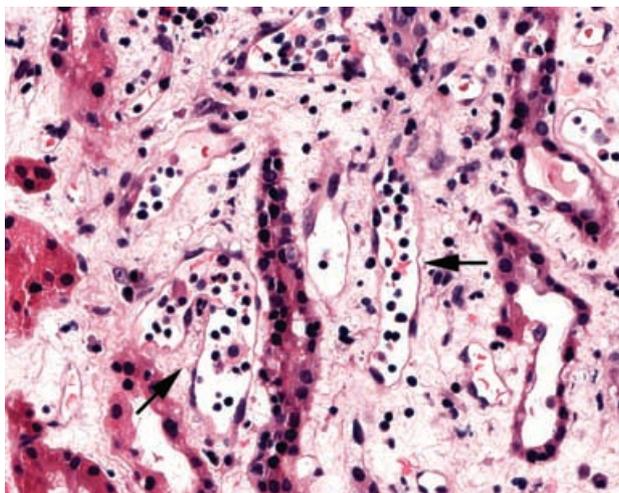
Figure 3: Aggregates of mononuclear inflammatory cells in dilated peritubular capillaries, scored as ptc3 (Hematoxylin and Eosin, original magnification ×265).

Figure 4: Transplant glomerulitis with infiltrating mononuclear inflammatory cells (arrows) within capillary loops (PAS, original magnification ×400).

endothelial cell injury and repair (15,17–22,27–32). Initially, Monga et al. (28,29) described splitting and multilayering of PTC basement membranes in renal allografts in association with TG. Ivanyi et al. (30) have reported moderate (5–6 layers) and severe (≥7 layers) PTCBMML in 16% and 12% of allograft biopsies and in 21% and 38% of failed transplant nephrectomy specimens with chronic rejection, respectively. Recently, Regele et al. (16) associated endothelial C4d deposition with TG, PTCBMML and accumulation of mononuclear inflammatory cells in PTC. Similarly, Mauiyyedi et al. (33) correlated marked PTCBMML with the presence of C4d in PTCs; they found 4.7 ± 1.8 layers in C4d+ cases versus 1.9 ± 1.2 layers in those that were C4d−. Thus the association of C4d deposition and alloantibody with TG and PTCBMML in some cases suggests AMR as a pathogenesis in at least a subset of patients. On the other hand, the precise definition of PTCB-MML is critical when comparing studies describing associations with PTCBMML. For instance, Drachenberg et al. (34) showed that TG was mostly associated with severe PTCBMML (more than 6 layers), whereas lesser degrees of these changes (mostly 2–3 layers) were observed in transplants with other types of glomerulopathies and in native kidneys with various types of immune complex glomerulonephritis, diabetes, and hypertension. A representative picture of marked PTCBMML is shown in Figure 2.

The thickening and lamination of PTC basement membranes might be appreciated on periodic acid-Schiff or silver stains at least in advanced cases (15) and sometimes in Toluidine blue stained EM thick sections but would not allow one to define the severity of lesion, that is, count the layers. The question of whether electron microscopy should be routinely done on every or some subset of renal allograft biopsies remains open and should be addressed
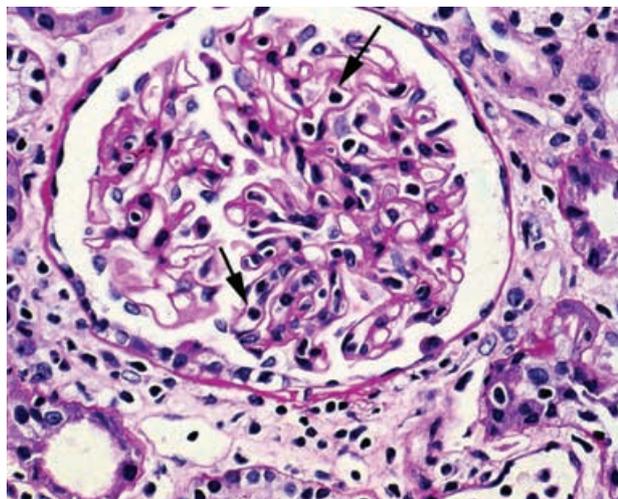
at the next Banff meeting along with the feedback from transplant physicians.

The pathogenesis of C4d negative TG and PTCBMML is unclear. In contradiction with the previous observations, three recent studies found no significant correlation between TG and C4d deposition in PTC (35–37) or in glomerular capillaries (36). Akalin et al. (36) showed glomerular infiltration by CXCR3+ ICOS+ activated T cells in grafts with TG/CAN, but not in CAN alone, suggesting an ongoing effector T-cell response to glomerular antigens can result in TG. At the 2005 Banff meeting, Dr. Colvin suggested possible causes of TG that is not associated with C4d staining: (1) technical/sampling error in AMR (e.g. PTC may disappear with allograft fibrosis); (2) residual injury from prior episodes of AMR; (3) T-cell-mediated TG or (4) non-alloimmune causes of TG (such as thrombotic microangiopathy). PTCBMML also appears to be a non-specific regenerative response to various types of injury both in transplants and native kidneys, including obstructive uropathy, thrombotic microangiopathy, analgesic nephropathy, various types of glomerulonephritis and radiation nephritis (30,32,34). Thus, definitive diagnosis of 'chronic' AMR requires a combination of morphologic changes (e.g. TG and/or PTCBMML and/or IF/TA and/or chronic arterial changes), with positive C4d immunostaining, and demonstration of DSA.

Category 5 in the Banff classification now includes only those cases for which no specific etiologic features can be defined (see Table 2). Quantitation of these changes is based on the percentage of cortex involved by IF/TA. Another change in the updated schema is the replacement of 'cellular rejection' with 'T-cell-mediated rejection'. Cellular rejection is associated with a primarily T-cell infiltrate, although the other inflammatory cells including

Table 3: Changes from Banff '97 and '01 diagnostic categories

Category 2. Antibody-mediated rejection now includes 2 subcategories:

Acute antibody-mediated rejection

Chronic active antibody-mediated rejection
Category 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 mild tubulitis (t1) and at least i1. It is now defined more clearly that t2, t3 with i0 or i1 is also under the borderline category.
Category 4. Acute/active cellular rejection is now replaced with T-cell-mediated rejection and includes two subcategories:

Acute T-cell-mediated rejection

Chronic active T-cell-mediated rejection
Category 5. Chronic/sclerosing allograft nephropathy 'CAN' is now replaced with:

Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology
Category 6. Other, changes not considered to be due to rejection-acute and/or chronic. The specific diagnoses responsible for chronic allograft injury, given in Table 1, are represented under category 6.

macrophages/monocytes, B cells, NK cells and plasma cells could also present in the graft and might contribute to the alloimmune response. However, we think that the more definitive term 'T-cell mediated' should be regarded to be similar to the antibody-mediated category as indicating the immunological component that is specifically recognizing the alloantigens. It should also be emphasized that both rejection types have cellular participation (macrophages/monocytes, etc.). Thus the term of 'cellular rejection' is now replaced with 'T-cell mediated rejection' as category #4 with subcategories of 'acute T-cell mediated rejection' and 'chronic active T-cell mediated rejection'. Major changes from the previous Banff schema are summarized in Table 3.

The Pathology of Antibody-Mediated Rejection

Complement deposition as a mediator and/or marker for AMR was discussed in the context of kidney, liver and heart allografts. Method standardization and guidelines for interpretation of complement staining were provided by Drs. Collins and Colvin, summarized elsewhere (38). In the kidney, PTC staining appears quite specific for alloantibody using either monoclonal antibody with immunofluorescence detection on frozen tissue, or polyclonal antibody with immunoperoxidase (IP) detection on paraffin sections; the for-

mer, however, is more sensitive (39). Glomerular capillary staining may be a marker for alloantibody effects using polyclonal antibody and IP staining in paraffin embedded tissue (19), but it can also be caused by immune complex deposition in glomeruli. At this time the clinical significance of C4d deposition in a graft with normal histology is unknown. In contrast to patients with anti-HLA antibody, diffuse PTC staining for C4d is commonly detected in well-functioning allografts in patients with anti-A or -B blood group antibodies, without histological evidence of injury (40). The complexity of control of the complement cascade, and resistance to injury with possible arrest of the cascade as a marker for 'accommodation' were emphasized (41). However, recipients with positive cross-match (HLA-incompatible) were recently shown to have increased risk for TG one year after transplantation in comparison to ABO-incompatible and conventional allografts (22% vs. 13% vs. 8%, respectively), and prior AMR appeared as an independent determinant for development of TG (42).

Capillary margination of inflammatory cells is an important histological marker of AMR in kidney and heart allografts, and acute capillaritis in lung allografts may be an equivalent process. Marginating neutrophils are more specific for AMR (43), but both neutrophils and mononuclear cells/monocytes have been associated with PTC C4d staining (16,44). Aggregation of mononuclear cells in PTC is shown in Figure 3. Given the importance of PTC

Table 4: The proposal of quantitative criteria for peritubular capillary margination of inflammatory cells ('ptc') score[1]

ptc0—no significant cortical peritubular inflammatory changes
ptc1—cortical peritubular capillary with 3–4 luminal inflammatory cells
ptc2—cortical peritubular capillary with 5–10 luminal inflammatory cells
ptc3—cortical peritubular capillary with >10 luminal inflammatory cells

[1]Use asterisk (*) to indicate only mononuclear cells and absence of neutrophils.

margination of inflammatory cells as a histological feature of AMR, Ian Gibson proposed a scoring method for quantitation ('ptc' score) at the Banff 2003 conference and reviewed this at the 2005 conference. The proposal focuses on the most severely involved PTCs, in analogy to other inflammatory rejection features such as tubulitis (Table 4). The number of luminal inflammatory cells includes all types (neutrophil, monocyte/macrophage and lymphocyte), with an asterisk (*) used to indicate only mononuclear cells and absence of neutrophils. The extent of the PTC inflammation in the biopsy should be documented, either as focal (<50% of cortical area) or diffuse (>50% of cortical area). The presence of associated PTC dilatation may also be noted. Areas affected by acute pyelonephritis or necrosis, and subcapsular cortex with non-specific inflammation should not be scored. Inflammatory cells within PTC must be distinguished from interstitial inflammation by careful examination of basement membrane stains (PAS, silver). Inflammatory cells within veins and medullary vasa recta should not be scored.

Several groups represented at the Banff 2005 conference indicated that they are using this scoring system. It is particularly applicable to comparison of sequential biopsies from the same graft, for example, in assessing responses to rejection treatments, as well as for documenting biopsy features in clinical trials. Some provisional reports using the peritubular capillaritis scoring system have been published (45,46), confirming its applicability, and showing high 'ptc' scores associated with AMR, and that lower 'ptc' scores can be associated with progressive chronic graft injury (46). It must be emphasized that the 'ptc' score alone does not equate with any specific diagnosis, and ongoing reproducibility and diagnostic studies are required, but the 'ptc' score helps to direct the pathologist to careful examination of the PTC.

B Cells in the Renal Allograft

The role of B cells in allograft rejection and ischemic injury was also highlighted at the 2005 Banff conference. Memory B cells and long-lived plasma cells in bone marrow may persist for years. Initial B-cell activation leads to the formation of short-lived plasma cells that provide the first burst of antibody. Long-term antibody responses, however, are maintained by non-dividing, long-lived plasma cells that produce high-affinity antibody. It should be noted that the B cells or plasma cells reside in lymphoid compartments during AMR and antibodies enter the graft as the effector molecules of humoral immunity (47). 'Lymphoid neogenesis' has been described in renal allografts with prominent lymphoid aggregates (48), though not all lymphoid aggregates are associated with acute rejection (AR) (49). In other contexts (e.g. rheumatoid arthritis, SLE), such aggregates can locally secrete tissue-specific pathogenic antibodies. B cell tolerance may also be possible, as reviewed by Dr. Cascalho (50).

The presence of molecular markers associated with B cells has also been identified in a subset of clinical cases of AR; immunostaining of allograft biopsy tissue confirmed significant numbers of B cells in the inflammatory infiltrates (51). The presence of B cells/markers was associated with worse outcome in this series. However, the frequency of B cell infiltrates in allografts in either AR or non-specific injury has not been extensively studied, nor has the association of allograft B cell infiltrates and AMR/presence of DSA. Recent interest in B cells in allografts has been spurred by the availability of anti-B cell therapies such as rituximab. A few centers have begun to routinely perform immunohistochemistry for B cells in allograft biopsies that have inflammatory infiltrates, for quantitative assessment and pattern of localization. B cell-rich infiltrates should be denoted with an asterisk on the 'i' score in the Banff scoring system. In the short term, these observations could guide therapy for those cases of AR that are B cell-rich and resistant to standard immunosuppression. However, evidence is lacking at this point whether anti-B cell therapy can reverse a resistant episode of B cell-rich rejection. In the long term, detection of B cell markers will provide important data in regard to incidence of significant B cell infiltrates, effects of same on response to therapy, clinical correlates and effect on outcome.

Genomics Markers in Solid Organ Transplantation

Molecular approaches and techniques were the subject of a pre-meeting symposium as well as sessions during the 2005 Banff conference. Techniques discussed included gene expression profiling using high density and DNA microarrays, transcriptome (gene chips) or quantitative PCR, metabolomics and proteomics. The importance of a 'biological' approach was emphasized, correlating gene expression array data with RT-PCR and Western immunoblotting and other proteomic technologies that can validate the actual levels of differentially expressed proteins as well as their post-translational modifications, such as phosphorylation that determine activation and molecular network signaling. It was considered equally important to cross-validate the expression levels of both gene transcripts and proteins, and with the biopsy pathology and clinical data to derive the fullest possible picture. Potential applications of array-based data include definition of disease mechanisms, identification of targets for pharmacological intervention, calibration of indicator systems for drug development, revision of new end points for trials, and development of new diagnostic and monitoring systems that could be applied to blood, fluids (urine, bile) or tissue specimens. The importance of using these strategies to focus on 'real' clinical issues was emphasized, with cluster analysis to identify clinically relevant genetic information.

An ultimate aim is to develop a genomics supported 'Banff classification' for diagnosis and grading of rejection and

other processes in allografts. The potential pitfalls in using genomics markers exclusively for differential diagnosis of acute and chronic dysfunction in allografts were discussed, based on the burgeoning literature in this area (52). Studies are often based on small cohorts of patients and may not include individuals with allergic drug reactions, systemic or intragraft infections or other inflammatory processes. Therapeutic regimens may alter findings and correlations, as has been shown for steroid treatment (53). Many studies of molecular markers for AR have not addressed discrimination between subtypes of AR (tubulointerstitial vs. vascular, cell- vs. antibody-mediated). Sarwal reported that C4d-positive cases of AR fall in each of the 3 AR categories defined by gene profiling. In addition, no obvious differential gene expression could be defined in cases of allograft fibrosis due to different causes (51). At the molecular level, the predominant signatures for gene expression may reflect the predominant pathological mechanisms in the biopsied tissue, for example, fibrosis or inflammation. If this is the case, then a specific cause may be difficult to connect directly to a particular molecular profile without additional data including clinical and histopathological data.

Identification of a few relevant diagnostic markers may be more useful and reasonable for diagnostic application in the near future, particularly, since array data often need to be markedly pruned in order to provide discrimination between patient groups (54). Currently molecular screening of blood and urine represent a promising alternative to invasive biopsy procedures for surveillance to detect early AR, but do not provide enough discriminatory power. At the present time, the assays are not statistically robust enough for clinical guidance. At least for the foreseeable future, the biopsy remains the 'gold standard' for definitive allograft assessment, though exciting alternatives are on the horizon.

Acknowledgments

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