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Meeting Report

Banff 2003 Meeting Report: New Diagnostic Insights and Standards

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The Seventh Banff Conference on Allograft Pathology was held June 14–18, 2003 in Aberdeen, Scotland representing the latest iteration of the international consensus meeting, which develops worldwide standards for interpretation of allograft biopsies. The meeting is an important force behind standardized slide interpretation to strengthen endpoints in international clinical trials. Of participants polled 87% reported that they would alter clinical practice as a direct consequence of the meeting and its content. Advances were made in many areas including tubulitis mechanisms, real-time polymerase chain reaction (PCR) gene analysis and microarrays in rejection diagnosis, tolerance/accommodation/immunomodulation, the role of monocytes and macrophages in rejection and C4d as a marker for antibody-mediated rejection. A provisional scoring system for peritubular capillary inflammatory cell accumulation in antibody-mediated rejection was presented for testing, as well as plans for a nephrectomy study to determine specificity of vascular lesions of rejection. Future meetings are planned for 2005 (Edmonton), 2007 and 2009, with active ongoing Internet discussion between meetings.

Key words: Banff classification, central slide review, scoring

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Introduction

The Seventh Banff Conference on Allograft Pathology was held June 14–18, 2003 in Aberdeen, Scotland (near Banff, Scotland), representing the first time this standard-setting, consensus conference has been held outside Canada. The meeting was attended by 190 participants from all over the world including pathologists, clinicians, surgeons and basic scientists. A remarkable 87% of participants polled reported that they would alter clinical practice as a direct consequence of their experience at the meeting.

As has been true in all previous Banff meetings dating back to 1991, the Aberdeen meeting reviewed progress in the understanding of mechanisms of rejection lesions in solid organs, in setting standards for diagnosis of rejection and creating conceptual structures that will enable important future research. This meeting report will highlight breakthroughs made in the area of tubulitis as a marker for rejection of renal allografts, and then summarize the presentations at the meeting relating to real-time gene analysis and microarrays in rejection diagnosis, tolerance/accommodation/immunomodulation, the role of monocytes and macrophages in rejection and C4d as a marker for antibody-mediated rejection. The following is not intended as a complete account of the meeting, but highlights a number of selected areas, which are of conceptual importance. Many of the PowerPoint presentations from the meeting as well as the full program, images and other details can be found at the meeting website http://cybernephrology.ualberta.ca/Banff/2003.

Tubulitis

Tubulitis—the presence of mononuclear cells in the basolateral aspect of the renal tubule epithelium—is a defining lesion of T-cell-mediated rejection in kidney transplants. Intimal arteritis is less common than tubulitis and thus most rejection episodes are defined by tubulitis. Tubulitis with its accompanying interstitial infiltrate is a key lesion driving clinical consequences of graft rejection.

Philip Halloran (Canada) presented data on recently described mouse models of tubulitis (1) that have permitted the examination of the rejection process in gene knockout mice. In this model the tubulitis can be correlated with changes in transcription and protein expression, to create an emerging picture of the mechanisms involved. A model has recently been described in which mice differing by full MHC (major histocompatibility) plus non-MHC differences receive a right kidney transplant, replacing the right kidney but leaving the left native kidney in place. This sustains renal function and permits the examination of the pathology as it evolves over several weeks in the absence of immunosuppression. In this model, the key lesions of rejection

develop at different rates. Using immunoglobulin knock-out mice, it can be shown that there are three different patterns of lesions. All of these phases are T-dependent, that is, do not occur in transplants into mice lacking T cells. First, there is MHC induction, which is highly IFN (interferon)-γ dependent and is associated with interstitial infiltration. This appears by day 5 and is associated with interstitial edema, presumably reflecting capillary leak. At this time, there is no tubulitis or intimal arteritis. These lesions (edema, interstitial infiltration, MHC induction and IFN-γ effects) peak at day 5–day 10 and then stabilize or regress slightly over the next 21 days. The second phase is tubulitis, which is completely T-dependent and is unaffected in immunoglobulin knock-out mice. The third set of lesions is late (day 21) with progression in edema, congestion, necrosis and immunoglobulin deposition. These lesions are absent in immunoglobulin knock-out mice and presumably reflect the effects of antibody on the graft.

Thus in this model, one has MHC induction, inflammation/invasive lesions of tubulitis and progressive antibody-mediated lesions. Tubulitis emerges as the principal correlate of T-cell-mediated effects independent of antibody. Endothelial arteritis in this model appears to be a combination of antibody-mediated and T-cell-mediated effects. It is reduced in severity in immunoglobulin-deficient hosts, but nevertheless does occur. This model shows spontaneous homeostasis in that the interstitial infiltration actually stabilizes or regresses spontaneously after day 7 even though the tubulitis progresses. The results also suggest independent homeostatic control of inflammation/IFN-γ effects, invasive tubulitis, endothelialitis and antibody-mediated effects (1).

Halloran presented data on the traffic response, which is the systemic evidence of the alloimmune response to the graft. In the host kidney, systemic effects could be seen, including not only effects of IFN-γ (intense MHC class I and II induction) but also expression of granzyme B, A, perforin and other inflammatory genes. This systemic expression peaked at day 5 in most cases and then regressed spontaneously. This suggests that what is being monitored in the contralateral kidney is not simply a reflection of the systemic effects of the events in the graft, but actually a separate event, namely the traffic of antigen-expressed cells from their site of activation (presumably the lymphoid organs) through the tissues of the host.

Margaret Jonker (Netherlands) reviewed data on serial biopsy analysis and rejecting and non-rejecting Rhesus monkeys with renal allografts. The kidneys are transplanted and immunosuppression is carried out with either cyclosporine/sirolimus or T-cell depletion and the kidneys are then observed with serial biopsies for varying times before the organs are ultimately examined. The grafts are rejected around day 7 without immunosuppression, but last for varying times with different immunosuppressive protocols, often beyond 100 days. Interstitial infiltration and tubulitis are frequent in kidneys of monkeys on cyclosporine therapy alone. When anti-CD40 therapy is used, the monkeys show considerable early graft pathology, more than when the anti-CD40 was combined with anti-CD86. The anti-CD40 plus anti-CD86 prevented the appearance of tubular atrophy and interstitial fibrosis. Cells positive for CD4, CD8 and CD2 can be found in tubulitis lesions in the monkeys on anti-CD40.

Overall, these primates' studies suggest that the interstitial cell infiltrates are similar in animals with different forms of immunosuppression, and that anti-CD40-treated monkeys survive longer after discontinuation of immunosuppression despite having more tubulitis than anti-CD40- plus anti-CD86-treated monkeys. While in both groups rejection is dominated, as in the mouse, by CD8 cells, the majority of the tubule infiltrating cells (in tubulitis lesions) in the anti-CD40 group are CD4 positive in contrast to the tubulitis in the monkeys treated with anti-CD40 plus anti-CD86. The possibility exists, therefore, that a CD4 regulatory cell can be demonstrated in tubulitis. Latent TGF (transforming growth factor)-β is abundant in the early graft interstitial infiltrates, which do not seem to be particularly aggressive. Active TGF-β is also present in rejected grafts, localized mainly in the infiltrating cells rather than on the renal epithelial components. The data would suggest that TGF-β has complex roles, possibly influencing T regulatory cells despite a strong association with rejection.

Gregg Hadley (USA) presented data that islet allografts under the kidney capsule are not rejected in mice lacking CD103. The CD103 presumably interacts with the E-Cadherin at the basolateral aspect and at the adherens junction of the epithelium. CD103 and E-Cadherin thus emerge as a key ligand system to allow T lymphocytes to interact with the epithelium (like 'tubulitis' in kidney), analogous to ICAM1 (intercellular adhesion molecule 1) and lymphocyte function-associated antigen-1 (LFA1) in lymphocyte interaction with some inflammatory cells. It remains to be seen whether human tubulitis requires interactions between CD103 and E-Cadherin.

Steven Sacks (UK) reviewed the many potential roles for complement, particularly the complement, which is produced in the renal cells. A number of complement components can be produced locally in kidney, both in the epithelium itself and in the inflammatory cells. Kidneys from hosts lacking C3 show impaired rejection (2,3). Some data suggest that this may be due to a role of complement factors in regulating the immune system itself. However, it is also possible that complement has a more conventional role as an effector for antibody-mediated injury in the graft.

A non-conventional role of T cells has been documented recently with the demonstration that impairment of B7-CD28 interaction suspends or reduces renal injury in experimental models of ischemia reperfusion injury in the kidney (4). Hamid Rabb (USA) presented evidence that T cells are involved in the pathogenesis of ischemia reperfusion in

rodent kidneys (5). One potential explanation for this effect could be the expression of B71 in the renal microcirculation. Marc Debroe (USA) presented data on inducible expression of B71. It is not clear whether this represents a point of difference between humans and experimental animals. In particular, the results of renal function studies in humans receiving CTLA4 Ig in the current clinical trial will be awaited with interest.

Real-Time polymerase chain reaction (PCR) Gene Analysis and Microarrays in Transplantation

This session provided an overview and examples of the use of these technologies in transplantation. Examples included applications in a variety of organ allografts, in acute and chronic rejection, in donor graft assessment and in ischemia-reperfusion injury. Allan Kirk (USA) described practical application of real-time PCR to provide data in a few hours that can be used for real-time patient management. Minnie Sarwal (USA) described microarray profiling in acute rejection (6), and cited the web address http://genome-www5.stanford.edu/ (published data) at which the array data could be retrieved for further investigation, an admirable template for sharing of these large data sets for 'mining' by the transplant community. Her data included a number of conceptually important observations, including the point that there is a significant B cell/plasma cell signature in graft rejection. Moreover, some episodes considered rejection by the pathologist do not have the typical pattern of T-cell activation, suggesting that some of the pathology of rejection may be incorrectly interpreted, or that other less conventional mechanisms for rejection exist.

Tolerance/Accomodation/ Immunomodulation

A review of mechanisms of tolerance by Robert Lechler (UK) provided a background for discussion, with a brief review of current and planned clinical trials of 'tolerance' protocols. Dr. Eleanor Ramos (USA) represented the Immune Tolerance Network, reviewing the two major mandates of the network, support of clinical trials to test novel agents and protocols and development of mechanistic assays to measure tolerance. The structure and application-review process for trials proposals, and ongoing trials supported by the network in transplantation, autoimmunity and allergy and asthma were reviewed, and the community was encouraged to take advantage of this mechanism. Immunodepletion and blockade of co-stimulation signals are successful strategies, but should move forward with important guiding principles as pointed out by Dr. Alan Kirk:

1. types of T cells are different, which must be taken into account in anti-T-cell protocols,
2. it is not just T cells but also other cells, which may be effectors and
3. context-based therapy is needed, recognizing that alloimmunity includes naïve and memory cell responses.

Discussion of NIH immunodepletion protocols by Dr. Roz Mannon (USA) provided several key lessons learned in use of these protocols to successfully reduce use of standard immunosuppression. Modification of the initial protocols was required to reduce incidence of rejection, and protocols should be designed to be flexible enough so that adjustments can be made as necessary and even unsuccessful protocols should be shared with the transplant community, so that all can benefit from lessons learned in the context of these trials. At the tissue level, Dr. Robert Colvin (USA) reviewed the appearance of processes in the graft that cause or predict graft acceptance, either regulatory tolerance or accommodation, compensatory changes in the graft conferring resistance to persistent alloimmune processes. In general, these processes are inflammatory, differing from rejection somewhat in cytokine profile and cell turnover. Care must be taken in all anti-rejection protocols to avoid perturbation of these adaptive processes. As a final point, overall, while potentially costly to initiate, these protocols have the potential to provide great cost savings by avoidance or reduction of the cost of maintaining immunosuppression and associated complications.

Monocytes and Macrophages

Michael Fishbein (USA) outlined the role of macrophages in diagnostic interpretation of endomyocardial biopsies. Macrophage markers are useful in distinguishing between acute rejection and Quilty lesions in cardiac allograft biopsies. Quilty lesions contain B cells and T cells but very few macrophages, while there are many macrophages in acute rejection.

Alex Magil (USA) provided data that macrophage infiltration may be useful in the diagnosis of humoral rejection in the kidney. Glomerular and tubulo-interstitial macrophages are associated with C4d deposition. Presence of macrophages is associated with poor outcomes.

Jeremy Hughes (UK) reviewed the role of macrophages in disposal of both necrotic and apoptotic cells. There are multiple receptors responsible for uptake of apoptotic cells, including the phosphytidyl serine receptor. Uptake of apoptotic cells induces an anti-inflammatory phenotype in macrophages, overwhelming the normal mechanisms of disposal, which results in inflammation.

David Kluth (UK) discussed the various functional types of macrophages and transfection experiments showing that anti-inflammatory macrophages can have profound effects

in vivo. He described the well-recognized macrophage 'phenotypes'—activated, innate-activated, alternatively activated, type 2 regulatory and anti-inflammatory. Evidence that transfected macrophages expressing either IL-4 or IL-10 but not TGF-β reduce acute experimental glomerular injury was presented. These macrophages localize only to the kidney in which they are injected but inflammation is reduced in both kidneys. Introduction of macrophages with blocked NF-κB are also anti-inflammatory, but only in the kidney into which the cells were injected. Clearly regulation of macrophages is complex, and of potential relevance to the allografted kidney.

Tim Johnson (UK) discussed the role of tissue transglutaminases in cell death and in matrix accumulation. He presented intriguing data that transglutaminase is the switch between inflammation and scarring in chronic allograft nephropathy. He demonstrated that all models of chronic renal scarring are associated with increased expression of tissue transglutaminases. Expression is associated with increased cross linking of collagen and with increased TGF-β expression. Tubular cells were the source in transglutaminases models of scarring in native kidneys, but interstitial macrophages were the source in chronic scarring in allografts.

Andrew Rees (UK) summarized the conclusions from this session: macrophages are numerous in all forms renal injury, and that macrophages have numerous different functions that may be injurious or reparative. Key issues for the future are to devise a more complete understanding of the range of macrophage activities, how to identify macrophage 'phenotypes' *in vivo* and how to manipulate macrophage function as therapy. All of these issues have relevance to allografted organs.

Standardization of Scoring of C4D Staining in Renal Allograft Biopsies

Consensus sessions moderated by Robert Colvin discussed standardization of scoring of C4d staining in renal allograft biopsies. Positive immunostaining for complement split product C4d has been found to be a sensitive and specific marker for antibody-mediated rejection in kidney allografts, and is a criterion for the various morphological subtypes of antibody-mediated rejection (5). However, terminology issues and differing antibodies and immunostaining techniques make definition of positive staining problematic. The following consensus were reached regarding C4d staining in renal allograft biopsies:

1. Because antibody-mediated rejection is a significant problem, and because C4d staining is an independent prognostic factor for graft outcomes, C4d staining should be done on all diagnostic renal transplant biopsies.
2. Criteria for C4d positivity in cryostat frozen sections using monoclonal antibody and immunofluorescence—widespread strong linear circumferential peritubular capillary staining in either cortex or medulla, excluding scarred or necrotic areas. A concurrent positive control must be run, essential if a negative result is being reported. Extent and intensity of staining should be reported.
3. Criteria for C4d positivity in paraffin-embedded sections using polyclonal antibody and immunohistochemistry—widespread linear circumferential peritubular capillary staining in either cortex or medulla, excluding scarred or necrotic areas. However, strong staining is not required for a positive reading using this technique, as the type of tissue pre-treatment influences the staining intensity, and may be variable. Glomerular basement membrane/mesangial staining in the sample cannot be used as a control, as this appears to be non-specific, but membranous glomerulonephritis (GN) is satisfactory as a positive control. Weak and/or focal is suggestive of the presence of antibody/complement-mediated endothelial injury, but is not as definitive. In some cases focal staining precedes widespread staining, or it may persist as focal or disappear without specific treatment. To enable refinement of determination of specificity, reports should include the percentage of non-scarred parenchymal area with peritubular capillary staining for C4d, as well as staining intensity. Capillary C4d staining may be correlated with capillary injury, dilatation and margination of inflammatory cells as well as with donor specific antibody in serum, which remains the 'gold standard' for diagnosis of antibody-mediated rejection.

Kim Solez also moderated consensus sessions to develop a new score for accumulation of inflammatory cells in peritubular capillaries (a feature of antibody-mediated rejection). The scoring schema developed (Table 1 below) will be tested in research projects over the next 2 years with the goal of possibly incorporating this new score in the Banff classification in 2005.

Consensus discussions moderated by Kim Solez also developed a proposal for nephrectomy studies to define the relationship between vascular lesions and antibody-mediated versus cell-mediated rejection.

Notes: Inflammatory cells include polymorphonuclear leukocytes (PMNLs), lymphocytes and monocyte/macrophages. Avoid scoring in just subcapsular cortex, or just around areas of necrosis or infarction, or as part of pyelo. Peritubular capillaries (PTCs) are characteristically dilated. Inflammmatory cell aggregates are often diffuse, indicate extent when not diffuse. Scoring should be done on PAS or silver stain. Ideally, those cells within PTC should be excluded from the score.

Table 1: Proposal—Banff ptc score

• ptc 0—no significant cortical peritubular inflammatory changes
• ptc 1—cortical peritubular capillary with 3–4 luminal inflammatory cells*
• ptc 2—cortical peritubular capillary with 5–10 luminal inflammatory cells*
• ptc 3—cortical peritubular capillary with >10 luminal inflammatory cells*

*Asterisk given if cells are mononuclear only.
Numbers refer to highest number seen in all types of inflammatory cells.
Peritubular capillary accumulation of polymorphs has been shown to be more specific for antibody-mediated rejection (6).

Proposal—Nephrectomy Study

Examine circulating anti-class I and class II donor reactive antibody by solid phase or flow assay (pre- and post-nephrectomy, at least 3-weeks post-nephrectomy) and V3 lesions of patients with nephrectomy for refectory acute rejection or chronic allograft nephropathy where immunosuppression has been withdrawn or tapered before nephrectomy. A study looking at the 'natural unmodified rejection' that occurs under these circumstances, asking the question of whether transmural arteritis and fibrinoid change are inexorably linked or are separable and of different significance. Is transmural arteritis per se a feature of antibody-mediated rejection, or is fibrinoid change the arterial lesion linked with this entity? What is the role of intimal arteritis (V1, V2) in this setting?

If possible, a portion of nephrectomy should be frozen for immuno studies in the same fashion as is done for percutaneous biopsies. Markers for C4d, immunoglobulins and CD68, CD20, CD79a, CD138 and CD3 should be used.

Future Banff Allograft Pathology meetings are planned in Edmonton, Canada (2005); Edinburgh, Scotland (2007) and Banff, Canada (2009).

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