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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=0.00[27]).

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 cytotluorometric 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 pericalyceal 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 itu* hybridization for Epstein-Barr virus encoded small RNA (EBER R A). CD2O (B-cell marker) and 0D3 (T-cell marker) immunohistoc emistry 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 frequently 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?