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?

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 interstium 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 histocombatibilty complex (MHC)-class II [humanlymphocyte antigens (HLA)-DR] and intercellular adhesion molecule-i (ICAM-1); both molecules, which are normally not expressed in tubular cells. However, HLA-DR and ICAM-1 can be upregulated by interferon-y, 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 to 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 1 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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