Interleukin 2 receptor expression in renal biopsies and the diagnosis of acute allograft rejection

Anila Mathan, Sarah Kuruvilla, Georgi Abraham

Abstract: The aim of the study is to test the diagnostic usefulness of assessing interleukin 2 receptor (IL2R) expression in infiltrating lymphocytes in renal biopsies from patients with suspected acute renal allograft rejection and to compare the NIH-CCITT and the Banff 97 systems of classifying the histopathologic changes in acute renal allograft rejection. The expression of interleukin 2 (IL2) and IL2R, as shown immuno-histochemically, is the final step in T cell mediated acute renal allograft rejection. Renal biopsies obtained from 40 patients clinically suspected to have early acute allograft rejection were examined histologically to diagnose acute allograft rejection and classified by the two systems. Frozen sections of the biopsies were stained with specific antibody for the presence of IL2R. 31 of the 40 patients were histologically and clinically confirmed to have acute allograft rejection. There was significant correlation with this diagnosis and the demonstration of IL2R on infiltrating lymphocytes. The CCITT system of grading correlated better with the presence of IL2R and the confirmed diagnosis of acute allograft rejection. The immunohistochemical demonstration of IL2R is a useful adjunct in the evaluation of biopsies suspected to show changes of acute cellular rejection. Since IL2 expression reflects the relative proportion of activated lymphocytes in the cellular infiltrate, it is proposed that the degree of IL2 expression may reflect the response of the use of monoclonal antibodies (Humanised/Chimaerised) as anti rejection therapy.

Key Words: interleukin 2 receptor, acute cellular rejection, kidney transplantation

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Introduction

The deterioration of renal allograft function due to acute allograft rejection is still the leading cause of loss of transplanted kidneys, despite significant advances in immunosuppressive therapy. Careful histo-pathologic evaluation of renal biopsies is essential for confirmation of the diagnosis of acute allograft rejection. The characteristic diagnostic findings are an influx of inflammatory cells, predominantly T lymphocytes into the stroma leading to interstitial inflammation, tubulitis and intimal arteritis. The two methods of classification of acute allograft rejection currently used are the Banff 97 and the National Institute of Health – Cooperative Clinical Trials in Transplantation (CCITT) Classification. Although semiquantitative criteria have been established in both these systems of classification, the skill and experience of the pathologist is a crucial factor. Alloantigen triggered T cell activation, recognising the donor kidney as “foreign” and the subsequent infiltration into the graft by activated CD4 and CD8 T cell clones are the key events in acute allograft rejection. The final step in T cell activation by alloantigens is the induction of interleukin 2 and the expression of interleukin 2 receptor (IL2R) on the surface of T cells, which then undergo clonal expansion. Since it is possible to demonstrate and quantitate the expression of IL2R by immunohistochemical methods, we decided to study the utility of this procedure in the morphologic diagnosis of acute cellular rejection of renal allografts. Both the Banff 97 and the CCITT systems of grading were used to classify and grade the changes of acute allograft rejection. Since CCITT was the system routinely employed at this center a part of the study was to compare the usefulness of the two systems of grading the changes.

Materials and Methods

Percutaneous needle biopsies of the kidney were available from 40 post renal transplant patients with graft dysfunction. From each patient there were at least two evaluable cores, one snap frozen at -70°C and the other formalin fixed and embedded in paraffin. An evaluable biopsy core had at least two areas of cortex with 7 glomerular and a minimum of one arteriolar profile. Biopsies from 9 other patients who were not
transplanted, but had other renal pathology were also evaluated. The paraffin sections (4-5 microns thick) were stained with haematoxylin and eosin, periodic-acid-Schiff (PAS) and Masson's trichrome by standard methods as part of the regular routine work up.

The frozen core was sectioned on a cryostat at -20°C and ribbons of at least three serial sections (5-8 microns) were placed on each slide, air dried and fixed in acetone for 10 minutes at -20°C and immunohistochemical staining was done for interleukin 2 receptor. A sandwich technique was used, with primary mouse anti human monoclonal antibody for CD25 (the alpha peptide of human interleukin 2 receptor), a rabbit antimouse polyclonal serum as the sandwich antibody and the alkaline phosphatase-antialkaline phosphatase (APAAP) complex for developing colour, with naphthol AS-MX phosphate as substrate. Levamisole was used in the last step to inhibit endogenous phosphatases. The primary mouse monoclonal antibody was IL-2 Rec, CD25, clone ACT-1, Code MO731, Lot/Ch.B.025 (501). The kit for this was obtained from DAKO Corporation, Carpinteria, California, USA. The procedure given by the manufacturer was strictly followed. Sections from lymph node and tonsil were used as positive controls and sections from renal biopsies for diseases other than allograft rejection were used as negative controls. The slides for immunohistochemistry were batch processed and immediately read under the light microscope. The activated lymphocytes positive for IL2R showed a purple pink colour and were graded as described in the results.

The H&E sections were examined by 2 observers, one of whom was an experienced renal pathologist. The slides were classified as showing acute allograft rejection, with or without other renal pathology. Acute allograft rejection was typed using both the currently available schemes, CCITP and Banff 1997.

Observations

Clinical findings: Renal biopsies were obtained from 40 transplant recipients who were suspected to have acute allograft rejection as the cause of graft dysfunction. The patients in this study ranged in age from 11 years to 63 years and 16 were in the 30 to 49 year group. There were 28 males and 12 females. All patients had been given standard triple immunosuppressive regimen, the first dose on the day of transplant and a second dose 4 days later. The mean time from transplant to the biopsy in these 12 patients was 147 days (range 3-665 days, median 45 days) compared to the other patients where the mean number of days post transplant to rejection was 24 days (range 1-120 days, median 8 days). This difference between the two groups was statistically significant at the 5% level (Fischers exact test).

Histologic findings: Mononuclear cell infiltrate of the interstitium, tubulitis and vasculitis were the parameters evaluated to arrive at a diagnosis of acute cellular rejection (Fig. 1). Oedema, tubular atrophy and mild glomerulitis are additional features useful in arriving at the diagnosis of acute allograft rejection. A pleomorphic interstitial infiltrate of lymphohblasts, small lymphocytes, monocytes and occasional basophils occupying more than 5% of the area of the section was present in 31 of the 40 patients. This was associated with tubulitis or mononuclear infiltrate of tubular sections in all 31 patients and intimal arteritis with elevation of the endothelium from the basement membrane in 16 patients. The extent of mononuclear infiltration was more than 10% of the area of the section in only 23 patients. One patient had fibrinoid change with a homogenous eosinophilic fibrin like material replacing the intima and media of the arterioles. Other morphologic features such as glomerulitis, arteriolar hyaline thickening; interstitial fibrosis, tubular atrophy etc., that have occasionally been associated with acute cellular rejection were not present in any of the biopsies. Associated pathology in the 31 patients with acute cellular rejection and in the 9 patients without evidence of rejection are shown in Table 1.

<p>| Table 1 |</p>
<table>
<thead>
<tr>
<th>Associated Pathology</th>
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<tr>
<td>Patients with cellular rejection</td>
</tr>
<tr>
<td>Acute cellular rejection</td>
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<tr>
<td>Cytomegalo virus toxicity</td>
</tr>
<tr>
<td>Acute tubular necrosis</td>
</tr>
<tr>
<td>Pyelonephritis</td>
</tr>
<tr>
<td>Cytomegalo virus infection</td>
</tr>
<tr>
<td>Focal segmental glomerulosclerosis</td>
</tr>
<tr>
<td>Minimal change disease</td>
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<tr>
<td>No pathology</td>
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Morphologic classification of acute cellular allograft rejection: The CCTT system of classification is routinely used in this department. Biopsies from 16 patients with mononuclear cell infiltration involving more than 5% of the area, tubulitis and at least one of the following features, activated lymphocytes, oedema or tubular atrophy/degeneration were classified as Type I rejection. An additional 15 biopsies showed these features as well as arterial or arteriolar endothelialitis and were therefore considered to be Type II. Fibrinoid arteriolar degeneration was present in only one biopsy, which was classified as Type III. There was no evidence of acute cellular rejection in biopsies from 9 patients with early graft dysfunction. The Banff 97 system of classification is comprehensive covering all causes of allograft dysfunction, but by definition, if less than 10% of the area of the biopsy only shows infiltration with activated lymphocytes, a definitive diagnosis of rejection is not entertained. In the present instance 8 of the patients who were diagnosed as acute cellular rejection by the CCTT criteria were classified as Borderline by the Banff 97 criteria, since only 5-9% of the area showed infiltration, without arteriolar involvement. The remaining 8 patients classified as type I by CCTT were placed in Banff 97 grade IA, the 15 biopsies graded as CCTT grade II in Banff 97 grade IIA and one biopsy was graded as III in both systems.

Immunohistochemical demonstration of interleukin 2 receptors: The frozen sections of the renal biopsies stained for IL2R by the sandwich technique with APAAP showed activated lymphocytes with purple pink stained granules, indicating the presence of interleukin 2 receptors (Fig. 2). The positive and negative control sections included in each batch for staining gave appropriate reaction.

Grading system used for IL2R expression:

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<th>Grade</th>
<th>No. of cells showing intracytoplasmic positivity for IL2</th>
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<tr>
<td>0</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>+</td>
<td>5-33%</td>
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<tr>
<td>++</td>
<td>33-66%</td>
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<tr>
<td>+++</td>
<td>&gt;66%</td>
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The extent of IL2R staining was graded as 0 in 7 of the 9 biopsies not showing morphologic evidence of acute allograft rejection and in one biopsy with CCTT grade 1 change. In 30 of 31 biopsies diagnosed as acute allograft rejection by CCTT criteria as showing changes diagnostic of acute allograft rejection IL2R was 1+ or more. Six of these biopsies categorised as 3+, 11 biopsies were graded as 2+ and the remaining 13 biopsies as 1+. Two of the biopsies without morphologic evidence of acute allograft rejection had 1+ and 2+ expression of IL2R. Four of the 9 biopsies from patients who had not had a renal transplant and had conditions other than acute cellular allograft rejection were positive for IL2R. Three of these were graded as 1+ with less than 10% of the cells showing the positive stain. The fourth patient was 2+ and was a case of acute tubular necrosis. The association of IL2R expression was statistically significantly associated with the morphologic diagnosis of acute allograft rejection (p<0.001, chi square test) (Table 2). All the 8 biopsies classified as Borderline by the Banff 97 criteria showed IL2 expressivity in the activated T lymphocytes.

Discussion

The morphologic confirmation of acute allograft rejection in renal biopsies is critical to the diagnosis and management of early graft failure in the transplanted patients. In this study, thirty one of the forty (77.5%) patients, clinically suspected to have acute allograft rejection with biopsy confirmation, is similar to the findings in other reported series. The presence of an interstitial infiltrate of activated lymphocytes (lymphoblasts), tubulitis and intimal

![Fig. 2. Photomicrograph showing IL2R with scattered activated lymphocytes showing 3+ positivity (APAAP, x450).](image-url)
arteritis were the primary criteria for the diagnosis of acute cellular allograft rejection. Additional diagnoses were present in 5 of the patients with rejection, as well as in 7 of the 9 transplant recipients without rejection.

Acute cellular allograft rejection is a T cell mediated process triggered by the presentation of alloantigens from the donor kidney to the recipient's T cells by antigen presenting cells. Binding of the processed alloantigen to T cell receptors triggers a cascade of intracellular events leading to activation and transcription of the Interleukin 2 gene and the over expression of IL2R on the cells. IL2 and IL2R are central to the immunobiology of acute allograft rejection and this has led to the development of chimaeric and humanised monoclonal antibodies which are designed to target specific receptors which may be used as an induction in immunosuppressive therapy. Attempts have also been made to utilise assays for IL2R in diagnosing rejection. An association between the expression of IL2R and non-vascular rejection in renal biopsies and in renal aspirates has been reported. However the estimation of soluble IL2R in urine or serum was not found to be useful in diagnosis. Our findings show that the demonstration of IL2R on the surface of activated lymphocytes in renal biopsies from patients suspected to have acute allograft rejection may be a useful adjunct for the pathologist faced with this diagnostic challenge and is similar to the findings of Yang and colleagues. Expression of IL2 receptors indicates also the relative proportion of activated lymphocytes in the mononuclear infiltrate and we would like to postulate that this may have a bearing on the effectiveness of Anti IL2 monoclonal antibodies which are being used in the antirejection regime. A prospective study is underway in this center on a larger number of cases with acute allograft rejection who are being treated by monoclonal antibodies against IL2 receptors along with a close followup of the clinical outcome.

The CCTA and the Banff 94 criteria have been evolved by experienced renal pathologists to ensure uniform criteria of diagnosis and comparability between different centers. The CCTA system has been used regularly in this department and has been found to be a simple and straightforward way to classify the changes in biopsies with acute allograft rejection. The Banff 97 method of classification includes all pathology related to early allograft failure and relies on a semiquantitative scoring system to measure interstitial infiltrate, tubulitis and vasculitis. A major difference between the two systems is that, while CCTA requires that a minimum of 5% of the area of the biopsy should be infiltrated by lymphocytes, the latest published Banff 97 criteria defines the minimum area of infiltrate as 15% for the diagnosis of rejection. However at the Banff 97 web page this has now been reduced to infiltration of 10% of the interstitium. Therefore patients without vasculitis, in whom a lymphocytic infiltrate covers 5 to 10% of the interstitium would be classified as borderline patients to be confirmed by clinical criteria and follow up if the Banff 97 criteria are followed, while the CCTA system would classify them as type I acute allograft rejection. The diagnosis would have significant impact on the initiation of aggressive anti rejection regime.

The results presented here show that immunohistochemical demonstration of IL2R on activated lymphocytes in the renal interstitium correlated significantly with the clinical and histological diagnosis of acute cellular rejection by the CCTA criteria. All the patients categorised as Borderline by Banff 97 criteria were IL2R positive. Their clinical course and response to therapy also confirmed this diagnosis. We therefore suggest that the histochemical demonstration of IL2R on activated lymphocytes in the renal interstitium is a useful adjunct in the diagnosis of acute cellular rejection in renal allografts and may be a good indicator of the patient's response to anti-IL2 antibodies being used in the treatment protocols.

Acknowledgement

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References

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CME on Endoscopy biopsy Correlation

18th and 19th August 2006

Venue: Nair Hospital, Auditorium, T N Medical College, Mumbai, INDIA.

Endoscopy is an established procedure for the diagnosis of luminal lesions. A good clinicopathological correlation definitely improves diagnostic ability. This CME will cover all types of endoscopies with histopathology in different fields of medicine including common ones like the GI endoscopy, bronchoscopy, cystoscopy, laparoscopy and other less common scopies related to ENT, joints, brain and breast. It should be of interest to endoscopists and pathologist both trainee and established histopathologist, who evaluate endoscopic biopsies in their practice. The faculty consists of expert clinicians and histopathologists who will address all issues regarding the diagnosis, problems and pitfalls related to endoscopic biopsies.

For further details, contact

Dr Anjali Amarpurkar (or)

Dr Vinaya B Shah
Department of Pathology
BYL Nair Ch Hospital &
T N Medical College,
Mumbai-400008

Contact Nos:-

9820519610 / anjali_1963@hotmail.com
9869058584 / shahvinaya@yahoo.com