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Immunohistological analysis of renal allograft biopsies from cyclosporin-treated patients

Induced HLA-class II antigen expression does not exclude a diagnosis of cyclosporin nephrotoxicity

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Abstract. Differentiation of cyclosporin nephrotoxicity from renal allograft rejection is often difficult. Induction of tubular HLA-class II antigens and elevated levels of leucocyte infiltration are associated with allograft rejection but their association with cyclosporin nephrotoxicity is unclear. In order to determine these relationships, transplant biopsies ($n=32$) from patients considered to have cyclosporin nephrotoxicity, allograft rejection or stable graft function were stained with monoclonal antibodies specific for HLA-class II antigens and infiltrating leucocytes. Leucocyte infiltration was elevated during rejection but not in cyclosporin nephrotoxicity or stable graft function. While HLA-class II antigen expression was induced in 71% of the biopsies obtained during clinical rejection, no increased expression was found in the other 29%. Induced antigens were detected in five of the nine biopsies obtained in the presence of cyclosporin nephrotoxicity 90 days after transplantation. In four of these, induction was attributed to prolongation of increased class II expression following previous rejection episodes. Thus, the presence of induced class II antigens in the renal allograft does not exclude a diagnosis of cyclosporin nephrotoxicity.

Key words: Kidney biopsy - HLA-class II expression, in kidney biopsies - Cyclosporin nephrotoxicity.

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Despite the success of cyclosporin immunosuppression in clinical renal transplantation [6, 9, 26, 27], nephrotoxicity remains a major side effect of therapy [25]. The differentiation of nephrotoxicity from rejection is often difficult as there are no morphological changes on conventional histology that are diagnostic of cyclosporin nephrotoxicity [3].

The various histological abnormalities that have been attributed to cyclosporin nephrotoxicity include glomerular capillary and arteriolar thrombosis [28, 33], tubular microcalcification and giant mitochondria [24], isometric tubular vacuolation [3], arteriolar hyalinosis [24, 36] and focal mononuclear cell infiltration [29, 34]. Immunohistological evaluation of the transplant biopsy has suggested that the absence of MHC class II antigen induction in the presence of allograft dysfunction may be indicative of cyclosporin nephrotoxicity [1].

In this present study we have examined the interrelated immunohistological parameters of leucocyte infiltration and HLA-class II antigen expression. Renal allograft biopsies were obtained from recipients treated with cyclosporin during periods of graft rejection and at 3 months in the presence of cyclosporin nephrotoxicity or stable graft function. HLA-class II antigens and infiltrating cells were identified with monoclonal antibodies and cellular infiltration was quantitated by morphometry.

Patients and methods

Needle-core biopsies ($n=32$) were obtained after transplantation from renal transplant recipients receiving short-term cyclosporin. In this regimen oral cyclosporin was administered at a dosage of

17.5 mg/kg per day for the first 30 days; it was later reduced to 15 mg/kg per day until day 60 and then to 12.5 mg/kg per day until day 90. Following biopsy at day 90, patients were converted to an immunosuppressive regimen of azathioprine and low-dose prednisolone [26]. Cyclosporin levels were determined using a radioimmunoassay (Sandoz).

The clinical function of patients at the time of the day 90 biopsy was assessed. Cyclosporin nephrotoxicity was diagnosed in those patients in whom there was a reduction in serum creatinine greater than 25% of the day 90 level, 2 weeks after cyclosporin withdrawal ($n=9$). In nine other patients the creatinine reduction was less than 25% and they were therefore considered to have stable function. The mean time of biopsy in each group (±SEM) was 91.6±0.8 days and 91.2±1.0 days, respectively.

A third group of biopsies, taken during rejection episodes, was included in the study ($n=14$). Rejection was diagnosed using the clinical criteria of graft tenderness, a decrease in urine output, a sustained rise in serum creatinine, pyrexia (in the absence of infection) and improvement in these features after a treatment with a course of intravenous methylprednisolone (0.5 g daily for 3-5 days). A minimum of three positive criteria were required for the diagnosis of rejection [22]. The mean time of biopsy was 26.1±7.9 days. All biopsy specimens were snap-frozen in liquid nitrogen and stored at −80°C.

Cryostat tissue sections of the biopsies were stained with monoclonal antibodies using an indirect immunoperoxidase technique, as previously described [12]. HLA-class II antigens were detected with NFK1 [11] and HLA-DQ antigens with Tu22 [39] and anti-Leu10 [7]. Infiltrating leucocytes were identified with F10-89-4, an antibody directed against the leucocyte-common antigen [8], and T-lymphocytes with the CD3 antibody, anti-Leu4 [20]. F3-20-7 (anti-dog Thy-1; [21]) was used as a negative control.

Leucocyte infiltration was quantitatively assessed using a morphometric point counting technique [22] and results expressed as the percentage area of the tissue section occupied by infiltrating cells (±SEM). In addition, the distribution of the leucocyte infiltrate was assessed as "focal" or "diffuse".

Table 1. Serum biochemistry in patients with stable function and cyclosporin nephrotoxicity. Statistical comparisons were done using the Mann-Whitney U test

	Stable function	Nephrotoxicity	*P*
Preconversion creatinine (mmol/l±SEM)	146±19	215±23	<0.05
Postconversion creatinine (mmol/l±SEM)	133±16	128±14	NS
Creatinine reduction (mmol/l±SEM)	13± 5	87±14	<0.001
Serum cyclosporin (ng/ml±SEM)	144±18	144±42	NS

Table 2. Distribution of leucocyte infiltration in biopsies from renal allografts from cyclosporin-treated patients

Graft status	Focal infiltration	Diffuse infiltration	Total
Stable function	3	6	9
Cyclosporin nephrotoxicity	3	6	9
Rejection	0	14	14

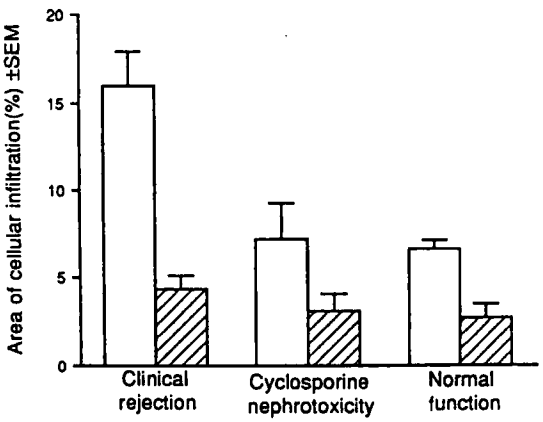

Fig. 1. Leucocyte and T-lymphocyte infiltration in biopsies from cyclosporin-treated patients during clinical rejection ($n=14$), cyclosporin nephrotoxicity ($n=9$), or stable graft function ($n=9$). □, Leucocytes; ▨, T-lymphocytes

The nature and intensity of class II antigen staining was assessed on renal tubules, glomerular endothelium and mesangium, intertubular structures and, where present, large vessel endothelium. Biopsies were graded as having no induction (normal), induction within all the renal tubules (generalised induction) or focal induction of tubular class II antigens [11-13].

Results

The mean serum creatinine in patients with stable graft function at the time of conversion to azathioprine/prednisolone ($n=9$) was 146±19 mmol/l; 2 weeks after conversion it was 133±16 mmol/l (NS; Table 1). In contrast, in patients with cyclosporin nephrotoxicity ($n=9$), the mean serum creatinine fell from a preconversion level of 215±23 mmol/l to 128±14 mmol/l postconversion ($P<0.02$). The mean postconversion serum creatinine levels in patients with stable function and cyclosporin nephrotoxicity were similar. The mean trough serum cyclosporin concentrations in patients with cyclosporin nephrotoxicity and stable graft function were 144±42 ng/ml and 144±18 ng/ml, respectively.

The mean area of leucocyte infiltration in biopsies from grafts with stable function was 6.6%±0.5%, which was similar to the 7.2%±2.0% infiltration found in cyclosporin nephrotoxicity (Fig. 1). However, in biopsies from grafts undergoing rejection, leucocyte infiltration was 15.9%±2.0%, which was significantly greater than that in kidneys with cyclosporin nephrotoxicity ($P<0.01$) or in kidneys with stable graft function ($P<0.01$). This pattern was also found with T-lymphocytes where the level of infiltrate in biopsies taken during rejection (4.4%±0.7%) was greater than those obtained dur-

ing cyclosporin nephrotoxicity (3.1% ± 1.0%; NS) or in periods of stable function (2.7% ± 0.8%; $P < 0.05$).

The distribution of cellular infiltration in each biopsy was similar for total leucocytes and T-lymphocytes. In all biopsies from grafts undergoing rejection, a diffuse pattern of cellular infiltration was observed (Table 2). In biopsies from grafts with stable function and cyclosporin nephrotoxicity, both diffuse and focal infiltration were seen and thus no distinct distribution was associated with cyclosporin nephrotoxicity.

The expression of HLA-class II (HLA-DR and -DQ) antigens found in the 32 transplant biopsies is shown in Table 3. In biopsies with normal expression HLA-DR antigens were expressed on glomerular endothelium and mesangium, intertubular capillaries and interstitial leucocytes. Large vessel endothelium was either weakly positive or negative. Cytoplasmic HLA-DR antigen was detected in the proximal but not distal tubules. In contrast, while HLA-DQ antigens were expressed on the glomeruli and intertubular structures, all of the renal tubules were HLA-DQ-negative. In the biopsies with induced HLA-class II expression, the renal tubules strongly expressed cytoplasmic and membrane HLA-DR and -DQ antigens and, when present, large vessel endothelium was also positive. Focal induction of HLA-DR and -DQ antigens was usually associated with an area of leucocyte infiltration. While HLA-class II antigens were induced in 10 of the 14 biopsies obtained during clinical rejection episodes (7 generalised, 3 focal induction), normal levels of class II antigen expression were found in the remaining 4 biopsies.

Normal class II antigen expression was seen in five of the nine biopsies from patients with stable graft function at the time of conversion. Focal induction was seen in two and generalised induction in the remaining two biopsies (Table 4). In three of these four patients with induced class II expression, induction had been observed in biopsies obtained during the 1st month after transplantation in association with clinical rejection.

Normal class II antigen expression was found in four of the nine biopsies obtained from patients who were considered to be nephrotoxic, but induced antigens were present in the other five [two focal, three generalised induction (Table 5)]. One of the two patients with focally induced class II antigens had induced antigen in a previous biopsy in association with rejection. Early transplant biopsies were unavailable from the other patient, but the focal induction at day 90 preceded generalised induction at day 171. Two of the three patients with generalised induction during nephrotoxicity had induction in earlier biopsies in association with rejection. In the third patient there was no history of overt clinical rejection, but no earlier biopsy material was available.

Table 3. HLA-Class II antigen expression in transplant biopsies

Graft status	No increase	Focal increase	Generalised increase
Rejection ($n = 14$)	4	3	7
Cyclosporin nephrotoxicity ($n = 9$)	4	2	3
Stable function ($n = 9$)	5	2	2

Biopsies from patients with normal function and cyclosporin nephrotoxicity were taken at day 90, immediately before conversion from cyclosporin to azathioprine and prednisolone immunosuppression. The mean day of biopsies taken during clinical rejection (± SEM) was 26.1 ± 7.9 days after transplantation

Table 4. Patients with stable graft function at day 90

HLA-class II expression	Day 90 biopsies *(n)*	Clinical history	Previous biopsies – HLA expression *(n)*		
			Induction	No induction	No data
Normal	5	4 Previous rejection	0	2	2
		1 No previous rejection	1	0	0
Focal induction	2	1 Previous rejection	1	0	0
		1 No previous rejection	0	1	0
Generalised induction	2	2 Previous rejection	2	0	0

Table 5. Patients with cyclosporin nephrotoxicity at day 90

HLA-class II expression	Day 90 biopsies *(n)*	Clinical history	Previous biopsies – HLA expression *(n)*		
			Induction	No induction	No data
Normal	4	3 Previous rejection	2	1	0
		1 No previous rejection	0	1	0
Focal induction	2	2 Previous rejection	1	0	1
Generalised induction	3	2 Previous rejection	2	0	0
		1 No previous rejection	0	0	1

Discussion

The major difficulty in the management of renal transplant recipients immunosuppressed with cyclosporin is the differentiation of cyclosporin nephrotoxicity from other causes of allograft dysfunction. Unfortunately, the histopathological

changes attributed to cyclosporin nephrotoxicity are not specific to the condition [3], and the identification of nephrotoxicity therefore often becomes a diagnosis by exclusion. Other techniques that have been employed to diagnose cyclosporin nephrotoxicity include cyclosporin blood levels [35, 36], intrarenal hydrostatic pressures [14, 32] and immunohistology of graft aspirates and transplant biopsies using monoclonal antibodies directed against the cyclosporin molecule [19, 37]. However, none of these has proved to be universally successful.

In this present study we have investigated the role of immunohistology in the diagnosis of renal allograft dysfunction. An association between HLA-class II antigen induction and renal allograft rejection was first described by Hall et al. [15] and later confirmed by others [16, 17]. In a previous study of biopsies from patients treated with azathioprine/prednisolone and cyclosporin, we demonstrated that most biopsies taken during rejection expressed induced tubular class II antigens. There was, however, a small, but significant, number of biopsies from rejecting grafts with normal class II antigen expression [12, 23]. Thus, the correlation between rejection and induction of class II antigens is not absolute. This could be the result of early administration of methylprednisolone pulse therapy during incipient rejection or, alternatively, it may reflect the potent immunosuppressive qualities of cyclosporin.

There is a significant association between induced class II antigens and leucocyte infiltration [12]. It is probable that T-lymphocyte-derived lymphokine interferon-gamma, a potent inducer of class II antigen expression, may stimulate local induction of class II antigens within the graft. Cyclosporin inhibits the production of interferon-gamma from activated cells [31] and thus may provide an alternative explanation for normal levels of class II antigens in some biopsies obtained during rejection. Class II induction was also noted in biopsies from grafts with good function. In vitro interferon-gamma causes induction of class II expression on renal tubules that disappears within days of removal of interferon-gamma [4]. In the clinical situation, the kinetics of decay are difficult to study, but class II antigens, once induced, may persist for several weeks [2, 12]. It is therefore not surprising that induced class II antigens can be detected during periods of stable graft function. Class II antigen induction may reflect previous rejection or viral infection [38] and may even precede later graft rejection.

The absence of induced HLA-class II antigen expression in allograft biopsies obtained during cyclosporin nephrotoxicity has been reported [1, 5]. Thus, it is possible that class II antigen expression may be used in the differentiation of cyclosporin nephrotoxicity from rejection. However, in this present study, in biopsies from grafts considered to be exhibiting nephrotoxicity as judged by a substantial improvement in renal function on withdrawal of cyclosporin, class II antigens were induced in five of nine biopsies examined (56%), and in four of these the induction was associated with previous rejection episodes. Similarly, in biopsies from patients with stable graft function, increased levels of class II antigens were found in four out of nine (44%) biopsies, three of which were associated with previous allograft rejection. Although the nephrotoxicity defined in this study does not truly reflect the acute nephrotoxicity that provides the diagnostic dilemma, usually seen between 2 and 6 weeks after transplantation [25], there is no question that at 90 days there was significant evidence of cyclosporin nephrotoxicity. It is possible that at an earlier stage in the course of the graft, in the absence of previous rejection episodes, class II induction may not have been noted in the presence of nephrotoxicity. However, the data also show that in cyclosporin-treated patients, HLA-class II antigens were not invariably induced during clinical rejection; 71% of the biopsies showed increased levels of class II antigen expression, but in the other 29% no evidence of induction was found. Thus, the data reported in this study show that the presence of induced class II antigens in the renal allograft does not exclude a diagnosis of nephrotoxicity. Nor does the absence of induced class II antigens exclude a diagnosis of rejection.

The similar levels of infiltrating mononuclear cells in grafts with stable function and chronic cyclosporin nephrotoxicity indicate that cyclosporin-related damage does not contribute to the density of the interstitial infiltrate. Indeed, Platt et al. [30] and Farnsworth et al. [10] found sparse infiltration in cyclosporin nephrotoxicity. Thus, the significantly greater levels of cellular infiltration seen during rejection may permit the differentiation of nephrotoxicity from rejection in the patient with clinical dysfunction.

Other investigators have equated focal mononuclear cell infiltration with both stable graft function and cyclosporin nephrotoxicity and diffuse infiltration with rejection [34, 36]. However, a diffuse pattern of cellular infiltration may also occur in well-functioning grafts [22]. Although diffuse infiltration was commonly found in rejection in this present study, both diffuse and focal patterns of infiltration were identified in stable function and in cyclosporin nephrotoxicity.

The value of serum cyclosporin levels in the diagnosis of chronic cyclosporin nephrotoxicity is uncertain. The mean cyclosporin level in patients with cyclosporin nephrotoxicity was not markedly greater than in patients with stable renal function. Although nephrotoxicity is more common with high cyclosporin levels [18], the occurrence of nephrotoxicity with therapeutic cyclosporin levels is well recognised [26].

This study has assessed the closely related immunohistological parameters of tubular HLA-class II induction and leucocyte infiltration in the diagnosis of cyclosporin nephrotoxicity. While evaluation of class II antigen expression may theoretically distinguish nephrotoxicity from rejection, in practice this is not possible as the level of class II antigen expression is dependent on previous intragraft events. Thus, HLA-class II antigen expression may only be of value in the first episode of graft dysfunction or when the previous biopsy history is available.

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