Original Article

HLA-DR class II and ICAM-1 expression on tubular cells taken by fine-needle aspiration biopsy in renal allograft dysfunction

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Abstract

Background. Percutaneous biopsy is the method of choice for differential diagnosis of renal allograft dysfunction, although it is not risk-free. The use of less aggressive methods for diagnosis should limit the need for percutaneous biopsy to some specific situations.

Methods. We analysed 42 fine-needle aspiration biopsies from 36 kidney allograft recipients immunosuppressed with quadruple sequential therapy who suffered renal allograft dysfunction. Seven cases with stable renal function were used as controls and included as non-rejection cases in the analysis. In all aspirates the Corrected Increment was calculated and an immunocytochemical analysis of renal tubular cells with the monoclonal antibodies HLA-DR and ICAM-1 was performed.

Results. The Corrected Increment was increased in 13 out of 18 acute rejection cases and in one out of 31 non-rejection cases. HLA-DR expression was found in more than 30% of tubular cells from the aspirates in 16 out of 18 acute rejection cases and in eight out of 31 cases without acute rejection (P<0.001). ICAM-1 expression was detected in more than 30% of tubular cells in 14 out of 18 cases with acute rejection, and in four out of 31 cases without acute rejection (P<0.001). Interestingly, all acute vascular rejection cases (n=6), and six out of 12 acute cellular rejection cases expressed both, HLA-DR and ICAM-1, in more than 30% of tubular cells. On the other hand, none of the non-rejection allograft dysfunctions or control samples showed more than 30% of tubular cells immunostained with both HLA-DR and ICAM-1 antibodies.

Conclusions. The immunocytochemical analysis of HLA-DR and ICAM-1 on renal tubular cells taken by fine-needle aspiration biopsy, allows the diagnosis of acute cellular rejection and acute vascular rejection even when the Corrected Increment is not increased. Moreover, the risk of a core renal biopsy can be avoided when both tests are negative since an acute rejection is a remote possibility.

Key words: fine-needle aspiration biopsy; immunocytochemical analysis; renal allograft dysfunction; renal transplantation.

Introduction

Fine-needle aspiration biopsy (FNAB) allows morphological and immunocytochemical analysis of both parenchymal and inflammatory cells in the transplanted organ without any risk to the kidney graft [1-3]. In the morphological analysis, an increase in lymphocytes and monocytes into the graft with the presence of blast cells has a high sensitivity and specificity for the diagnosis of acute cellular rejection. However, this method is of little value in the diagnosis of acute vascular rejection or chronic rejection [1,4]. In our experience, immunocytochemical analysis of both inflammatory and tubular cells allows the diagnosis of acute rejection with a high sensitivity and specificity [2,5].

De novo expression of HLA-DR and ICAM-1 by renal tubular cells has been demonstrated in core biopsies of the rejecting kidney allograft [6-9]. The expression of these molecules during rejection is presumed to be secondary to the presence of cytokines in the microenvironment of the allograft. It has been demonstrated that IL-1, IFN-gamma, and TNF stimulate the expression of HLA class II molecules and ICAM-1 on various cell types [10-11]. The induced ICAM-1, together with HLA class II antigens, may render the graft more susceptible to damage mediated by cytotoxic T lymphocytes. The aim of the present study was to analyse the value of the expression of both molecules on tubular cells taken by FNAB in the differential diagnosis of renal allograft dysfunction, particularly in that of acute vascular rejection.

Materials and methods

Patients

The study group included 36 recipients of cadaveric renal allograft (34 first transplant, and two second transplant)
seven of whom had more than 75% of panel reactivity antibodies. They were immunosuppressed with quadruple sequential therapy (ALG/CsA + Pred + Aza) as previously reported [2]. All suffered an acute decrease in allograft function between 6 and 186 days after transplantation. According to our customary protocol they were subjected to FNAB and core renal biopsy as soon as graft dysfunction was detected, or when dialysis was needed for longer than one week after transplantation, and always before an anti-rejection therapy was instituted. Forty-two consecutive and non-selected FNAB performed to these patients were included in the present study. Seven first transplant patients with stable allograft function at the time of discharge from the hospital were used as control group (<1 month after transplantation) (C). Rejection episodes were treated with pulses of methyl-prednisolone, and either ALG or orthoclone OKT3, for steroid-resistant cases.

Final diagnosis was reached in each case by FNAB independent means. Acute or chronic rejection were diagnosed according to histological parameters by core renal biopsy [12]. Acute cellular rejection (ACR) (n=12) showed a significant interstitial infiltration with tubulitis. Acute vascular rejection (AVR) (n=6) showed severe intimal or transmural arteritis with occasional fibrinoid changes or interstitial haemorrhages, and they all were classified as >IIb using the Banff criteria [12]. All AVR and five ACR episodes were diagnosed in the first two weeks after transplantation. Chronic rejection (CR) (n=2) showed the characteristic interstitial fibrosis with fibrous intimal thickening of the arterial wall. The diagnosis of acute tubular necrosis (ATN) (n=4) was established if hemodialysis was required after transplantation followed by the spontaneous recovery of renal function without additional immunosuppression. An increased plasma creatinine that became normal after CsA dose reduction was considered to be CsA nephrotoxicity (CsAT) (n=14). Cytomegalovirus infection (CMV) (n=3) was diagnosed by clinical, biochemical and serological parameters. One case of allograft dysfunction was due to urinary obstruction (UO) as ascertained by anterograde pyelography.

Two allograft dysfunction cases were not subjected to core renal biopsy: one CsA toxicity case due to high levels of Cs A in the course of flucanazole therapy, and another patient with obstructive uropathy ascertained ultrasonography. In both cases, renal function improved after appropriate measures were performed with no increase in immunosuppression. The seven patients with normal renal function were not subject to core renal biopsy for ethical reasons and none of them showed a graft dysfunction within 4 weeks following the FNAB. Histological diagnosis was established using the Banff criteria in the remaining cases [12].

**Fine-needle aspiration biopsy**

FNAB was performed according to previously published protocols [1-3]. Briefly, a 25G needle was used to aspirate about 50 μl samples of the allograft into 3 ml of cell culture media. A similar amount of blood was obtained from the fingertip. In order to assure that the sample was representative of the allograft infiltrate [1], only those FNABs that showed seven or more tubular cells per hundred inflammatory cells were included in the present study. Cytospins were obtained from FNABs and blood samples, and processed for both May-Grunwald-Giemsa staining and immunocytochemistry.

**Morphological analysis**

The technique described by Hayry and von Willebrand [1,13,14] was employed to process and evaluate FNABs. The corrected increment (CI) was calculated from the slides stained with May-Grunwald-Giemsa. Briefly, a white-cell differential count is performed on both the FNAB specimen and the blood specimen, and the blood values are subtracted from the FNAB values. The numerical differences for each cell type is multiplied by a correction factor and the sum of the different corrected increment values describes the intensity of inflammation. A CI greater than 3 was considered as suggestive of acute rejection [1,13,14].

**Immunocytochemical analysis**

Cytospins were fixed with 4% paraformaldehyde for 5 min. After washing with phosphate buffered saline (PBS), aliquots of cells were incubated overnight with a 1:50 dilution of the monoclonal antibodies against the non-polymorphic domain of HLA-DR or the ICAM-1 antigen (Dako; Denmark). After a 15 min wash with PBS, cytospins were incubated with rabbit anti-mouse IgG serum for one hour. Next, slides were washed with PBS for 15 min and incubated with alkaline phosphatase-anti alkaline phosphatase following instructions provided by the manufacturer (Dako; Denmark). After washing with tris buffered saline, cells were incubated with Napthol-Red chromogen, dehydrated and mounted.

**Scoring of positive cells**

FNABs with more than 30% of tubular cells immunostained with the monoclonal antibodies (HLA-DR or ICAM-1) were considered positive (Figure 1). This was considered as the cut-off value, according to the findings of Bishop et al. for the HLA-DR expression on tubular cells [6], and because it represents the mean±2 SD of our control group values (13±8 and 11±9 for the HLA-DR and ICAM-1, respectively).

Statistical methods included t-test and Fisher's exact probability test. A P value <0.05 was considered significant.

**Results**

Forty-nine FNABs obtained from 44 patients were divided into eight final diagnosis groups. Eighteen samples corresponded to acute rejection (AR), twenty-four to other causes of allograft dysfunction, while seven samples constituted the control group with stable renal function (Table 1).

Acute rejection cases showed CI values significantly higher than those with non-rejection allograft dysfunction and controls taken together (3±1 vs 1.4±0.8; P<0.001). A total of five patients with acute rejection (3 AVR and 2 ACR) gave CI values below 3, while only one patient without acute rejection (1 case of ATN) showed a CI above 3 (P<0.000007). Thus, the CI test yields a 72% sensitivity and 97% specificity for the diagnosis of acute rejection. Although ACR cases showed CI values higher than those of AVR (3.2±0.8 vs 2.6±1.6) these differences were not significant.

HLA-DR expression on tubular cells was variable. While 10 out of 12 ACR cases and all AVR cases (n=
Fig. 1. (A) ICAM-1 immunostaining on tubular cells from a representative FNAB in a patient undergoing acute allograft rejection. (B) A large tubular cells expressing the HLA-DR in a patient with acute tubular necrosis (anti-HLA-DR alkaline phosphatase-antialkaline phosphatase procedure. Haematoxilin counterstaining) (\(\times 100\)).

6) showed a positive immunostaining, it was also positive in all CR (\(n=2\)) and CMV (\(n=3\)) cases. On the other hand, only two out of 14 CsAT cases, and one out of seven control cases showed a positive staining (Table 1). Taken together, HLA-DR immunostaining was positive in 16 out of 18 cases with acute rejection, and in only nine out of 31 cases without acute rejection (\(P<0.00005\)). Thus, HLA-DR immunostaining has a 89% sensitivity and 71% specificity for the diagnosis of acute rejection.

ICAM-1 expression on allograft tubular cells was less common than HLA-DR expression. More than 30% tubular cells showed ICAM-1 immunostaining in all six AVR cases and in eight out of 12 ACR cases. On the other hand, only five cases without acute rejection (2 ATN, 2 CsAT and 1 control) showed ICAM-1 expression (\(P<0.00002\)) (Table 1). Thus, ICAM-1 immunostaining yields a 78% sensitivity and 84% specificity for the diagnosis of acute rejection.

When both HLA-DR and ICAM-1 expression are considered together, four possible patterns of immunostaining can be observed (Figure 2). All AVR cases and 50% of ACR showed both HLA-DR and ICAM-1 immunostaining. The other 50% of ACR cases were positive for either HLA-DR (\(n=4\)) or ICAM-1 (\(n=\)

Table 1. Corrected increment (CI), HLA-DR and ICAM-1 expression on allografts tubular cells from the different final diagnosis groups

<table>
<thead>
<tr>
<th>CI &gt;3</th>
<th>ACR</th>
<th>AVR</th>
<th>CR</th>
<th>ATN</th>
<th>CsAT</th>
<th>CMV</th>
<th>UO</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DR (+)</td>
<td>10 (83%)</td>
<td>3 (50%)</td>
<td>0 (0%)</td>
<td>1 (25%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ICAM-1 (+)</td>
<td>8 (67%)</td>
<td>6 (100%)</td>
<td>0 (0%)</td>
<td>2 (25%)</td>
<td>2 (14%)</td>
<td>1 (100%)</td>
<td>0 (0%)</td>
<td>1 (14%)</td>
</tr>
</tbody>
</table>

ACR = acute cellular rejection; AVR = acute vascular rejection; CR = chronic rejection; ATN = acute tubular necrosis; CsAT = CsA nephrotoxicity; CMV = CMV infection; UO = urologic obstructions; C = controls.
2). Conversely, none of the non-rejection allograft dysfunctions or control samples showed both HLA-DR and ICAM-1 immunostaining. Interestingly, all AR cases with the corrected increment below 3 (3 AVR and 2 ACR), were positive for both, HLA-DR and ICAM-1 immunostaining. Taken together, the finding of both, HLA-DR and ICAM-1 positive immunostaining on allograft tubular cells is highly suggestive of an acute rejection (specificity and positive predictive value 100%).

Discussion

The immunocytochemical analysis of renal tubular cells obtained by FNABs was helpful in the differential diagnosis between acute rejection and non-rejection causes of renal allograft dysfunction in this study. The morphological analysis of the allograft infiltrate yields the CI score, which is highly sensitive and specific for the diagnosis of acute rejection [1,3,4]. However, in some situations such as acute vascular rejection, the CI score is not considered reliable [1,4]. In our study, 17% of ACR cases, mostly late rejections, and 50% of AVR did not show diagnostic CI values.

HLA-DR and ICAM-1 expression was abundant (over 30% of tubular cells immunostained) in 89% and 78% of acute rejection FNABs, respectively, while the corresponding figures in non-rejecting cases were only 29% and 16%. Several immunocytochemical studies of the HLA-DR and ICAM-1 expression in renal tubular cells from allograft core biopsies have been recently published [7–9, 15–17]. These studies demonstrated that the expression of HLA-DR and ICAM-1 are induced on renal tubular cells after transplantation, particularly during acute rejection episodes. ICAM-1 expression in normal kidneys has been detected in the entire vascular endotelium as well as in the glomerular mesangium [8,9,10]. On renal tubular cells, ICAM-1 expression has been reported to be intense, mainly along the proximal tubule [8,9], but others have found a very weak [17] or no expression in normal or non-rejecting kidneys [15,16]. These differences may be explained by technical reasons, ischemic damage or perfusion injury to the allograft. As a matter of fact, abundant ICAM-1 expression along the proximal tubule has been found in biopsies taken either before implantation or a few minutes after revascularization [8,9]. However, in this study we evaluated tubular cells present in FNABs performed several days after transplantation. Although ICAM-1 expression varied among different FNAB samples, proximal tubule cells showed the strongest immunostaining (Figure 1).

ICAM-1 expression has also been studied in urine cytologies [18], where 24 ±4% of renal tubular cells are ICAM-1 positive in ATN patients, and 53 ±4% in patients with acute rejection. These authors showed that ICAM-1 immunostaining of over 35% renal tubular cells is a sensitive and specific test for the diagnosis of acute rejection. More recently, von Willebrand et al. have reported that ICAM-1 and HLA-DR expression was present in 3 ±4% and 4 ±3%, respectively, of tubular cells from FNABs of patients without allograft rejection, while ICAM-1 and HLA-DR immunostaining was detected in 21 ±7% and 50 ±10%, respectively, in acute rejection samples [19]. These authors concluded that ICAM-1 is induced early during acute rejection on renal tubular cells, and the induction of class II antigens is slightly slower but quantitatively stronger. In the present study, we reach a similar conclusion, that is, HLA-DR and ICAM-1 antigens are highly expressed during acute allograft rejection. The percentage of ICAM-1 immunostained tubular cells found in our study is closer to that reported by Chan et al. [18] in urinary cytologies than in that reported by von Willebrand et al. [19] in FNAB samples. These differences are more likely due to technical reasons, and further studies are needed in order to investigate the optimal cut-off value for ICAM-1 immunostaining on renal tubular cells taken by FNAB.

We herein report that the combined immunocytochemical analysis of HLA-DR and ICAM-1 in FNABs allowed the diagnosis of acute rejection in cases that would have been false negative by conventional cytoligic analysis. All acute vascular rejection and half of acute cellular rejection episodes expressed both HLA-DR and ICAM-1 in over 30% of tubular cells. As a consequence of the substantial number of false positives, HLA-DR expression by itself is a poor diagnostic marker. However, when combined with ICAM-1 expression, which is less sensitive but more specific for acute rejection, the immunocytochemical test yields a high diagnostic value. Our study suggests that, when both HLA-DR and ICAM-1 are present in over 30% of tubular cells, a more severe form of allograft rejection might be underway which should be confirmed by core biopsy and treated with more aggressive measures than the sole use of methylprednisolone boluses. When both HLA-DR and ICAM-1 are negative acute rejection can reasonably be ruled out as the cause of allograft dysfunction, and a core biopsy, which is not risk-free, can be avoided.

Acknowledgements. This work was supported in part by a Grant 112/08.03.90 P.I from Gobierno Autónomo de Canarias.

References


Received for publication: 22.11.94
Accepted in revised form: 17.7.95