Expression of inducible lymphocyte costimulatory molecules in human renal allograft

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Abstract

Background. CTLA–4/CD28–B7 and CD40–CD40L interactions constitute two key costimulatory pathways in lymphocyte signalling during experimental allograft rejection. Studies on the expression of these molecules in human transplant rejection are still lacking.

Methods. The immunohistochemical study was performed on renal biopsies obtained for various clinical complications from 25 renal transplant patients. Expression of B7–1 and B7–2 and their counter-receptor CTLA–4, and of CD40 and its counter-receptor CD40L was examined.

Results. In acute rejection a focal intense infiltration of B7–1⁺ and B7–2⁺ cells (mainly CD20⁻ CD14⁺) and of CTLA–4⁺ T lymphocytes (mainly CD8⁺) was present. In contrast, CD40 and CD40L were rarely expressed. Accumulations of T lymphocytes were found in the interstitium in the same area containing B7–1⁺ and B7–2⁺ cells. The scattered CD40L⁺ cells found in the T-cell infiltrate exhibited the CD4⁺ phenotype.

In chronic rejection only a few B7–1⁺, B7–2⁺ or CTLA–4⁺ cells were detectable. In contrast, several CD40L⁺ CD4⁺ cells were present both in the interstitium and in glomeruli. Moreover, an intense expression of CD40 on the endothelium was observed. In patients with cyclosporin nephrotoxicity cells positive for B7–1, B7–2, CTLA–4, CD40, or CD40L were absent.

Conclusions. These results demonstrate a differential expression of costimulatory molecules in renal biopsies of allograft recipients undergoing acute or chronic rejection. Moreover, their detection may prove useful to discriminate rejection from cyclosporin nephrotoxicity.
The aim of this study was to evaluate the expression of the inducible B7–1, B7–2, CTLA–4 and CD40L in the biopsy tissue of patients receiving renal transplantation.

Subjects and methods

Patients

Twenty-five recipients of cadaver kidney transplants (16 males, 9 female, mean age 43±9.8 years) entered this study. The underlying renal disease was chronic glomerulonephritis in three, polycystic kidney disease in two, Berger’s disease in one, Schönlein–Henoch nephropathy in one, nephroangiосclerosis in one, nephritis in one, membranoproliferative glomerulonephritis in three, glomerulonephritis in two, chronic pyelonephritis in two, post-streptococcal acute glomerulonephritis in one, and undefined in seven. Immunosuppression was maintained in 11 patients with steroids and azathioprine and in 14 patients with a protocol including also cyclosporin A (CsA).

A retrospective study of costimulatory molecules was performed on selected patients with biopsy-proven graft complications. Patients were categorized on the basis of Banff classification of kidney transplant pathology [11] into three groups as follows: (1) recipients with acute rejection (14 patients, 9 Banff grade II, 5 Banff grade III); (2) recipients with chronic allograft nephropathy consistent with chronic rejection (7 patients); (3) recipients with CsA nephrotoxicity (4 patients). The latter was defined as decreased renal function or increased serum levels of creatinine followed by a favourable response to CsA dosage reduction in the presence of a suggestive percutaneous renal biopsy, which excluded acute rejection, chronic allograft nephropathy, or acute tubular necrosis [11].

Reagents and immunofluorescence microscopy

Fluorescein-(FITC)-conjugated mouse anti-human CD4, CD8, CD20, CD14 were from Sigma Chemical Co. (St. Louis, MO). Monoclonal antibody anti-HLA class II (DP+DQ+DR) antigen was from Novocastra (Newcastle, UK). Goat anti-human CTLA–4, B7–1, B7–2, and rabbit anti-human CD40L and CD40 (Santa Cruz Biotechnology, Santa Cruz, CA) reacted to an epitope mapping at the carboxyl terminus and corresponding to amino acids 205–223 (CTLA–4), 269–288 (B7–1), 302–320 (B7–2), 239–258 (CD40L) and 258–277 (CD40). Preincubation of polyclonal antibodies with corresponding peptide antigen resulted in absence of staining in tissue sections expressing the receptors. Control FITC-conjugated isotype-matched monoclonal antibodies were from Pharmingen (San Diego, CA) and purified goat and rabbit IgG were from Cappel Laboratories (Downington, PA). FITC- and PE-conjugated goat anti-rabbit IgG and rabbit anti-goat IgG were purchased from Sigma. These secondary antibodies were absorbed with human IgG and its specificity was confirmed by absence of glomerular staining for IgG of a kidney biopsy from a patient with membranous glomerulonephritis.

Cryostat-cut 5-μm tissue sections were mounted onto slides and stained with the primary antibody (2 μg/ml) for 1 h at RT, washed with PBS and then incubated with FITC-conjugated second-step antibody. After washing several times in PBS, the sections, mounted in a solution of PBS-glycerol 1:1 containing 1% DMSO as anti-fading agent, were examined by Zeiss microscope (Oberkochen, Germany) equipped with epifluorescence optics and appropriate filters. Fifteen to twenty randomly selected microscopic fields of three non-consecutive sections were examined and scored at ×400 magnification (Plan-Neofluor 40/0.9 glycerol immersion objective, Zeiss) by two independent observers. Double-staining was performed by incubating sections with a directly PE-conjugated monoclonal antibody anti-CD4, CD8, CD20, or CD14, as third step.

The intensity of staining was graded on a scale from 0 to +4 (0, absent; +1, weak; +2, moderate; +3, marked; +4, bright).

Results

Ten control human renal tissues obtained from kidney nephrectomized for polar renal carcinoma or trauma, did not express any of the costimulatory molecules involved in this study, with the exception of CD40 which was weakly expressed on glomerular and peritubular capillary endothelial cells. In contrast, several cells positive for CTLA–4 with a typical intracytoplasmic pattern (Figure 1A), and for B7–1 and B7–2 were detectable in renal biopsies of patients with acute rejection (Figure 1C and D). In this condition, B7–1 was expressed at a low to moderate intensity (+1/+2) on cells infiltrating the tubular interstitial space. In contrast, absence of staining was observed in the glomerular tufts. Double staining immunofluorescence indicated that B7–1− cells were also CD14+ and CD20+, suggesting that the expression of B7–1 was mainly confined to monocyte/macrophages or dendritic cells rather than to B cells. Double staining for B7–1 or B7–2 and CD8 or CD4 was never observed. Moreover, no staining of renal resident cells was evident. B7–2 expression followed the same pattern of B7–1 (Figure 1D) and was confined mainly to CD4+ cells. Analysis of serial sections suggests that the number of B7–2+ cells was higher than B7–1+ cells (Figure 2A). However, cells expressing B7–2 (+3) frequently coexpressed also B7–1, but with a lower intensity (+1/+2). MHC class-II antigens were expressed not only on cells infiltrating the interstitium but also on vascular endothelial and tubular cells (Figure 1E). Likewise, CTLA–4 was detectable on the lymphocyte infiltrate in the tubular interstitial compartment (Figure 1A). The lymphocyte infiltrate contained both CD4+ and CD8+ lymphocytes (Figure 2A). However, CD8+ lymphocytes (Figure 1B) accounted for most of the CTLA–4+ cells (73–80% of CTLA–4+ cells double stained for CD8). Among the CD4+ cells only a few (18–20%) were found to express CD40L with a low intensity (+1) (Figure 1F).

In contrast in chronic rejection (Figure 2B) only a few CTLA–4+ (Figure 3A), or B7–1+, B7–2+ cells (Figure 3B) were detectable, whereas several CD40L+ cells were present both in the interstitium around vessels (Figure 3C) and in glomeruli (Figure 3D). The CD40L expression was particularly bright (+3). Of CD40L+ cells, 75–80% were CD4+; whereas the remaining cells were CD8+. In chronic rejection an
intense and diffuse staining for CD40 was observed on peritubular capillaries and on glomerular endothelium, whereas CD40 was only minimally and focally expressed on glomerular and peritubular capillary endothelial cells in acute rejection (Figure 4).

In patients with CsA nephrotoxicity cells positive for B7–1, B7–2, CTLA–4, CD40L or CD40 were virtually absent (Figure 2C).

Discussion

The present study demonstrates a differential expression of costimulatory molecules in renal biopsies of allograft recipients undergoing acute or chronic rejection. In acute rejection a focal intense infiltration of B7–1 " and B7–2 " cells and of CTLA–4 " T lymphocytes was present. In contrast, CD40 and CD40L were infrequently expressed. In chronic rejection only few B7–1 " , B7–2 " or CTLA–4 " cells were detectable, whereas several CD40L"CD4 " cells were present both in the interstitium and in glomeruli. Moreover, intense expression of CD40 on the endothelium was observed.

In patients with cyclosporin nephrotoxicity, cells positive for B7–1, B7–2, CTLA–4, CD40 or CD40L were absent.

Several experimental studies identified the interaction between CD28/CTLA–4 on T cells with their
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ligands B7–1 and B7–2 on APC as the primary costimulatory pathway involved in specific T-cell activation during allograft and xenograft rejection [2–4]. Interestingly, CTLA–4 is neo-expressed mainly on activated CD8+ [1] and its role seems to counteract T-cell activation by competing 10 time more efficiently with CD28 for the binding to B7–1 and B7–2 [1]. It has been suggested that CTLA–4 transduces a negative signal involved in the downregulation of immune response initiated by the CD28 engagement [1]. Its privileged binding affinity to B7s in respect to CD28 was exploited by researchers for developing recombinant reagents able to therapeutically interfere in this pathway. Indeed, a soluble CTLA–4Ig fusion protein composed of the extracellular portion of CTLA–4 and an immunoglobulin IgG constant region was shown to be a potent immunosuppressant [4]. Several reports indicate that CTLA–4Ig prolongs cardiac [12], skin [13], renal [14], and pancreatic islet allograft survival [2] and prevent xenorejection of human islet cells in mice, with induction of donor-specific tolerance [2]. This therapeutic approach was also beneficial in various experimental models of renal immunological damage, such as HgCl2-induced autoimmune disease [15], and anti-GBM glomerulonephritis [16].

The receptor–ligand pair composed of the B-cell-associated receptor CD40 and its T-cell ligand CD40L has been shown to play a key role in the regulation of T-cell-dependent antibody production [17]. The physiological importance of the CD40–CD40L interaction was discovered through the elucidation of the underlying defect in a severe form of human immunodeficiency known as the hyper-IgM syndrome [10]. Beside its role in T–B-cell interaction, it now appears evident that this receptor pair is deeply involved in the basic mechanisms of T-cell activation and cell-mediated immune response. Experimental studies on allograft rejection recently showed that anti-CD40L therapy induced prolonged survival of murine cardiac allograft in both naive and sensitized hosts [18]. In addition, in combination with B7 blockade or with pretreatment with donor APC, CD40L blockade seems to evoke immunological tolerance to skin and cardiac allograft [13], and islet allograft [19] respectively. Combined blockade of the CD40–CD40L and CD28–CTLA4–B7–1/B7–2 pathways is also protective versus chronic vascular rejection of primarily vascularized allograft [13].

In the present study we provide evidence for the local expression of inducible costimulatory molecules such as CD8+ and CD4+ in acute rejection. The results obtained by double staining immunofluorescence indicated that CD8+ T-cells were observed in the areas of B7–1+/B7–2+ cell infiltration, suggesting a cell-to-cell interaction through the CD28/CD8+ lymphocytes which are known to play an effector role in acute rejection [20,21]. These results are consistent with the demonstration that CTLA–4 gene expression is increased in acute rejection [22]. It is well established that CD4+ T lymphocytes initiate acute rejection [23,24]. Detection of CD4+ CD40L+ cells may reflect specific activation of T-helper lymphocytes. However, the results of the present studies...
Fig. 3. Representative indirect immunofluorescence staining of costimulatory molecules in chronic rejection. Detection of CTLA–4 (A, ×250), B7–1 (B, ×250), and CD40L in the interstitium surrounding small vessels (C, ×250) and in a glomerulus (D, ×400).

Fig. 4. Micrographs representative of indirect immunofluorescence staining of CD40 in acute rejection (A, ×400), chronic rejection (B, ×250; C, ×400), and cyclosporine nephrotoxicity (D, ×250). A, slight staining for CD40 was observed on the endothelium of glomerular (G) capillary loops and focally in the interstitium (head-arrow) of an acute rejection. B and C, intense and diffuse staining for CD40 in a glomerulus (G) and in peritubular capillaries in a patient with chronic rejection. D, negative staining of renal biopsy from a patient with CsA nephrotoxicity.
indicate that only a few CD4+ cells express costimulatory molecules. This is consistent with the contention that only a few committed CD4+ lymphocytes may initiate and orchestrate the acute rejection by recruiting non-antigen-specific CD4+ and CD8+ lymphocytes [23]. Moreover, no expression of costimulatory molecules by renal resident cells was observed. Renal tubular epithelial are unlikely to initiate direct activation of infiltrating allospecific lymphocytes, despite their inducible ability to express potentially immunogenic levels of class II MHC antigens [25]. In vitro experiments indicated that this failure was due to lack of B7–1 or B7–2 expression [25]. Our demonstration of a local expression of B7–1 and B7–2 by infiltrating macrophages, in concomitance with tubular expression of class-II MHC antigens, suggests that they may provide the costimulatory signal to alloreactive T lymphocytes, whereas resident tubular, endothelial cells or fibroblasts may provide the cognate allospecific one.

In contrast to acute rejection, chronic rejection is characterized by the abundance of CD40L+ bright, CD4+ cells. Such population is detected not only in the interstitium and around vessels but also within glomeruli. Conversely, only a few CTLA–4+, B7–1+, B7–2+ cells were detected. This seems to be distinctive with respect to the pattern of distribution of costimulatory molecules observed in acute rejection. A role for CD40L in the pathogenesis of chronic rejection is suggested by several lines of evidence. (1) The importance of this pathway in antibody immune response, which is indeed involved in chronic rejection. (2) The observation that CD40 is also expressed on endothelium and its interaction with CD40L induces endothelial cell activation [8]. This event may potentially be involved in the genesis of the chronic vascular rejection. (3) In experimental allograft, anti-CD40L therapy helps preventing the chronic vascular rejection of primarily vascularized cardiac allograft [13]. In agreement with the experimental evidence on the role of CD40 in chronic rejection, we observed an intense and diffuse expression of CD40 on the endothelium of peritubular capillaries and focally on the endothelium of glomeruli of chronic but not acute rejection. Recent studies suggested a role of CD40/CD40L interaction in both renal interstitial [26] and glomerular [27] inflammatory processes.

In striking contrast to acute and chronic rejection, total absence of staining for costimulatory molecules was found in CsA nephrotoxicity. This immunohistochemical diversity may be exploited as valuable criteria for the differential diagnosis.

In conclusion, these results provide evidence for the local expression of the most relevant inducible lymphocyte costimulatory receptors in renal biopsies from patients undergoing acute and chronic rejection. This evidence support the contention that the costimulatory pathways, previously described in experimental animal transplant models, play a relevant role also in human pathology.

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