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Insensitivity to cyclosporine may explain the HLA-DRw6 recipient effect

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Abstract. Clinical as well as experimental studies have found an interindividual variability in the immunosuppressive effect of cyclosporine (CsA). In renal transplant patients treated with CsA and prednisolone alone, biopsy-verified rejections were significantly more frequent in DRw6-positive than in DRw6-negative graft recipients. The relative risk for developing a graft rejection independently of the CsA blood levels increased in HLA-DRw6-positive transplant patients. Although no statistical significance of the CsA levels within different DR phenotypes could be assessed, HLA-DR2-positive graft recipients with biopsy-verified rejection episodes had significantly lower CsA levels than DR2-negative patients (P = 0.01). Our results would indicate a very low CsA sensitivity of HLA-DRw6-positive graft recipients and might explain previous results describing an increased incidence of rejection and decreased graft survival rates in these patients.

Key words: Cyclosporine sensitivity – HLA-DRw6 phenotype – Kidney transplantation – Incidence of rejection

Studies of renal transplant patients who used cyclosporine (CsA) as part of their immunosuppressive regimen reported an overall increase in renal allograft survival rate of 15% compared with conventional immunosuppression [9]. The selection of the appropriate CsA dose which produces immunosuppression but not toxicity is complicated by marked inter- and intraindividual variability in the drug pharmacokinetics [2, 6, 8]. Recently, we were able to demonstrate differences of in vitro sensitivity of mixed lymphocyte culture (MLC) to CsA among healthy individuals according to the HLA-DR phenotype of responder cells [12]. Based upon these results, the aim of the present study was to evaluate a possible genetic influence on CsA sensitivity in vivo.

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Patients and methods

We investigated 144 consecutive kidney transplant patients receiving baseline immunosuppression for a possible relationship of HLA-DR phenotype and CsA sensitivity. Some 22 patients were eliminated because of nonhistologically proven rejection episodes and infectious complications. The remaining 122 graft recipients were treated either with prednisolone and CsA (n = 73) or prophylactically with antithymocyte globulin (ATG) and/or OKT3; 31 patients received azathioprine in addition. Rejection episodes between days 5 and 13 after transplantation were observed in 70 patients and were proven histologically, whereas an uncomplicated follow-up was seen in 52 patients [13]. The patients were treated with CsA and corticosteroids, and rejection episodes were initially treated with 500 mg methylprednisolone for 3 days. The baseline immunosuppressive therapy consisted of CsA (5 mg/kg day intravenously for 3–4 days followed by oral administration) and methylprednisolone (200 mg at surgery, afterwards reduced to 15 or 10 mg/day). CsA levels were measured for dose adjustment in whole blood, using high-performance liquid chromatography (HPLC) as described previously in detail [3]. An average of the CsA blood levels from 4 consecutive days before starting antirejection therapy was calculated. As patients with and without rejection episodes received an identical immunosuppressive protocol, dosages were comparable in both groups.

HLA-A, -B, -C and -DR antigens were determined according to standardized serological methods. The NIH test was used for HLA-A, -B, -C typing and two-color fluorescence for HLA-DR typing. The rejection frequency in patients with different haplotypes was evaluated χ^2 analysis. Representative CsA levels in patients with and without rejection were correlated to the absence or presence of particular HLA haplotypes using a non-parametric analysis of variance (Kruskal-Wallis). Levels of significance were determined using two-tailed tests.

Results

To approach the matter of CsA sensitivity, we compared graft recipients receiving CsA and prednisolone by specific HLA-DR haplotype for the incidence of rejection episodes and found significantly (P = 0.045) more rejections in HLA-DRw6-positive (77%, 20/26) than in DRw6-negative greft recipients (53%, 25/47). We observed no statistical significance if transplant patients receiving additional immunotherapy, e.g., OKT3, ATG, azathioprine, were evaluated (Fig. 1).

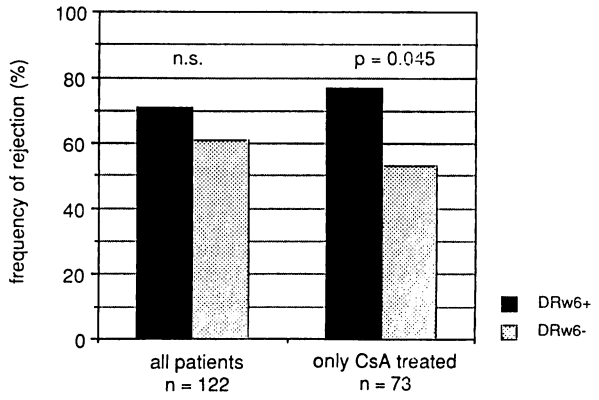

Fig. 1. Incidence of rejection (DRw6+ vs DRw6−) according to HLA-DR haplotype and type of therapy (CsA, cyclosporine)

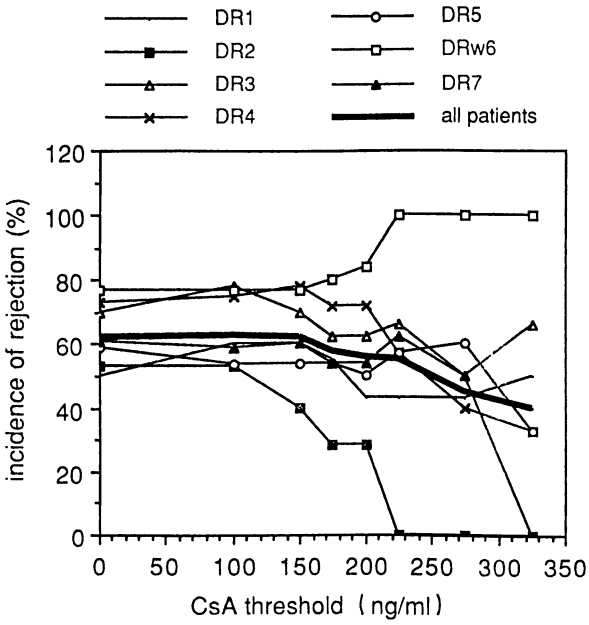

Fig. 2. Incidence of rejection according to HLA-DR haplotype and amount of CsA administered

Upon estimating the frequency of rejection with increasing CsA levels, we found that the relative risk for DRw6-positive graft recipients increased continuously because in this group the incidence of rejection was not influenced substantially by higher CsA levels, whereas in DRw6-negative graft recipients, rejections were less frequent at higher CsA levels (Fig. 2). In contrast, the relative risk for DR2-positive individuals of graft rejection decreased as a function of increasing CsA levels. No difference could be observed concerning the other class II haplotypes. Comparing patients with and without rejection, we found no significant difference in CsA dose between the groups (180 ± 85 ng/ml in rejection vs. 201 ± 91 ng/ml in uncomplicated courses). The mismatch of HLA phenotypes (A, B, DR) was significantly increased ($P = 0.015$) in patients with verified graft rejections (median 3; 0–4) compared with those with an uncomplicated posttransplant course (median 2; 0–4). Since the respective groups were matched similarly, the higher sensitivity of HLA-DR2-positive and the apparent insensitivity of DRw6-positive patients could not be explained by differences in the degree of alloreactivity.

Discussion

To evaluate previous findings that the sensitivity to CsA in vitro might be correlated to the HLA-DR phenotype of the responding cell population in MLC, we investigated whether or not such a relationship could be confirmed in clinical practice [10, 12]. Variation in the immunosuppressive effect of CsA has been observed in transplanted patients [4–8]. Some graft recipients were reported to present with rejection episodes even at higher CsA levels, while others had excellent graft survival despite low CsA levels. An association with superior graft survival has been described for HLA-DR1-, -DR2-, or -DR3-positive patients [1, 9]. DRw6 has been associated with a high responsiveness to transplant antigens as evidenced by more frequent rejection episodes or low graft survival rates in these patients [1, 4, 5]. In the present in vivo study we tested an influence of the HLA-DR phenotype on CsA sensitivity in renal transplant recipients. CsA sensitivity in vivo was determined either by evaluating the rejection frequency in patients with a particular HLA phenotype or by comparing CsA levels within the rejecting or nonrejecting group in patients positive or negative for a particular haplotype. Our findings indicate that the rejection frequency in DRw6-positive patients could not be influenced by increased the CsA levels. Nevertheless, if transplant patients receiving OKT3, ATG, or azathioprine additionally were also evaluated, the DRw6 effect on the incidence of rejection could no longer be observed. DRw6-positive transplant patients who received their graft between 1982 and 1985 and whose immunosuppressive protocol was mainly based on CsA and prednisolone had significantly lower graft survival rates compared with DRw6-negative patients. In patients transplanted between 1986 and 1989 when other immunosuppressive agents (OKT3, ATG, azathioprine) were used more frequently, no such difference could be found (unpublished data). Previous results showed an increased rejection frequency in DRw6-positive patients with high dose prednisolone therapy. Additional ATG treatment improved the graft survival significantly [5]. These results are in good accordance with our findings that more aggressive immunosuppressive agents may overcome the DRw6 effect.

In conclusion, we have demonstrated that individual sensitivity to CsA in vivo might be linked to the class II histocompatibility antigens. At present, it cannot be concluded that CsA therapy should be discountinued in patients possibly insensitive to the drug; however, it might be helpful to use other immunosuppressive agents more frequently.

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