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Evidence that cyclosporin A prevents clinical cardiac allograft rejection by blocking both direct and indirect antigen presentation pathways

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Abstract Monitoring for the responses to alloantigens presented either by the direct or the indirect presentation pathway have been reported to be of clinical value after kidney transplantation. Amongst others, the level of these responses may be dependent on the immunosuppressive treatment. We studied both presentation routes in peripheral blood mononuclear cells (PBMC) of cardiac transplant patients, who experienced episodes of rejection, and related them to the *in vivo* cyclosporin A (CsA) levels in plasma. PBMC of the recipients were stimulated with irradiated donor cells to determine the direct presentation pathway. As a method for the activation of the immune response via the indirect pathway, PBMC were stimulated with tetanus toxoid. Both immune responses increased when CsA levels inadvertently decreased to inadequate concentrations and histological rejection was diagnosed. After clinical heart transplantation, CsA may prevent rejection by blocking both the direct and the indirect antigen presentation pathway.

Key words Clinical transplantation · Presentation pathways · Cyclosporin A · Rejection

Introduction

Both direct and indirect presentation pathways for alloantigens have been reported to be involved in rejection after kidney transplantation [5, 6]. Recently, it was suggested that the indirect pathway is more sensitive to immunosuppression than the direct route of alloantigen presentation [1, 3–5]. The latter presentation pathway involves direct activation of the recipient T-cells by donor antigen-presenting cells (APC). For the indirect alloantigen presentation pathway, allogeneic major histocompatibility complex (MHC) molecules are processed into peptides and presented on recipient APC by MHC molecules of the recipient. This pathway is similar to the one by which nominal antigens, such as tetanus toxoid (TET), are presented.

In order to test whether the use of the different presentation pathways is dependent on the immunosuppressive load [1, 3–5], we monitored both routes in PBMC of human cardiac transplant recipients and related them to the *in vivo* cyclosporin A (CsA) levels measured in plasma and to acute rejection. PBMC from transplant recipients were stimulated by spleen cells derived from the donor to measure the direct alloantigen presentation route in a mixed lymphocyte culture (MLC). Recently, we have shown that in MLC, complete removal of donor APC from the stimulator population is not a suitable tool for determining indirect presentation of donor antigens [1]. Therefore, we measured the indirect presentation route by stimulating PBMC with the nominal antigen, TET.

Materials and methods

PBMC sampling

We selected blood samples from three cardiac transplant recipients, who had experienced one or more periods of rejection during

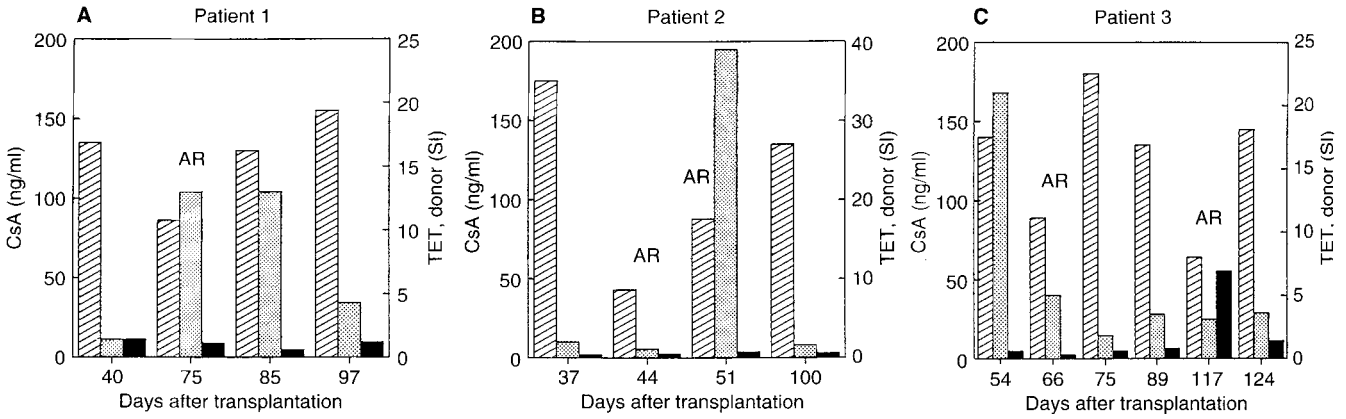

Fig. 1 Relationship of acute rejection *(AR)* and cyclosporin A *(CsA)* levels in plasma (▨) with the proliferative capacity of peripheral blood mononuclear cells to directly presented donor antigens (□) and indirectly presented antigens tetanus toxoid *(TET)* (■)

the first 4 months after heart transplantation when CsA levels were inadvertently decreased to inadequate concentrations. Rejection was diagnosed histologically in endomyocardial biopsies (ISHLT grade 3 or more) [2].

PBMC were isolated from heparinised blood by density gradient centrifugation using Ficoll-Isopaque ($\delta = 1077$). Spleen cells were obtained by mechanical dissociation of small pieces of spleen derived from the organ donor as previously described [1].

Proliferation tests

For MLC and TET stimulation, 100 µl of a PBMC suspension of 5×10^5 cells/ml in culture medium (RPMI 1640-DM supplemented with 4 mM L-glutamine, 100 IU/ml penicillin and 10 % heat-inactivated human AB serum) was added to 5×10^4 irradiated (60 Gy) donor spleen cells (direct pathway); TET at 7.5 If/ml (RIVM, Biltthoven, The Netherlands) final concentration (indirect pathway); phytohaemagglutinin M (PHA, 1 : 100 final dilution) to control the viability of the cells; and culture medium. After 6 days of incubation at 37 ° in a humified atmosphere of 5 % CO_2 in air, cell proliferation was measured by the incorporation of [^{3}H]thymidine (0.5 µCi/well) added during the last 8 h of culture. The mean counts per minute (cpm) were determined and expressed as the stimulation index (SI). The SI is the ratio of the cpm obtained in the presence of antigen to the cpm obtained in the absence of antigen.

CsA levels

CsA trough levels were measured in plasma from the same blood samples as described above using a radioimmunoassay with [^{125}I]labelled CsA and CsA-specific monoclonal antibodies (Cyclo-Trac SP; Incstar, Stillwater, Minn., USA). CsA plasma levels were maintained at therapeutic concentrations of 100–150 ng/ml after transplantation. Levels below 100 ng/ml were considered inadequate.

Results

Patient 1 (Fig. 1 A) experienced a rejection episode on day 75 after transplantation. On this day, a decrease in the level of CsA was found, while at the same time the response to the direct donor antigen presentation pathway increased. In contrast to the situation 1 day before transplantation (SI = 11), the indirect pathway became and remained undetectable (SI < 2) after transplantation. After anti-rejection therapy with steroids and increasing the dose of CsA, the response to the direct presentation route slowly decreased when the CsA levels normalised.

Patient 2 (Fig. 1 B) had two successive biopsies with histological signs of acute rejection on day 44 and day 51 after transplantation, when the CsA plasma levels proved to be inadequate. After treatment with steroids and adjusting the CsA dose on day 44, only a small increase in the CsA plasma level was detected on day 51, while the PBMC strongly responded to the directly but not to the indirectly presented antigens. A second anti-rejection therapy with rabbit anti-thymocyte globulin and a higher dose of CsA proved to be necessary. Thereafter, the CsA concentrations in plasma reached normal levels, the direct pathway became undetectable and no further histological signs of rejection were found.

Patient 3 (Fig. 1 C) experienced the first rejection period 66 days after transplantation during a drop in CsA concentration in plasma, while 12 days before a high reactivity of PBMC to the directly presented donor antigens had been found. After anti-rejection treatment with steroids and increasing the CsA dose, the response to the direct pathway decreased. On day 117 after transplantation a second rejection episode was observed, accompanied by a combination of another fall in the CsA level in plasma and an increment of the response to the indirectly presented antigens. After anti-rejection therapy consisting of steroids and increasing the dose of CsA, the CsA levels normalised and reduction of the response of PBMC to the indirect pathway was found.

Discussion

In the present study, we analysed four rejection episodes occurring at a time when the CsA trough levels were inadequate. During only one of these episodes the indirect antigen presentation pathway was functional (SI > 2) (Fig. 1 C), while during the other three rejection periods (Fig. 1) only the direct pathway was found to be intact. This finding is consistent with data described by Muluk et al. [5], who showed that the indirect presentation pathway is more susceptible to CsA therapy than the direct pathway. In agreement with these results, Clerici et al. [3] demonstrated that *in vitro* exposure of CsA to stimulated PBMC suppressed the indirect presentation pathway more strongly than the direct presentation pathway. Also Gallon et al. [4] confirmed that CsA inhibits proliferation of *in vivo*-primed T-cells to indirectly presented MHC peptides. In addition, recently, we have shown that a TET response (indirect pathway) was more frequently found before (75 %) compared to the first year after (13 %) clinical heart transplantation [1].

Others suggested that only the indirect presentation pathway correlates with acute rejection, independently of the direct pathway [3, 5]. In contrast, from our data we conclude that acute rejection after clinical cardiac transplantation in the presence of inadequate CsA levels may be accompanied by an increment in the responses of PBMC both to directly presented donor antigens or indirectly presented antigens. Therefore we conclude that after clinical heart transplantation CsA may prevent rejection by blocking both the direct and indirect presentation routes.

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References

1. Besouw NM van, Vaessen LMB, Daane CR, Jutte NHPM, Balk AHMM, Claas FHJ, Weimar W (1996) Peripheral monitoring of direct and indirect presentation pathways in clinical heart transplant recipients. Transplantation (in press)
2. Billingham ME, Cary NRB, Hammond ME, Kemnitz J, Marboe C, McCallister HA, Snovar DC, Winters GL, Zerbe A (1990) A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart Rejection Study Group. J Heart Lung Transplant 9: 587–593
3. Clerici M, Shearer GM (1990) Differential sensitivity of human T helper cell pathways by in vitro exposure to cyclosporin A. J Immunol 144: 2480–2485
4. Gallon L, Watschinger B, Murphy B, Akalin E, Sayegh MH, Carpenter CB (1995) The indirect pathway of allorecognition. The occurrence of self-restricted T cell recognition of allo-MHC peptides early in acute renal allograft rejection and its inhibition by conventional immunosuppression. Transplantation 59: 612–616
5. Muluk SC, Clerici M, Via CS, Weir MR, Kimmel PL, Shearer GM (1991) Correlation of in vitro CD4$^+$ T helper cell function with clinical graft status in immunosuppressed kidney transplant recipients. Transplantation 52: 284–291
6. Schulick RD, Weir MB, Miller MW, Cohen DJ, Bermas BL, Shearer GM (1993) Longitudinal study of in vitro CD4$^+$ T helper cell function in recently transplanted renal allograft patients undergoing tapering of their immunosuppressive drugs. Transplantation 56: 590–596