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PERIPHERAL MONITORING OF DIRECT AND INDIRECT ALLOANTIGEN PRESENTATION PATHWAYS IN CLINICAL HEART TRANSPLANT RECIPIENTS 1

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Abstract

It has been reported that the response to alloantigens presented by the direct and indirect pathway may be of differential relevance after human kidney transplantation. Accordingly, we monitored these routes in peripheral blood mononuclear cells (PBMC) of heart transplant patients from before transplantation and up to 2 years thereafter in an attempt to find a correlation with the clinical status of the patients.

Both before and after transplantation, comparable proportions of PBMC samples reacted in mixed lymphocyte culture to nondepleted donor spleen cells (direct route), but never to donor cells depleted for antigen-presenting cells (indirect route). In contrast, the latter route could easily be activated by a nominal antigen and persisted after transplantation, although the proportion of PBMC samples responding was significantly suppressed, irrespective of the occurrence of rejection. Consequently, complete removal of antigen-presenting cells from the stimulator population in a mixed lymphocyte culture with PBMC as responder is not a suitable tool for measuring indirect presentation of alloantigens, and therefore not relevant for monitoring the immunological status of heart transplant recipients.

The development of alloreactive effector cells participating in allograft rejection is dependent on the presence of T helper cells. These T helper cells may respond to alloantigens presented by antigen-presenting cells(APC *) via either the direct or the indirect pathway(reviewed by Sayegh) (1). The direct presentation pathway involves a direct activation of the recipient T cells by donor APC (dAPC). For the indirect pathway, donor MHC molecules are internalized by recipient APC (rAPC), subsequently processed into peptides which are bound by recipient's MHC class II molecules, and finally presented at the cell surface of the rAPC to self-MHC-restricted, allopeptide-specific responder cells. This pathway is identical to the presentation of nominal antigens, e.g., tetanus toxoid(TET).

Both presentation pathways were found to be able to induce an alloresponse by peripheral blood mononuclear cells (PBMC) of healthy volunteers (2). In renal transplant recipients with stable graft function, the direct pathway was not different from that of healthy donors, whereas these patients showed no response to antigens presented via the indirect pathway. When the latter reactivity was detected, a correlation with acute or chronic rejection was reported (3,4). In

addition, it was demonstrated that the indirect pathway was more sensitive to immunosuppression than the direct route of alloantigen presentation (4,5).

In the present study, we monitored the presence of both antigen presentation pathways in PBMC of human cardiac transplant recipients, by measuring the proliferative response of their cells to alloantigens in mixed lymphocyte cultures (MLC). PBMC were collected longitudinally from the time of transplantation to 2 years thereafter. The presentation pathways were investigated in an attempt to find a correlation with the immunological status of the patients and possibly to obtain a relevant tool to monitor the effect of tapering of immunosuppression on the donor-directed immune response.

PBMC were isolated from heparinized blood by density gradient centrifugation using Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden). The PBMC were collected from the interphase, washed twice with HBSS (Gibco BRL, Scotland, UK), and frozen at -140°C until use. Spleen cells were obtained by mechanical dissociation of small pieces of spleen through a sieve of stainless steel in RPMI 1640-DM supplemented with 4 mM l-glutamine, 100 IU/ml penicillin, 100µg/ml streptomycin (all from Gibco, Paisley, Scotland), and 10% pooled heat-inactivated human AB serum (i.e., culture medium). DNase (10µg/ml, Boehringer Mannheim GmbH, Germany) was added to prevent aggregation of cells. Subsequently, the cell suspension was filtrated through a 70-µm cell strainer (Falcon, Franklin Lakes, NJ) and washed. Thereafter, the cells were centrifuged over a Ficoll-Paque density gradient and stored at-140°C.

To test the response to alloantigens presented via the direct or the indirect presentation route, we stimulated PBMC from the transplant recipient with spleen cells derived from the heart donor (direct route) or with donor spleen cells depleted for dAPC (indirect route). The response to TET (RIVM, Bilthoven, the Netherlands) served as positive control for the indirect presentation pathway.

To remove all dAPC, 10^7 donor spleen cells (in 3.5 ml) were incubated with 125 µl of anti-CD14 (monocytes/macrophages; MY4, Coulter Immunology, Hialeach, FL), 75 µl of anti-CD19 (B-cells; Leu-12, Becton Dickinson, San Jose, CA), and 75 µl of anti-HLA-DR (dendritic cells; Becton Dickinson) in HBSS and 1% pooled heat-inactivated human AB serum. After an incubation period of gentle shaking for 30 min at 4°C, the cells were washed in HBSS. Thereafter, the cells were incubated with 210 µl of washed Dynabeads (M-450 sheep anti-mouse IgG, Dynal, Oslo, Norway) for 30 min of gentle shaking at 4°C. Subsequently, the dAPC were retained by a magnet and the nonadherent cells were transported to another tube. This depletion procedure was repeated twice before the nonadherent cells were processed further. The APC-depleted cells were washed with HBSS and were counted with trypan blue to determine the vitality before testing.

For MLC and TET stimulation, 100 µl of a PBMC suspension of 5×10^5 cells/ml in culture medium was added in triplicate wells in a round-bottom 96-well plate (Costar, Cambridge, MA) to 100 µl of (a) 5×10^4 irradiated (60 Gy) spleen cells derived from the donor; (b) 5×10^4 irradiated (60 Gy) dAPC-depleted spleen cells; (c) TET at 7.5 lf/ml final concentration; (d) phytohemagglutinin M (1:100 final dilution; Difco Laboratories, Detroit, MI) to control the viability of the cells; and(e) culture medium. After 6 days of incubation at 37°C in a humidified atmosphere of 5% CO_2 in air, cell proliferation was measured by incorporation of [^{3}H]thymidine (0.5 µCi/well) added during the last 8 hr of culture. The mean counts per minute (cpm) were determined and cell proliferation was considered significant when the ratio of the cpm obtained in the presence of antigen to the cpm in the absence of antigen was higher than 2(stimulation index >2).

The frequency of IL-2-producing T helper lymphocytes (HTL) was determined using the limiting dilution analysis assay (slightly modified) of Schanz et al. (6). In brief, 24 replicates of graded number PBMC responder cells were titrated in four-step double dilutions starting from 5×10^4 to 6250 cells/well and stimulated with 5×10^4 irradiated (30 Gy) spleen cells depleted or not depleted for APC. After 3 days of culture, 100 µl of the culture supernatant were harvested to test for IL-2 in a bioassay using the IL-2-dependent CTLL-2 cell line as indicator system.

The results of the MLC and TET stimulation are shown in Table 1. Most PBMC samples taken before heart transplantation (HTx) reacted to both nondepleted donor spleen cells (88%) and TET (75%), but never to spleen cells depleted for dAPC. Similarly, after HTx, 75% (45/60) of the PBMC samples did respond to spleen cells not depleted for dAPC, but not to the depleted population, independent of time after transplantation or occurrence of rejection (ISHLT grade 3 or more)(7). In


Table 1

contrast, the response to TET changed both after HTx compared with before HTx, and during the posttransplantation follow-up period. During the first year after HTx, only 13% (6/47) of the PBMC samples reacted to TET, which is significantly less than before HTx(P<0.001). During rejection and nonrejection episodes, no significant difference in the TET response was observed (P=0.33). Also, no difference between the first and the second year after HTx was found with regard to the TET response of samples taken during rejection. In contrast, in periods without rejection, a trend was observed in time after HTx, i.e., the TET response was more frequently observed during the second year (27%) compared with the first year (9%) after transplantation(P=0.15).

These results suggest that later after transplantation the response to nominal antigens tends to normalize, possibly as a consequence of the reduction of the immunosuppressive load given to the patient. The results obtained with the nominal antigen, TET, are in agreement with those found by Shearer's group (4,5), and Gallon et al.(8), who demonstrated that T helper cell responses to indirectly presented antigens are more sensitive to cyclosporine than responses to direct allogeneic stimulation. In contrast to Shearer's group (2-5), who found comparable positive responses both to indirectly presented nominal antigens and indirectly presented alloantigens in PBMC from kidney transplant recipients and healthy controls, we did not find any indirect response to alloantigens. Our results were confirmed by an unpublished study of PBMC from healthy persons which did not react to an APC-depleted stimulator population in MLC, while positive responses were found after stimulation with TET (F.H.J. Claas, Immunohematology and Blood Bank, Leiden, personal communication, 1995). Although, the present study was different from that of Shearer's group with respect to the organ transplanted and the immunosuppressive regimen, we think that the different results are due to the different methods used for APC depletion. Depletion of APC by plastic and nylon wool adherence may be incomplete, resulting in responses to suboptimal concentrations of dAPC, while the response was ascribed to alloantigens indirectly presented by rAPC. Dendritic cells are known as very potent APC, inducing MLC responses at low stimulator to responder ratios (1 dendritic cell to 1000 T cells)(9). Accordingly, a slight contamination of the stimulator population with dendritic cells can already induce T cell proliferation in an MLC. The reported correlation of dAPC-depleted MLC responses with clinical events may rather be associated with the presence of a few remaining dAPC in the tests than with the measurement of the indirect presentation route. We have checked for the efficiency of the depletion by FACS analysis and by performing two-way MLC with two depleted stimulator populations. After depletion, 0% $CD14^+$, <1% $CD19^+$, and <1% $HLA\text{-}DR^+$ were detected by analysis on FACS and in none of the cases was a response found in the two-way MLC (stimulation index <=2). From the present study, it is clear that no responses to alloantigens in MLC can occur in the absence of dAPC.

An explanation for the positive responses of PBMC to the nondepleted cells and the negative responses of PBMC to the dAPC-depleted population, however, remains that the direct presentation pathway is responsible for the strong proliferative responses seen in vitro by a high frequency of T cell precursors responding to allogeneic MHC molecules (10). The MLC used may not be sensitive enough for a detectable response to alloantigens presented via the indirect pathway to antigen-specific T cells that may be present at low frequencies. This was also reported by Watschinger et al.(11), who indicated that T cells primed in vivo by a vascularized cardiac allograft are able to proliferate in vitro to specific peptides but only when presented by APC, which were preincubated with the allopeptide. Therefore, we used a limiting dilution analysis assay to determine the HTL frequencies. This assay is thought to be more sensitive than MLC to measure responses to directly and indirectly presented antigens. We tested 4 pre-HTx samples, because the highest responses were expected at that point in time (2). All PBMC reacted clearly to their nondepleted donor cell population (57, 33, 77, and 56 HTL/10^6 cells), while responses after APC depletion were nondetectable or very low (6, 0, 17, and 0 HTL/10^6 cells, respectively). However, interference of backward stimulation (i.e., IL-2 production of stimulator spleen cells in response to PBMC), as described by Schanz et al. (6), could have played a role in case of low HTL responses. To avoid backward stimulation, generally, B cell lines are used as stimulator cells in the HTL assay, but these cell lines are not relevant for monitoring the indirect alloantigen presentation pathway, since they are excellent direct stimulators. Accordingly, like the MLC, the HTL assay with the APC-depleted cells as stimulator is not usable to measure responses of PBMC to indirectly presented alloantigens.

In summary, no difference in response of PBMC to alloantigens presented via the direct presentation pathway was detected before or after HTx, irrespective of the occurrence of rejection, and a reaction to indirectly presented alloantigens was never found. However, the response to TET remained detectable after HTx, although in significantly less cases, but again irrespective of rejection. We conclude

that monitoring the direct and indirect alloantigen presentation routes in PBMC is not useful as a tool for monitoring the immunological status of heart transplant recipients.

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Footnotes

This work was supported by grant 92.094 from the Netherlands Heart Foundation. [Context Link]

Abbreviations: APC, antigen-presenting cells; dAPC, donor antigen-presenting cells; HTL, IL-2 producing T helper lymphocytes; HTx, heart transplantation; MLC, mixed lymphocyte culture; PBMC, peripheral blood mononuclear cells; rAPC, recipient antigen-presenting cells; TET, tetanus toxoid. [Context Link]

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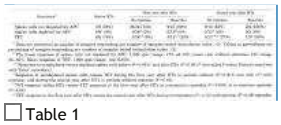

Table 1

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