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Relation of suppressor activity to lymphocyte subsets, in vitro IL-2R expression, and biopsy results in cardiac transplant patients

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Abstract. Nonspecific suppressor activity of peripheral blood mononuclear cells (PBMC) was determined from 8 patients preoperatively and 22 patients subsequent to heart transplantation. Whereas no correlation was found between any defined lymphocyte subsets, in vitro interleukin-2 (IL-2) production, and IL-2R expression, or biopsy-proven rejection and suppressor cell activity when data obtained on the same day as the suppressor assay were analysed, the phytohemagglutinin – but not the concanavalin A induced, in vitro IL-2 production – was significantly enhanced ($P < 0.02$) in patients with evidence of concomitant rejection. In contrast, a significant correlation between diminished, nonspecific suppression and rejection was found when the results of biopsies performed up to 2 ($P < 0.0075$) and 4 ($P < 0.0033$) weeks after the investigation of suppressor cell activity were compared. We conclude that periodic determination of the suppressor functional status may be useful to discriminate between patients at low or high risk for graft rejection after heart transplantation.

Key words: Nonspecific suppressor function – Interleukin 2 production – Rejection – Heart transplantation

Suppressor cells play important regulatory roles in the feedback control of immunological functions and, among other factors, a privotal role in transplantation tolerance. Nonspecific suppressor mechanisms have been demonstrated to act under a variety of experimental circumstances, including some that are of potential clinical relevance. Suppressor cells and specific and nonspecific suppressor functions were demonstrated using different cell systems in allograft recipients [3, 5, 14]. In addition, a reduced B-cell response after mitogenic stimulation, probably due to T suppressor cells, indicated a good graft acceptance in renal transplant patients [16]. Furthermore, after cadaveric kidney transplantation, cyclosporin-treated recipients showed a preserved suppressor function and a significantly lower incidence of acute rejection episodes when compared with patients on azathioprine and antilymphocyte serum [2].

In the course of monitoring the lymphocytes of cardiac transplant patients, we found that analysis of circulating cell subsets has only a restricted value in the diagnosis of rejection because of the high individual variability, although a high and constant level of CD57 and CD8 coexpressing cells showed a significant correlation ($P < 0.025$) with the number of rejection episodes (unpublished data). It was suggested that this natural killer (NK) cell subset represents an immature stage in the NK lineage, is much less efficient in the cytotoxic assay than $CD8^-CD57^+$ cells [1], and can also mediate suppression in different in vitro systems such as to act as suppressors of B-cell differentiation [13].

These results altogether prompted us to examine the inducibility of suppressor cell activity in the peripheral blood mononuclear cells (PBMC) of patients after heart transplantation. In addition, the correlation between cytoimmunological data, in vitro interleukin 2 (IL-2) production, and in vitro expression of IL-2 receptors (IL-2R) on T cells and the results of the suppressor assays in relation to graft rejection was analysed.

Materials and methods

A total of 144 consecutive biopsies were obtained from 22 heart allograft recipients. The patients received cyclosporin, azathioprine, and prednisone for baseline immunosuppression. At the time of the biopsy, venous blood samples were collected in heparinized tubes. Biopsy results were graded according to Kemnitz et al. [8].

Enumeration of lymphocyte subsets. Two colour fluorescence analysis was performed with the whole blood technique on a FACScan cytometer (Becton Dickinson) using monoclonal reagents directly conjugated either with fluorescein or phycoerythrin.

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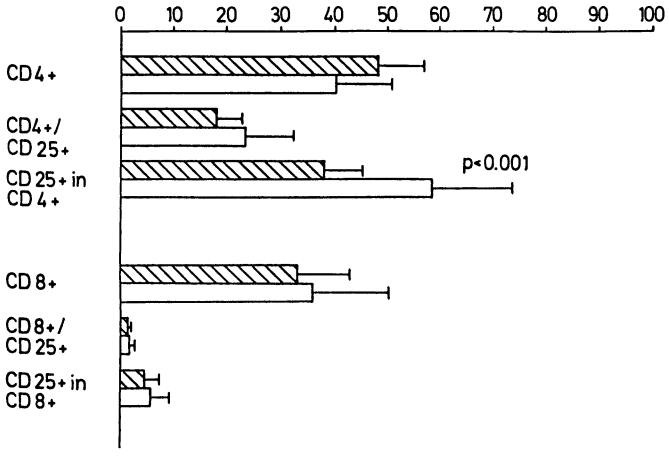

Fig. 1. Comparison of percentages (mean ± 1 SD) of CD4+, CD4+/CD25+, CD8+, and CD8+/CD25+ cells as well as the calculated proportion of the CD25+ cells within the CD4+ and CD8+ subsets in patients preoperatively ▨ and after heart transplantation □. Number of specimens analysed was 8 and 32, respectively

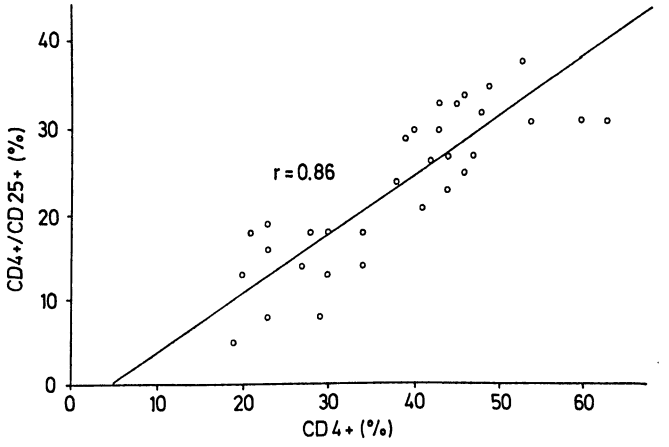

Fig. 2. Correlation between frequencies of the CD4+ cells and the IL-2R (CD25+)-expressing CD4+ cells

For cell culture methods PBMC were fractionated from the heparinized blood by Ficoll-Hypacue centrifugation. Cells were cultured in tissue culture medium consisting of RPMI 1640 supplemented with penicillin 100 U/ml, streptomycin 100 µg/ml, 2 m*M* glutamine, and 10% human AB serum.

IL-2 production and assay. For IL-2 production, PBMC (10^6/ml) were cultured in 12 × 75 mm culture tubes with and without 5 µg/ml phytohemagglutinin (PHA) or concanavalin A (ConA). After 24 h at 37°C in 5% CO_2 in air, the culture supernatant was harvested and frozen at −70°C until assayed. The indicator cells for measuring the IL-2 activity of the supernatants were of IL-2-dependent PHA blasts and prepared as previously described [11]. For the assay, indicator cells (100 µl; 4×10^5/ml) were placed in microtiter plates with a serial dilution of either the supernatants or recombinant IL-2 (Boehringer Mannheim, FRG). Cultures were incubated for 48 h at 37°C in 5% CO_2, and proliferation was measured by the incorporation of tritiated thymidine (Amersham) (0.5 µCi/well) over the last 16 h of the culture period. Results were expressed in U/ml by comparison with the standard curve.

Suppressor cell induction. Some 1×10^6/ml PBMC were treated with 20 µg/ml ConA and incubated in culture medium for 48 h and were compared with nontreated control cells. Autologous responder cells derived from the same sample were incubated during the induction phase of effector cells in culture medium. After the incubation period, ConA-activated and -nonactivated cells were washed three times with 30 m*M* α-methyl-D-mannoside (Sigma Chemical). Then, 50 µl of 1×10^6/ml responder and 50 µl of 1×10^6/ml effector cells were mixed and with and without 10 µg/ml PHA incubated at 37°C in 5% CO_2 in air for 3 days in microculture plates in quadruplicate. Controls, consisting of all cell populations alone with and without PHA were also prepared. Sixteen hours before harvesting, 0.5 µCi of tritiated thymidine was added to each well. The suppressor index (SI) was calculated according to the following formula:

$$SI = \frac{\text{cpm of responder cells with ConA – activated cells}}{\text{cpm of responder cells with ConA – nonactivated cells}} \times 100$$

Analysis of data and statistical methods. Mean values and standard deviations were calculated by Student's *t*-test and statistical analysis by χ^2 test.

Results

IL-2R expression on CD4+ and CD8+ cells

The analysis of the data obtained by cytoimmunological monitoring did not reveal any significant correlation between the different lymphocyte subsets and biopsy-proven rejection (data not shown). Correspondingly, in contrast to previous studies [4, 12], no correlation was found between the percentage of IL-2R-expressing T cells and rejection. As the preferential expression of IL-2R was demonstrated on CD4+ T cells in healthy adults [6], but only a few data are available on the distribution of the CD25 antigen-positive cells within the CD4+ or CD8+ T cells after heart transplantation [12], we studied the expression of this marker by means of two-colour fluorescence of the T-cell subsets in a group of patients before and after transplantation. Whereas an insignificant increase of CD4+ T cells was observed postoperatively, the proportion of IL-2R-expressing cells showed a significant increase from 38.0% ± 9.8% to 58.6% ± 15.7% ($P < 0.001$) within the CD4+ T cell subsets without a concomitant elevation of the CD25 antigen-expressing CD8+ cells (Fig. 1). Additionally, as shown in Fig. 2, there was a significant relationship of the percentage of CD4+/CD25+ cells to the percentage of CD4+ cells; thus, an enhancement of the IL-2R expression reflected only the relative rise of the inductor/helper cell subset.

In vitro induction of IL-2. Whereas the in vitro induction of IL-2R expression after PHA or allogeneic cell stimulation failed to correlate with a positive biopsy result (data not shown), the inducibility of IL-2 production after stimulation by PHA but not by ConA correlated significantly with biopsy specimens positive for acute rejection (Fig. 3).

Suppressor cell function. Some 84 suppressor cell inductions were carried out over 11 months, and the results were compared with data from the simultaneous examination of cell subsets, in vitro IL-2 production, in vitro IL-2R expression, and biopsies. No relationship could be established in any case between the grade of nonspecific

suppression and the results of the above investigations. In contrast, analysing the frequency of rejection episodes at 2 and 4 weeks after the suppressor cell assay, a significant correlation was found between biopsy-proven rejection and suppressor activity, when taking a cut-off value for the suppressor index (SI) of ≤ 60 % (Fig. 4).

Figure 5 shows the SI of 8 patients before transplantation and of the transplant recipients classified according to the grading of the histological findings. As in the case of a negative histological result and inconspicuous clinical state no biopsy was performed within 2 weeks, only a small number of SI data classified as 0 were evaluable, although 34 out of the 84 suppressor assays showed a SI > 60 %. Patients who did not experience rejection by up to 2 weeks exhibited a pronounced suppressor activity, and all patients with evidence of mild (A1, 2), moderate or severe rejection (A3a, 3b), except for one, showed a diminished suppressor cell activity. Furthermore, whereas a comparably lower suppressor activity was demonstrated when the patient's biopsy results were graded as in the early phase of resolving (5a) or in the late phase of resolving (5b), the distribution of SI found was similar to that in the group of patients tested preoperatively. Although the positive predictive value of a SI ≦ 60 % revealing a rejection of 3a or 3b was only 0.65 for up to 2 and 0.57 for up to 4 weeks, because the inducibility of suppressor cell activity was also diminished in the resolving phase of rejection, the negative predictive value was 0.93 and 0.94, respectively.

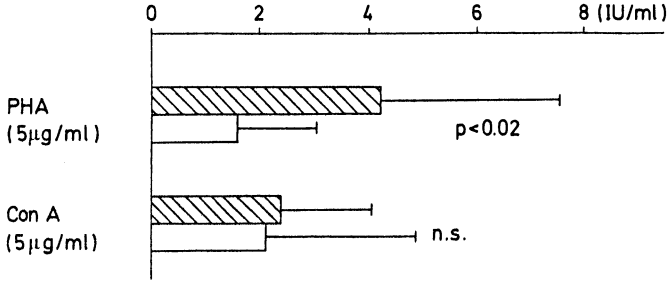

Fig. 3. Comparison of the mean values ± 1 SD of the in vitro IL-2 (U/ml) production induced by PHA and ConA in heart transplantation patients with (▨, $n = 11$) and without (□, $n = 12$) biopsy-proven rejection. Blood samples were taken on the day of biopsy

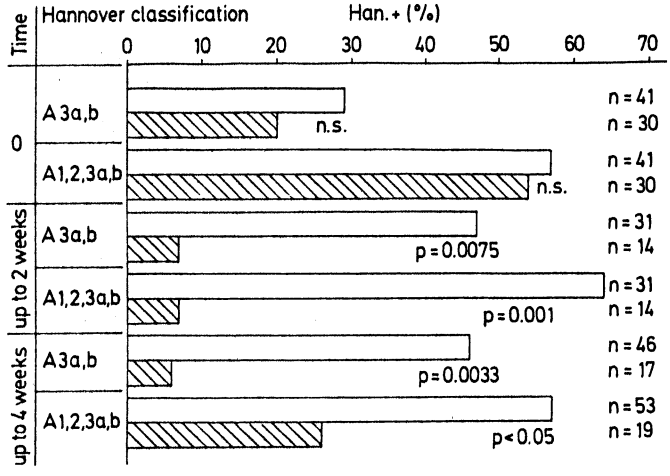

Fig. 4. Frequencies of biopsy-proven rejection and suppressor cell function. Patients were grouped according to the level of suppressor index (SI) > 60 % ▨ and ≤ 60 % □. Frequencies of biopsy results graded as A3a, b or A1, 2, 3a, b are shown in relation to the time intervals between the suppressor cell assay and examination of the biopsy

Discussion

Our observations demonstrate a better correlation between the histological findings and the investigation of cellular immunological functions, such as suppressor cell activity or IL-2 production, than the analysis of lymphocyte subsets in heart transplant patients. Previous studies have also failed to show a consistent correlation between different noninvasive techniques, such as cytoimmunological monitoring, expression of activation markers, or serum levels of sIL-2R, and rejection severity after solid-organ transplantation [7, 9, 17, 18]. The partially contradictory results can be explained by the intrinsic normal individual variability, in addition to the alterations due to processes other than rejection, e.g., infection. These disappointing findings suggest a poor and perhaps transient correlation between the features of circulating cells and immunological events in allograft.

Whereas neither the direct estimation nor the in vitro inducibility of IL-2R-expressing T cells correlated with the biopsy results, we found a significantly enhanced IL-2 production concomitant with a positive histological assessment in a restricted number of patients. Additionally, the analysis of IL-2R expression on T-cell subsets by means of the CD25-specific monoclonal antibody which detects the α-chain of the receptor revealed a close relationship of the proportion of IL-2R-expressing helper cells to the proportion of CD4 + cells. However, no correlation between the IL-2R-expressing cells and the rejection or suppressor function was established. Since IL-2

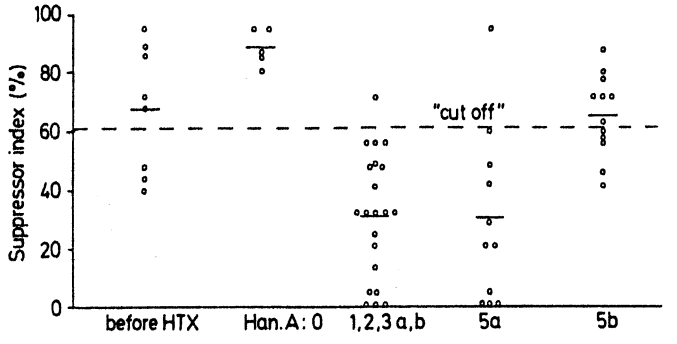

Fig. 5. Suppressor cell function from heart allograft patients. The SI results are shown for preoperative patients and are then grouped in accordance with rejection status as defined by biopsies examined between days 5 and 14 after investigation of suppressor function. *Dotted line* represents cut-off value for high (< 60 %) and low (≤ 60 %) SI

signal transduction is supposed to be transmitted via the β-chain but not the α-chain of IL-2R, evaluation of both high- and intermediate-affinity receptor-expressing cells might be more useful in monitoring transplant patients' lymphocytes, as discussed recently by Niguma et al. [10].

Although the relationship of mitogen-induced suppressor T cells to physiologically relevant suppressor function is unclear, heart allograft recipients displaying an efficient nonspecific suppressor acivity seemed to be protected from rejection in the subsequent 2–4 weeks. In addition, the failure of a correlation between biopsy-proven rejection and low SI (at time 0) suggests that the determination of different parameters such as circulating cells or humoral factors outside the allograft might not be helpful markers in the diagnosis of rejection.

To identify patients at risk of rejection, it seems to be more important to define the relative state of homeostasis between the recipient and allograft under conditions which favour tolerance induction, such as immunosuppressive therapy. The ability to display an effective hyporesponsiveness in the sense of a feedback downregulation of effector mechanisms demands to a certain degree the maintenance of immunoregulatory competence. This 'suppressor sparing effect' was postulated as one of the advantages of cyclosporine therapy [15]. In conclusion, we suggest that the periodic determination of the suppressor functional status may be useful to discriminate between patients at low or high risk for graft rejection after heart transplantation.

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