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IDENTIFICATION OF A HITHERTO UNDESCRIBED LEU-7/LEU-3 LYMPHOCYTE SUBSET IN LONG-TERM RENAL ALLOTRANSPLANT RECIPIENTS

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Summary

In long-term renal allotransplant recipients, the percentage of peripheral blood lymphocytes reacting with the monoclonal antibody anti-Leu-7 (HNK-1), a marker of 'Large Granular Lymphocytes' (LGL), is increased compared to normal controls. To further define this subset, we used two-colour flow cytometry analysis in 25 renal allograft recipients at risk for a mean of 79.8±55.2 months. We described three different phenotypes: Leu-7/Leu-1, Leu-7/Leu-2 and Leu-7/Leu-3. Within the Leu-7^+ subpopulation, the percentage of cells co-expressing either Leu-1 or Leu-2 is similar in both transplant and control groups. In striking contrast, the Leu-7/Leu-3 subset, normally barely detectable, is significantly elevated in transplant recipients (1.0±0.1% versus 6.8±1.3%, p=0.002). This newly described Leu-7/Leu-3 subset, sharing phenotypic characteristics of LGL and T helper/inducer cells has yet no known functional activity.

Introduction

Natural killer (NK) cells have been of increasing interest in transplantation studies since they are thought to play a major role in host defence mechanisms against viral infections and lymphoid malignancies [1]. These NK cells have been identified as a subset of mononuclear leucocytes residing within the Large Granular Lymphocyte (LGL) fraction of the peripheral mononuclear cell population and bearing surface receptors for the Fc portion of IgG [2]. The mouse monoclonal antibody (mab) anti-Leu-7 (HNK-1) is a marker of human LGL [3]. In long-term renal allotransplant recipients, the percentage of peripheral blood lymphocytes reacting with anti-Leu-7 has been shown to be significantly increased compared to a control population [4]. Using two-colour immunofluorescence and flow cytometry analysis, we have carried out a study to further define the phenotype of this Leu-7^+ lymphocyte subpopulation.

Patients and methods

We studied 25 patients (12 males, 13 females, mean age: 35.6±7.4 years) who received cadaveric renal allografts at the Royal Victoria Hospital between 1969 to 1983. The mean post-transplant follow-up was 79.8±55.2 (SD) months ranging from 12 to 180 months. All patients were on stable conventional immunosuppression (azathioprine and prednisone) and had normal renal function. The results were compared to a control population of 11 healthly volunteers (6 males, 5 females, mean age: 29.9±6.2 years).

Peripheral blood lymphocytes were isolated using density gradient centrifugation, washed twice in Hanks' Balanced Salt Solution and incubated with mab anti-Leu-7 directly conjugated with fluorescein isothiocyanate (FITC) for 30 minutes at 4°C. Cells were then washed twice and incubated for another 30 minutes with one of the following mabs conjugated with a new fluorochrome, phycoerythrin (PE): anti-Leu-1 (pan-T), anti-Leu-2 (suppressor/cytotoxic T), anti-Leu-3 (helper/inducer T). All reagents were kindly supplied by Dr A Saunders (Becton Dickinson Co).

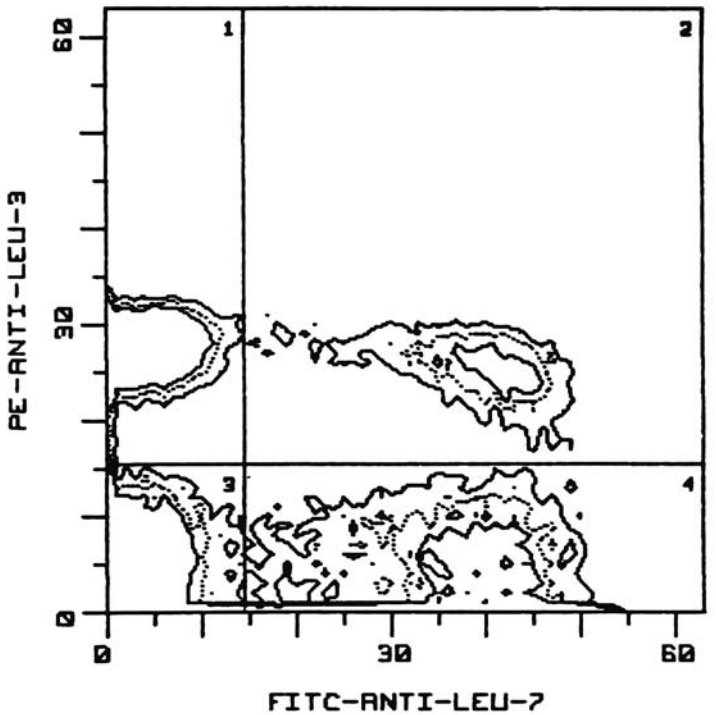

Figure 1. Two-colour flow cytometry analysis of Leu-7 and Leu-3 expression on human peripheral blood lymphocytes from a long-term renal transplant recipient. Results are shown as contours of green fluorescence (x axis, log scale) and red fluorescence (y axis, log scale)

Flow cytometry analysis was performed using a FACS Analyzer (Becton Dickinson Co, Mountain View, CA). Both fluorochromes, FITC and PE, were excited at a single wavelength (488nm) by a mercury arc lamp. The details of the procedure have been described previously [5]. Results are shown as 'contours maps' of green fluorescence (x axis, log scale) and red fluorescence (y axis, log scale) (Figure 1). The contour map was divided into quadrants to distinguish cells that demonstrated only red fluorescence (quadrant 1), both red and green fluorescence (quadrant 2), no fluorescence (quadrant 3) and only green fluorescence (quadrant 4). Absolute and relative percentage of cells in each quadrant were displayed. Statistics were performed using Student's 't' test.

Results

Percentages and absolute numbers of PBL reacting with anti-Leu-7 alone or with combinations of mabs are shown in Table I. As previously described [4], the per cent of Leu-7$^+$ cells is significantly increased in the transplant group. In

TABLE I. Relative per cent and absolute numbers of singly and doubly fluorescent lymphocytes

Monoclonal Antibody	Controls % Mean±SE (nb/mm^3 ±SE) n=25	Patients % Mean±SE (nb/mm^3 ±SE) n=11	t-statistic	p
Leu-7	18.4±2.6 (369±64)	38.0±4.7 (397±61)	2.713	0.005
Leu-7/Leu-1	3.9±0.9 (91±27)	11.3±1.9 (118±27)	2.115 0.549	0.021 0.297
Leu-7/Leu-2	6.57±1.6 (127±31)	15.7±2.4 (166±33)	2.545 0.768	0.008 0.273
Leu-7/Leu-3	1.0±0.1 (21±4)	6.8±1.3 (58±10)	3.241 2.509	0.002 0.009

addition, we found the percentage of the three following subsets to be significantly increased, i.e. Leu-7$^+$/Leu-1$^+$, Leu-7$^+$/Leu-2$^+$ and Leu-7$^+$/Leu-3$^+$. Because of the chronic immunosuppression, allografts recipients were lymphopenic (964±432/mm^3 vs 1995±521/mm^3). The absolute number of cells expressing the two phenotypes Leu-7/Leu-1, Leu-7/Leu-2 was similar in both transplant and control groups, indicating that the immunosuppressive regimen does not affect the number of these cells. Nevertheless, both relative and absolute number of cells expressing the Leu-7/Leu-3 phenotype are strikingly increased in allograft recipients.

Considering the percentage of cells within the Leu-7$^+$ subset, co-expressing another antigenic marker (Table II), it appears that there is either no (Leu-1) or a slight (Leu-2) difference between transplant and control groups. On the other hand, in allograft recipients, the percentage of Leu-7$^+$ cells co-expressing Leu-3 is markedly and statistically significantly increased.

TABLE II. Categorization of the Leu-7 positive lymphocyte subset (%)

Monoclonal Antibody	Controls % Mean±SE	Patients % Mean±SE	t-statistic	p
Leu-1	29.0±6.5	35.4±4.0	0.777	0.275
Leu-2	35.6±4.3	48.1±4.1	1.904	0.032
Leu-3	8.2±1.0	18.2±2.5	2.767	0.005

Discussion

Using a similar method, Abo et al [3] and Lanier et al [6] have described in a normal population, several phenotypes within the Leu-7$^+$ cell subpopulation. Some Leu-7$^+$ cells co-express antigenic markers of the T lineage, i.e. Leu-1 and Leu-2. These cells usually have weak NK cell activity. In contrast, a very minor subset of Leu-7$^+$ cells co-express the T helper/inducer marker Leu-3. In our studies of normal controls, the results are in agreement with these reports.

In the renal transplant population, although the per cent of cells co-expressing Leu-7 and Leu-1 or Leu-2 is increased, the relative proportion of these two phenotypes within the Leu-7$^+$ subset remains nearly unchanged. With regard to the Leu-7/Leu-3 phenotype which is barely detectable in our control group, it appears to be significant in the transplant group. This hitherto undescribed subset, sharing phenotypic characteristics of both LGL and helper/inducer T cells, has no known functional activity. These cells have morphological features of LGL [5]. Whether or not these cells have NK cell activity has still to be defined. Using functional assays [7,8], some authors have shown that NK cell activity is impaired in long-term renal allotransplant recipients, whether they receive conventional immunosuppression or Cyclosporin A. It is therefore possible to suggest that this unique subset defines a subpopulation of pre-NK cells. This would explain why there does not seem to be any correlation between Leu-7 expression and NK cell activity in transplant recipients, although one exists in the normal population [3]. Long-term renal allograft recipients are at increased risk to develop severe viral infections (CMV, hepatitis) and cancer [9]. The impairment of NK cell activity due to an increased number of pre NK cells might be one of the explanations.

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