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THE ROLE OF T-LYMPHOCYTE SUBPOPULATION IN RENAL ALLOGRAFT REJECTION

M. NOBAKHT, M.S.*, N. SIMFOROOSH, M.D.,** ALIMOHAMADIAN,*** AND B. AMIR-ANSARI, M.D.

*From the *Department of Histology, Tarbiat Modarress University, **Department of Kidney Transplantation, Shahid Labbafi-Nejad Medical Center, and the ***Pasteur Institute, Tehran, Islamic Republic of Iran.*

ABSTRACT

Twenty-two recipients of HLA-nonidentical living related and nonrelated renal allografts were studied for alterations in the relative percentage of OKT4-positive peripheral blood T-cells after transplantation. Characteristic shifts in the ratio of T-helper to T-suppressor/cytotoxic cells (TH/TS-C), but not absolute cell numbers, were demonstrated to correspond with the status of the allograft. Our results are indicative of a correlation between rejection episodes and the increase in OKT4:OKT8 ratios, that were characterized by a significant rise in the percentage of OKT4-positive cells (P=0.001), and a decrease in the percentage of OKT8-positive cells (P=0.001).

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INTRODUCTION

The recent development of monoclonal antibodies that recognize T-cell subsets has permitted the monitoring of various immunoregulatory cell populations in allograft recipients. Several investigators have reported the gradual development of suppressor cell activity shortly after transplantation, using function in vitro assay.[6,12,19,23] It is believed that the early post-transplant period represents the critical stage in the determination of graft acceptance.[6,11,17,22] Early administration of immunosuppressive agents to allograft recipients might be responsible for tipping the balance in favor of suppressive influence. In the present study, we utilized these reagents to serially analyze the ratio of T-helper to T-suppressor/cytotoxic cells (TH/TS-C) in the peripheral blood of recipients of related or nonrelated renal allografts.

MATERIALS AND METHODS

T-lymphocyte subpopulations were monitored for one month post-transplantation in twenty two recipients of HLA-nonidentical related (15 cases) and nonrelated (seven cases) renal allografts. All patients received at lease three blood transfusions prior to transplantation. A standard immunosuppressive protocol was administered to all patients. It included prednisolone at the initial dose of 1 gr/kg, tapering to 20 mg/kg by 30 days post-transplantation. Rejection was diagnosed by a 40% increase in the blood urea nitrogen, or a 25% increase in the serum creatinine level in conjunction with decreased urine output, hypertension, edema, and tenderness of the allograft; or an increase in urinary IgG excretion.

Rejection episodes were managed by IV administration of 1 gm methylprednisolone for three days, if

cytotoxic-positive plasma exchange was used.

Whole blood for T-cell analysis was obtained prior to surgery and on days 5,14, and 28 post-transplantation. These cells were analyzed for T-lymphocytes, and their subpopulations of helper/inducer and suppressor/cytotoxic cells, by indirect immunofluorescence. T-lymphocyte subsets were quantitated by monoclonal antibody stainings. Monoclonal antibodies (Ortho Diagnostic systems Inc., Raritan, New Jersey, 08869) used in this study were anti-OKT3 (mature peripheral blood T-cells), -OKT4 (helper/inducer T-cells), and -OKT8 (supressor/cytotoxic cells) antibodies.

The production and characterization of these monoclonal antibodies have been described elsewhere.[19-21] For the determination of T-cells, 100 μl of buffy coat preparations were collected from heparinized blood, using Ficoll-hypaque density gradient centrifugation. Then, 100 μl of these samples were reacted with 10 μl of fluorescein isothyocyanate-tagged monoclonal antibody and allowed to react at 4°C for 20 min. Erythrocytes were lysed with an ammonium chloride EDTA buffer for 10 min, and the leukocytes were washed twice in phosphate-buffered saline (PBS) soduim azide. Monoclonal antibodies defining all T-lymphocytes (OKT3), T helpers (TH;OKT4), and T-supressor-cytotoxic cells (TS-C; OKT8), and the percentage of reactive cells were identified by indirect immunofluorescence, using fluorescein isothiocyanate conjugated goat antimouse IgG (Nordic Immunology, Tilburg, The Netherlands) as the second antibody.

Lymphocytes were distinguished on the basis of their forward and right-angle light scatter properties as described elsewhere.[17] At least 100 cells were counted for flourescence, using a Leitz Ortholu II fluorescence microscope equipped with epi-illumination.

Preliminary studies establishing the accuracy and reproducibility of such counts were performed, using multiple counts. The levels of OKT3- and OKT8-positive lymphocytes were 83.9 ± 13.8, 68.10± 10.42, and 33.75 ± 8.37, respectively, with an OKT4/OKT8 ratio of 2.03±0.1 in normal healthy controls (n=20); and68.66± 7.34, 57 ± 4.39, and29.5 ± 4.5 , witha ratio of 1.95 ± 0.4, in hemodialyzed patients (n = 22).

Results were expressed as a precentage of total cells or as the ratio of TH:TS-C calculated as follows:

$$\text{TH:TS-C} = \frac{\%\ \text{OKT4-positive cells}}{\%\ \text{OKT8-positive cells}}$$

All preparations were read without prior knowledge of recipient status or cell subpopulation under investigation. The results expressed in Figure l did not include assays in which the percentage of OKT3-positive cells was equal or less than 10%; since TM:TS-C ratios are, most likely, irrelevant at such low precentages of T cells. All comparisons were statistically analyzed for significance, using a two-tailed student's t test.

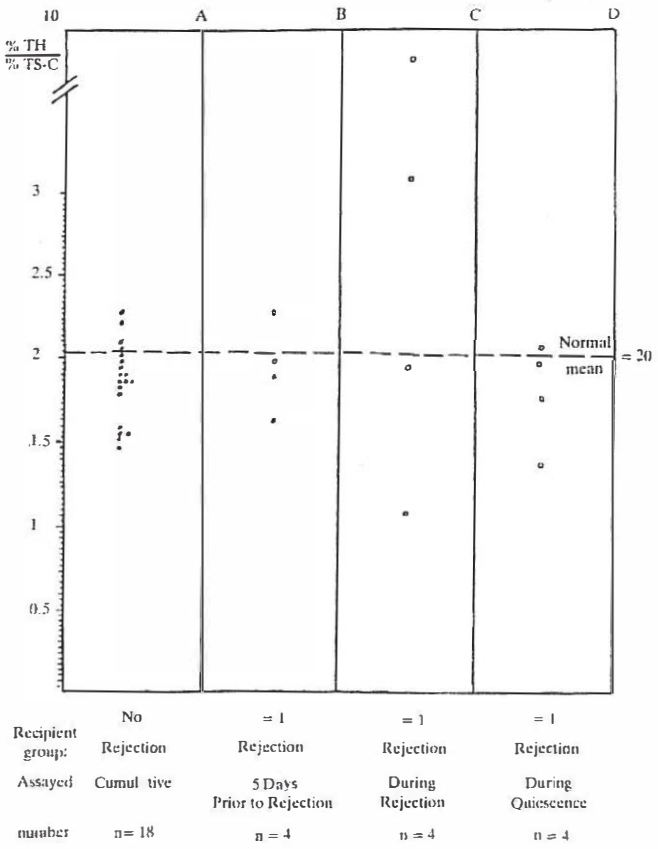

Figure 1. A scatter diagram of individual TH:TS-C measurements in 18 recipients with no rejection (O) and recipients experiencing one rejection episode (O) during the first 30 days post-transplantation. Measurements of ratios in patients with one rejection episode are subdivided into ratio measured 5 days before rejection (B), during rejection (C), or during quiescence (1). Points represent measurements obtained on days 5,14, or 28 post-transplantation; which were not associated with low values of OKT3+ cells. The number of recipients analyzed in each category is designated by the letter n.

The index of sensitivity and the index of specificity were calculated using the following formulas:

$$\text{Index of Sensitivity} = \frac{\text{no.of true positive tests} - \text{no.of false negative tests}}{\text{no. of true positive tests}} \times 100$$

$$\text{Index of Specificity} = \frac{\text{no. of true negative tests} - \text{no. of false positive tests}}{\text{no. of true negative tests}} \times 100$$

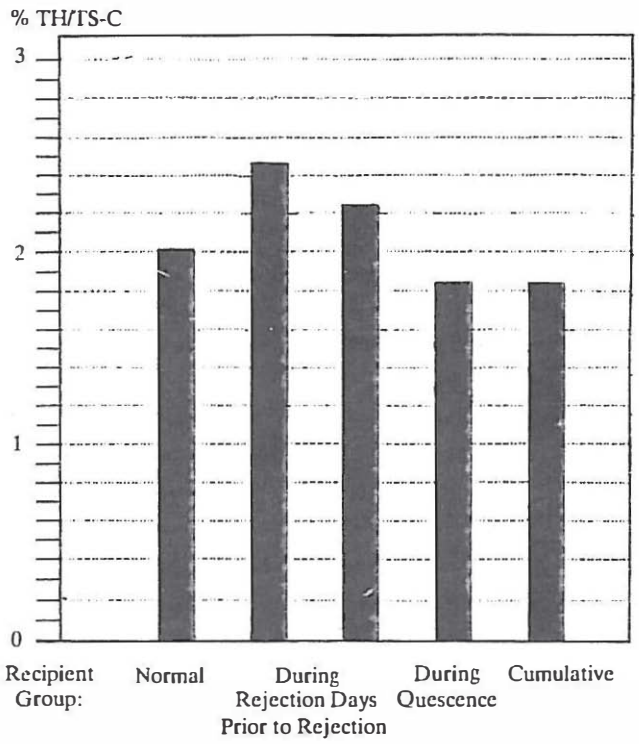

Figure 2. TH: TS-C ratios in 22 recipients of related renal allografts, 18 of whom experienced no rejection episodes during the first 30 days post-transplantation.

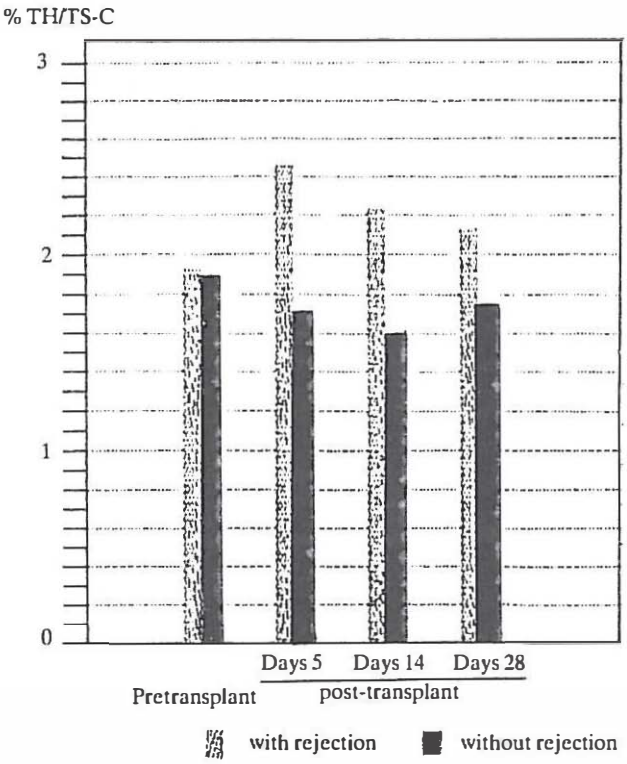

Figure 3. Comparison of alterations in the proportion of OKT4-reactive to OKT8-reactive cells in patients experiencing rejection and those in quiescence.

RESULTS

The immune response to allografts is directed primarily by thymus-derived (T) lymphocytes.

Subsets of T-cells with different functional capabilites have now been identified. T-cells that provide helper/inducer function may be responsible for the initiation of allograft rejection.[1,2,16] On the other hand, suppressor T-cells may be responsible for the maintenance of successful allograft.[7,18,23]

Analysis of total T-lymphocytes and their subsets was performed by indirect immunofluoresence prior to renal transplantation; and on days five, 14, and 28 post-transplantation.

The results indicate that ratios measured immediately before rejection were significantly higher that those obtained pretransplant or during quiescence ($P<0.01$) (Figure 2). TH/TS-C ratios were observed to be significantly decreased below normal values (2.03 ± 0.1), in recipients with functioning allografts (1.6 ± 0.7, $p= 0.001$). Analysis of recipients during rejection episodes demonstrated a normal (2.47 ± 1.38), or an increased TH/TS-C ratio (Figure 3); while, TH/TS-C ratios in recipients in quiescence were significantly decreased (1.6 ± 0.73, $p = 0.001$).

Therefore, our results, like those of other recent studies,[5,14,25] demonstrate a correlation between rejection and the increase in OKT4: OKT8 ratios.

DISCUSSION

The development of monoclonal antibodies against various immunoregulatory T-lymphocytes has permitted the determination of total T-cells, and the relative ratios of functional subsets during the development of clinical allograft rejection. Several studies have now indicated that alterations in the OKT4/OKT8 ratio appear to identify patients who are at risk for rejection.[8,9]

Binkley, et al, and Cosimi, et al,[3,9] determined that patients with a persistently high ratios (3.77) were at high risk of rejection, and those with low ratio demonstrated a low risk. Likewise, Ellis[13] reported that patients who did not reject or those during quiescence demonstrated low OKT4/OKT8 ratios. But ratios increased significantly during periods of rejection.

On the other hand, reports by several other groups have failed to find a correlation between rejection and an increased ratio.[15,26]

Although the number of patients available for analysis was small, the failure of a low ratio to be

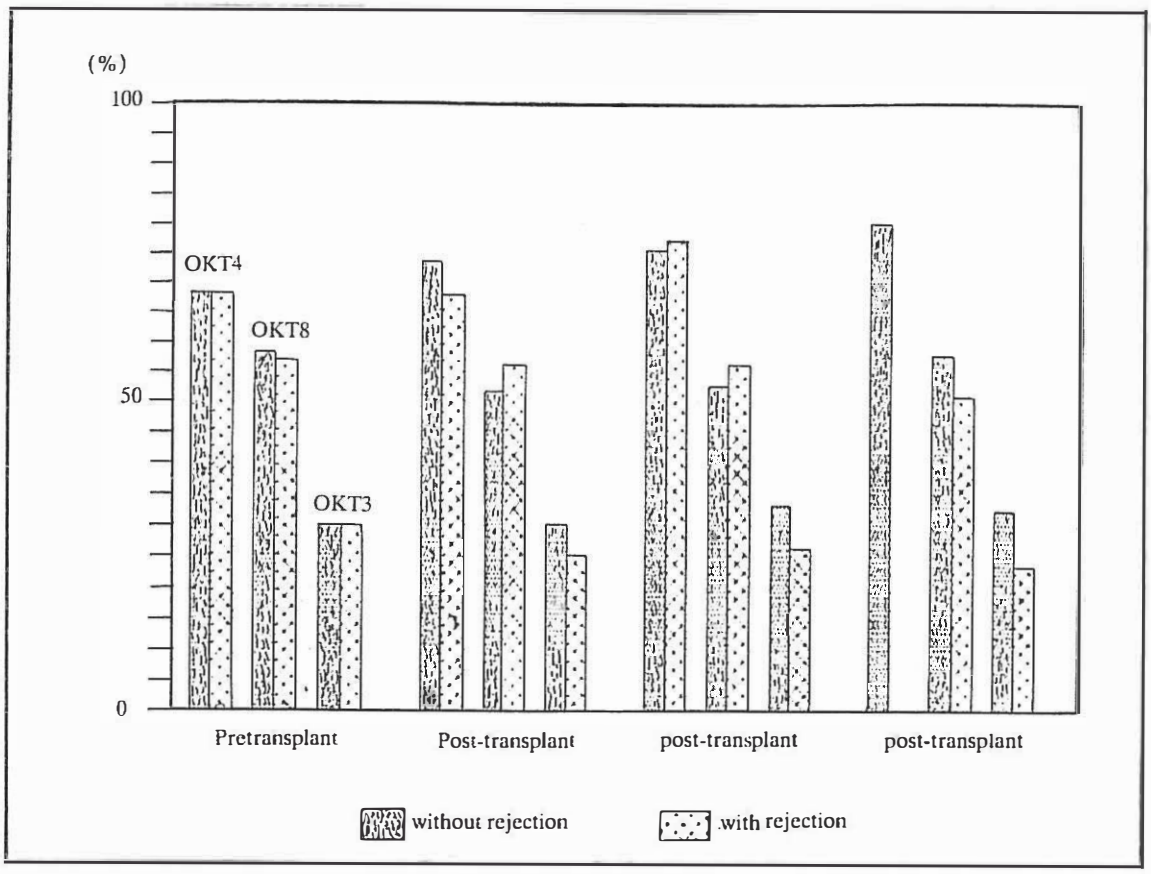

Figure 4.a. A comparison of the percentage of OKT4-, OKT8-, and OKT3-positive cells in patients experiencing rejection and those in quiescence.

predictive may be the result of different immunosuppressive protocols that were employed. Rabbit antithymocyte globulin, commerical equine antithymocyte globulin, cyclosporine, and ibuprofen have been used by investigators in some of the previous studies instead of, or, in addition to, a standard protocol employing azathioprine and corticosteroids.

The initiation of immunosuppressive therapy in our patients resulted in a significant reduction in the ratios of circulating T-lymphocytes.

The effect was not specific for a particular subset, because the concentrations of OKT3, OKT4, and OKT8-staining cells were all diminished to the same degree. Therefore, we could demonstrate significant changes in the OKT4/OKT8 ratio induced by the initiation of immunosuppressive therapy. SEveral studies have reported an increase in total circulating T-cells immediately before a during rejection.[4,10]

According to the studies of Ellis, et al,[13] using monoclonal antibodies, of significant alteration in the number of OKT3-,OKT4-, or OKT8- positive cells accurred during rejection (Figures 4a and 4b). These results indicate that the relative ratio of helper/inducer cells to suppressor/cytotoxic cells is apparently a more reliable indicator of immunologic events within the allograft, than the absolute number of lymphocytes. Although some previous reports indicated that a high OKT4/OKT8 ratio predicts the occurrence of rejection within the first 3 months post-transplantation, the reliability of this assay in diagnosing a specific rejection episode was not addressed. Therefore, the index of specificity and sensitivity of an increase in this ratio were determined in our patients. An increase of 0.3 was found to be a sensitive and specific indicator of a rejection episode. The sensitivity and specificity of the test were found to be 75% and 72%, respectively.

Our results demonstrate the effect of the OKT4/OKT8 ratio on the reversibility of rejectiona, as also demonstrated by Yan, et al.[26]

Our results supports the use of T-cell subset-monitoring in order to assist clinical decision-making in renal allograft.

However, several factors appears to limit the usefulness of the results of such monitorings. First, the immunosuppressive therarpy employed may change the helper/suppressor cell ratios in ways that remain to be identified. Second, certain patients may not respond in a helper/suppressor cell ratio that may reflect im-

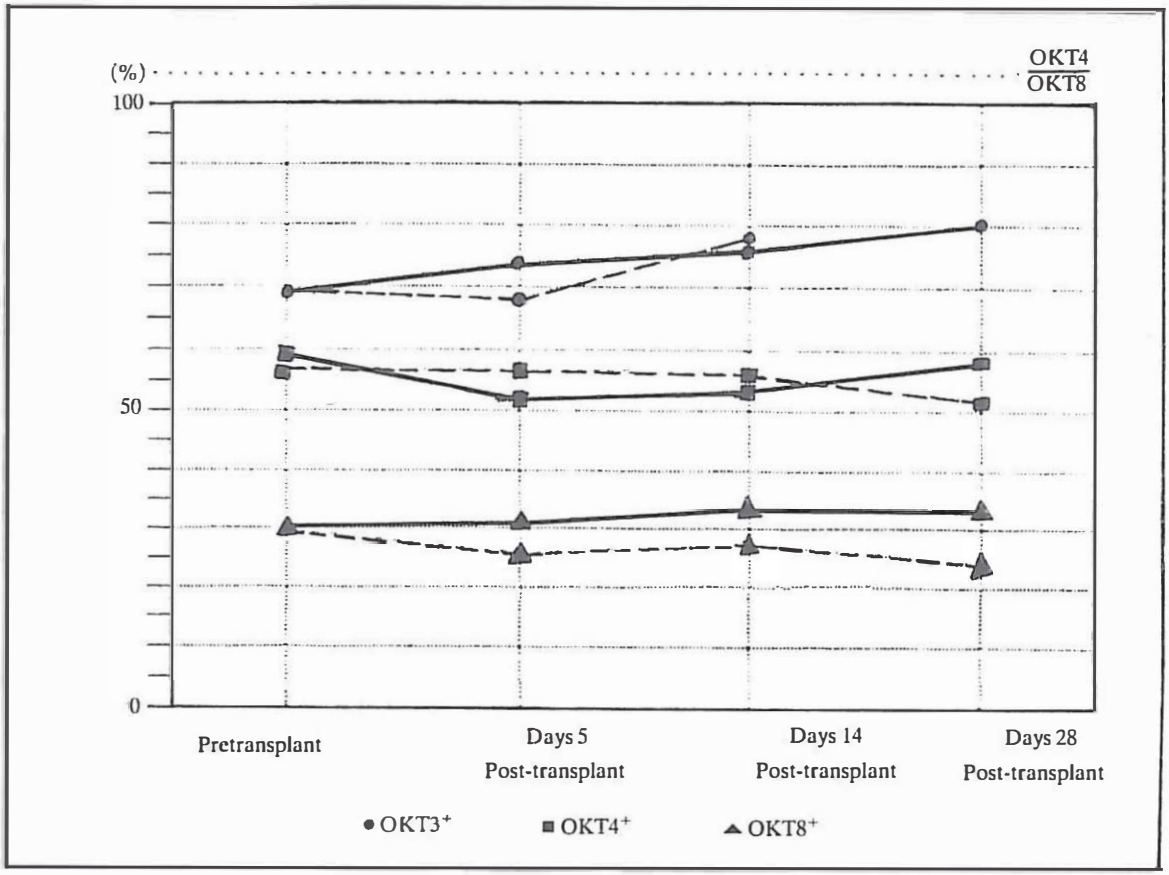

Figure 4,b.

munologic events not evident at the clinical level.The present study, in conjunction with the previously reported results, indicates that further investigation of the alterations in the helper/suppressor cell ratios in clinical circumstances and also under controlled experimental conditions will be required before the complete usefulness of this new technique will become apparent.

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