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Phenotypic T-Cell Changes in Thoracic Transplant Recipients Immunosuppressed With FK 506

R. Keenan, C. Kaufman, A. Zeevi, S. Yousem, T. Zerbe, J. Armitage, R. Kormos, R. Duquesnoy, B. Griffith, and T. Starzl

THE accurate diagnosis of rejection following pulmonary or cardiac transplantation presently requires the histologic interpretation of transbronchial or endomyocardial biopsies, respectively. Because these procedures are invasive, efforts have recently been directed toward finding alternative methods of determining the presence of active rejection episodes.

Using 2-color flow cytometry, we examined changes in T-lymphocyte populations in 3 pulmonary and 3 cardiac transplant recipients in whom FK 506 was the sole immunosuppressive agent used. These patients were chosen because cytolytic lymphocyte therapy was not used and so circulating lymphocyte counts remained consistent throughout the posttransplant period. This allowed early and sequential T-cell subset analysis and comparison with histologic data simultaneously obtained from biopsies.

MATERIALS AND METHODS

Three pulmonary transplant recipients (2 double lung and 1 heart–lung) received FK 506 as the only maintenance immunosuppressive agent in the posttransplant period. These patients were followed for 90 to 285 days during which time each patient underwent 4 to 6 bronchoscopies with bronchoalveolar lavage (BAL) and transbronchial biopsy (TBB). Three cardiac transplant recipients were also included, and they too, received only FK 506 for immunosuppression. Cardiac patients were followed for 48 to 120 days and each underwent 4 to 7 endomyocardial biopsies (EMB). The diagnosis of rejection was based on accepted histologic criteria for cardiac and pulmonary transplantation. Treatment of rejection episodes consisted of alterations in the dosage of FK 506 and/or a single bolus injection of corticosteroids.

Total cell counts and differentials were performed on BAL fluid and on peripheral blood (PBL) obtained at the time of lavage or endomyocardial biopsy. Cells (100 μL PBL or 1×10^5 BAL) were suspended in 100 μL of phosphate-buffered saline with 0.1% sodium azide and labeled with various combinations of fluorescein isothiocyanate (FITC) and phycoerythrin (PE)-labeled monoclonal antibodies at 4°C for 20 minutes. Red blood cells were then lysed using FACSlyse solution (Becton-Dickinson, Mountain View, CA) for 6 minutes. All cell suspensions were then centrifuged for 5 minutes at 500 rpm. Supernatants were discarded and the cells resuspended in 0.5 mL phosphate-buffered saline and 0.5 mL 1% paraformaldehyde. The following antibody combinations were used: Leukogate, nonspecific IgG, T/B, CD4/CD8, T/NK, CD4/DR, CD8/DR, T/$\alpha\beta$, T/$\gamma\delta$. (All antibodies were obtained from Becton-Dickinson, Mountain View, CA except for $\gamma\delta$ obtained from T Cell Sciences, Cambridge, MA). Flow cytometry (2-color) was performed on a FACScan (Becton-Dickinson, Mountain View, CA) equipped with a Consort 30 computer program. Student t tests for unpaired samples were used to analyze alterations in the various T-cell subsets during active rejection episodes as compared to normal or quiescent biopsies. There was no difference in values obtained when the diagnosis was acute cellular rejection or active obliterative bronchiolitis so they were considered together as active rejection episodes.

RESULTS

In pulmonary transplant patients, the proportions of $CD8^+DR^+$ and $CD4^+DR^+$ T cells (expressed as a percent of *total* CD8 and CD4) obtained from BAL were significantly higher ($P = .0015$ and $P = .0023$, respectively) when there was histologic evidence of rejection than were values obtained when the TBB was negative or showed resolving rejection (Fig 1). The proportion of DR^+ cells did not exceed 34% for $CD4^+DR^+$ and 21% for $CD8^+DR^+$ subsets when biopsies were negative or showed resolving rejection while respective values were all greater than 43% and 40% during active rejection. Two of these episodes were associated with active cytomegalovirus infections (pneumonitis). The proportions of $CD8^+DR^+$ and $CD4^+DR^+$ T cells obtained on these occasions were intermediate within the group of rejection values. Changes in the peripheral blood were less striking with only the proportion of $CD8^+DR^+$ T lymphocytes exhibiting significant increases during periods of active rejection ($P = .038$) when compared to negative biopsies or to pretransplant values (Fig 2). The separation in individual values for the $CD8^+DR^+$ subset was much less distinct in the PBL. The largest proportion of PBL $CD8^+DR^+$ T lymphocytes was seen in the 2 recipients with CMV pneumonitis coincident with the onset of active obliterative bronchiolitis.

In the cardiac recipients (Fig 3), similar increases in the proportion of $CD8^+DR^+$ T lymphocytes from PBL were associated with moderate (grade 2–3) rejection episodes ($P = .01$) when compared to values obtained at the time of negative or mild (grade 0–1) rejection biopsies. As with pulmonary transplant recipients, no significant changes in

From the Departments of Surgery and Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania.

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Address reprint requests to Dr T. Starzl, Division of Transplantation Surgery, Falk Clinic, University of Pittsburgh, Pittsburgh, PA 15261.

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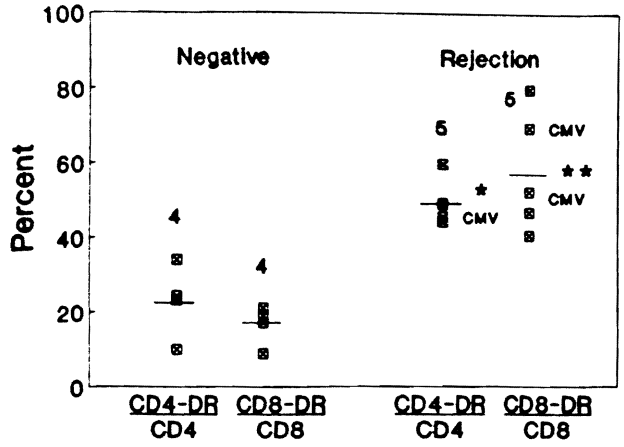

Fig 1. The proportion of each DR^+ T-cell subset obtained from BAL lymphocytes of pulmonary transplant recipients when transbronchial biopsy was negative or showed active rejection. The mean value is indicated by the straight line. The number above the symbols refers to the number of individual value points within each group. **CMV** beside the symbol refers to a measurement taken when CMV pneumonitis was also present. $^*P = .0023$, rejection vs negative for $CD4^+DR^+/CD4^+$; $^{**}P = .0015$, rejection vs negative for $CD8^-DR^+/CD8^+$.

the proportion of $CD4^+DR^+$ T cells were found in the peripheral blood.

No significant changes in the proportion or number of T B, CD4/CD8, or T/NK were found in either pulmonary or cardiac transplant recipients. The majority of T cells in PBL and BAL were $\alpha\beta$ receptor positive with few expressing the $\gamma\delta$ receptor.

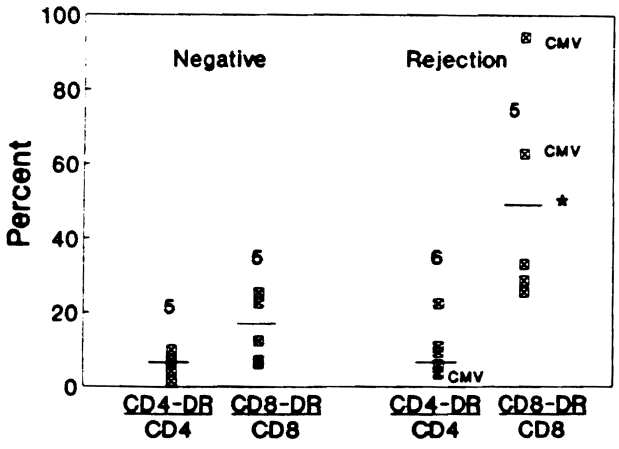

Fig 2. The proportion of each DR^+ T-cell subset obtained from peripheral blood lymphocytes from pulmonary transplant recipients when transbronchial biopsy was negative or showed active rejection. Refer to Fig 1 for explanation of straight line and **CMV**. $^*P = .038$, rejection vs negative for $CD8^+DR^+/CD8^+$.

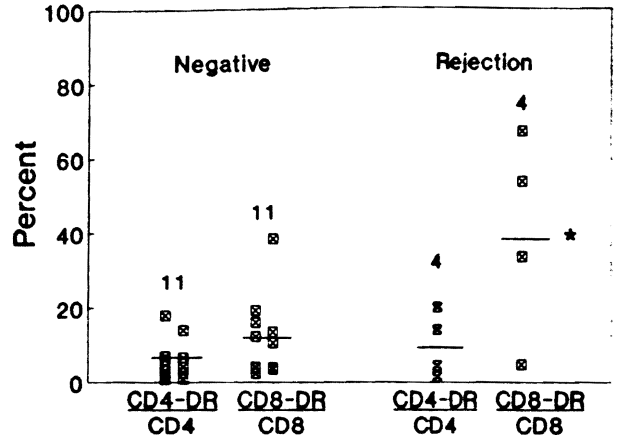

Fig 3. The proportion of each DR^+ T-cell subset obtained from peripheral blood lymphocytes of heart transplant recipients when endomyocardial biopsy was negative or showed active rejection. The mean value is indicated by the straight line. $^*P = .01$, rejection vs negative for $CD8^-DR^+/CD8^+$.

DISCUSSION AND CONCLUSIONS

These results suggest that graft-infiltrating T lymphocytes (BAL) exhibit a greater degree of activation during pulmonary rejection as measured by significant increases in the proportions of both $CD4^+DR^+$ and $CD8^-DR^+$ T-cell populations whereas lymphocytes recovered from peripheral blood express only $CD8^+DR^+$ activity. This also suggests that local events within the graft, particularly activation of the $CD4^+DR^+$ subset, are not fully reflected by changes seen in the peripheral blood. This is also the first report, in patients immunosuppressed with FK 506, to document an increase in DR^+ T cells with active rejection in pulmonary and cardiac transplant recipients. In renal transplantation, previous investigators using T lymphocytes obtained from fresh kidney biopsies, have also documented an association between $CD3^+DR^+$ or $CD8^+DR^+$ subsets and the presence of active rejection[1,2] but this is the first study to demonstrate that activated $CD4^+$ cells also accumulate during rejection episodes.

Cytomegalovirus infections are also known to cause lymphocyte activation with expression of the DR^+ marker resulting in nonspecific elevation of the $CD8^+DR^+$ T-cell subset in the peripheral blood of renal[3] transplant recipients during both rejection and infection. Similar increases in PBL $CD8^+DR^+$ T cells during infection were also seen in cardiac transplant recipients[4,5] although the association with rejection was less clear. In this study, 2 episodes of active obliterative bronchiolitis, accompanied by CMV pneumonitis, were associated with the largest increases in PBL $CD8^+DR^+$ T cells. Of interest is that values obtained from BAL lymphocytes, at the site of infection, were only intermediary within the range of values from rejection episodes. The proportion of $CD4^+DR^+$ T lymphocytes in either PBL or BAL did not appear to be affected by the presence of CMV. These studies support our observation

that use of peripheral blood T lymphocytes may not be sufficient to distinguish rejection from CMV infection and that analysis of graft-infiltrating cells more accurately reflects intragraft events.

The results from this study suggest that analysis of DR^+ subsets of T cells, particularly $CD8^+DR^+$, may be helpful in the routine monitoring of thoracic transplant recipients in that significant changes occur coincident with the histologic diagnosis of active rejection. Analysis of subpopulations of BAL lymphocytes shows that proportions of DR^+ cells greater than 40% are always associated with active rejection whether acute or chronic. Elevations in the $CD8^+DR^+$ subset of PBL lymphocytes indicates a probability of active rejection or CMV infection which, in pulmonary transplant recipients, would lead to further testing to obtain a diagnosis. Whether these tests will be useful as a screening tool for rejection is the subject of ongoing investigation.

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