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Effect of Cyclosporin-A on Hepatic and Renal Allograft Mononuclear Cell Infiltration

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A MONONUCLEAR cell infiltrate is the dominant histopathologic lesion of the allograft rejection process.[1] This infiltrate in renal allografts has been characterized previously by immunofluorescence. It consists predominantly of suppressor/cytotoxic T cells. Furthermore, there is no correlation between the degree of infiltration and the severity of the rejection process.[2] Using a new sensitive immunoperoxidase procedure, we quantitated the mononuclear cell infiltrate in hepatic and renal allografts and correlated the severity of the infiltrate with clinical abnormalities resulting from the rejection process.

MATERIALS AND METHODS

Tissue

Due to the clinical probability of allograft rejection, 25 tissue specimens were obtained from 20 patients who had liver transplants and 20 tissue specimens were obtained from 16 patients who had renal transplants. In the liver transplant group, 22 specimens came from 17 patients treated with cyclosporin-A, and 3 specimens came from 3 patients treated with azathioprine. One half of the renal specimens were from cyclosporin-A-treated patients, and the remainder were from azathioprine-treated patients. Tissue was embedded, frozen, and 4-μ cryostat sections were prepared for analysis. Tissue sections were fixed in acetone for 10 min and refrigerated at 4°C until processed.

Staining Procedure

Tissue was incubated for 15 min with a primary monoclonal antibody at the following dilutions: T11–total T cells at a 1:200 dilution; T4–T helper/inducer cells at a 1:100 dilution; T8–T suppressor/cytotoxic cells at a 1:150 dilution; B1–B cells at a 1:100 dilution (Coulter Electronics, Hialeah, FL); Leu-10–natural killer cells (NK) at a 1:100 dilution (Becton-Dickinson, Sunnyvale, CA); and OKM1–monocytes and macrophages at a 1:225 dilution (Ortho Pharmaceuticals, Raritan, NJ). Tissue sections were next incubated for 15 min with a biotinylated goat anti-mouse antibody at a 1:70 dilution (Vector Labs, Burlingame, CA). Tissue sections were then incubated for 15 min with an avidin-biotin-peroxidase complex (ABC) (Vector Labs, Burlingame, CA) at a 1:80 dilution for the avidin and a 1:80 dilution for the biotinylated peroxidase. Tissue sections were next incubated with the chromogen, 3-amino-9-ethylcarbazole, (Sigma, St. Louis, MO) for 10 min to develop the color reaction product. The tissue was counterstained with Mayer's hematoxylin (Sigma, St. Louis, MO) for 10 min and mounted with Aquamount (Lerner Labs, Greenwich, CT) for viewing with a light microscope.

Cell Enumeration

Immunostained cells developed a red-brown color reaction product on the cell membrane. The total number of immunostained cells for each cell population of the mononuclear cell infiltrate, enumerated in a specified region of each tissue specimen, defined the intensity of this infiltrate. For liver specimens, a 6 sq mm area in both hepatic triads and lobules was enumerated. For renal tissue, a 2 sq mm area in the cortex around glomeruli and blood vessels was enumerated. Immunostained cells were enumerated using a 7 × 7 mm grid in the ocular at 400× magnification. Two observers independently counted cells in the renal allografts, and the results of the observers were averaged.

Statistics

Once cell enumeration was accomplished, the tissue T-lymphocyte number and helper/suppressor (H/S) ratio were compared to the clinical parameters of the rejection process by linear regression analysis. Levels of significance were determined by the Student's t test, and a p value of less than 0.05 was regarded as significant.

RESULTS

Hepatic

The mononuclear infiltrate consisted primarily of T lymphocytes, the majority of which were in hepatic triads. The T lymphocytes consisted of approximately equal numbers of helper and suppressor cells so that the mean helper/suppressor (H/S) cell ratio was 0.9 ± 0.4. The tissue H/S ratio did not

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0041-1345/83/1503-0050$01.00/0

correlate with the intensity of the T-lymphocyte infiltrate. B cells, NK cells, and monocytes were always few in number and scattered throughout the tissue specimen. Only a scattering of T lymphocytes were found between hepatocytes within lobular tissue.

Neither the intensity of the T-cell infiltrate nor the H/S ratio in tissue correlated with the duration or dose of cyclosporin-A. There were too few patients treated with azathioprine to develop any conclusions. There was no correlation between the intensity of the T-cell infiltrate in hepatic triads and any clinical parameter of the rejection process, including: gamma GTP, SGOT, SGPT, alkaline phosphatase, bilirubin, (total, conjugated, and unconjugated), and the clinician's overall impression of rejection. The intensity of the T-cell infiltrate in hepatic lobular tissue did correlate directly with the serum alkaline phosphatase. The H/S tissue ratio, however, did correlate directly with the alkaline phosphatase and inversely with the bilirubin (total, conjugated, and unconjugated).

Renal

The mononuclear cell infiltrate consisted primarily of T lymphocytes located in the cortex around glomeruli and blood vessels. When the infiltration was intense, T lymphocytes were also found within the glomeruli and blood vessel walls. T lymphocytes were also scattered throughout the medullary interstitium. The T-lymphocyte infiltrate consisted of approximately equal numbers of helper and suppressor cells, so that the mean H/S tissue ratio was 1.2 ± 0.3. The tissue H/S ratio did not correlate with the intensity of the T-lymphocyte infiltrate. B cells, NK cells, and monocytes were scattered throughout the tissue and were always few in number.

The intensity of the T-cell infiltrate did not correlate with the duration or dose of cyclosporin-A or azathioprine. The intensity of the T-lymphocyte infiltrate did correlate directly with the serum creatinine and inversely with the urine output. This relationship was much stronger in the patients not on dialysis. Tissue H/S ratios did not correlate with any clinical parameter of the rejection process. There was no significant difference in the intensity of the T-lymphocyte infiltrate and tissue H/S ratios between the cyclosporin-A and azathioprine-treated groups.

The overall correlation between each observer's cell counting results was significant, with an $r = 0.78$.

DISCUSSION

Our results indicate that a T-lymphocyte infiltrate predominates in the hepatic allograft rejection process. There were no correlates between the intensity of the tissue T-lymphocyte infiltrate in hepatic triads and several clinical parameters of rejection. There was, however, a direct correlation between the intensity of the T-cell infiltrate in the hepatic parenchyma and the serum alkaline phosphatase. Of unknown significance are the relationships between tissue H/S ratio and alkaline phosphatase and the tissue H/S ratio and bilirubin. We found that a high H/S tissue ratio is associated with a high alkaline phosphatase and a low serum bilirubin (total, conjugated, and unconjugated).

In the renal allograft rejection process, our results reconfirm the presence of a predominant T-lymphocyte infiltrate.[1,3] Furthermore, our data demonstrate a statistically significant relationship between the tissue T-cell response and two clinical parameters of the renal rejection process. A low serum creatinine and high urine output were consistent with a mild degree of T-lymphocyte infiltration. This mild tissue infiltrate presumably reflects a milder degree of tissue rejection, with better preservation of renal function.

In both hepatic and renal allografts, the mean ratios of helper to suppressor lymphocytes were low regardless of the intensity of the lymphocyte infiltrate. Even though there was unexplained correlation between H/S ratios in hepatic allografts and alkaline phosphatase/bilirubin, this ratio of helper to suppressor cells does not seem to correlate with the clinical severity of the rejection process.

Indeed, based on experimental studies with skin allografts in mice, the composition of the infiltrates, as determined histologically, may not be related to the severity of rejection.[4] Functionally, however, the helper lymphocyte population appears to be the primary cell involved in experimental graft rejection.[5]

In summary, we found that the cellular rejection process in hepatic and renal allografts is quantitatively similar, regardless of the immunosuppressive regimen employed. As very few B lymphocytes, NK lymphocytes, and monocytes were present within the tissue, it is unlikely that these cells play a major role in the rejection process. The clinical correlates of this tissue response are unclear with hepatic allografts. However, with renal allografts, a clinical pattern has now been described that can predict the intensity of the lymphocytic infiltrate and presumably the severity of the rejection process.

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