1472 xp

Immunoreactivity of T Lymphocytes Propagated From Biopsies of Human Cadaveric Small Intestine Allografts: A Serial Study of Four Patients

M.E. Uknis, K. Abu-Elmagd, S. Suwan, G. Block, D. Van Thiel, J. Reyes, A. Tzakis, A.J. Demetris, S. Todo, T.E. Starzl, R. Duquesnoy, and A. Zeevi

RECENT success with clinical small bowel transplantation[1] has enabled further study of the complex immunology inherent to this allograft.[2] Previous histopathologic studies have been informative[3]; however, it is important to describe the functional role of graft lymphocyte populations. This is possible only by propagation of lamina propria associated lymphocytes for in vitro functional assay.

MATERIALS AND METHODS

Lymphocytes were propagated from serial proximal and distal mucosal biopsies by culturing divided biopsies for 2 weeks in RPMI 1640 plus 5% human AB serum, and 30 U/mL recombinant interleukin-2. Propagated lymphocytes were tested for primed proliferative activity (PLT) when challenged with irradiated donor splenocytes or host cells in a 3-day assay. Proliferative activity was measured as uptake of tritiated thymidine. Lymphocytes were also tested for cytotoxic activity against donor splenocyte targets or host cells labeled with ^{51}Cr in a standard 4-hour cell-mediated lympholysis (CML) assay. Propagated cells were then stained for immunophenotype analysis using two-color flow cytometric analysis.

RESULTS

Twenty-seven of 36 biopsies yielded T lymphocytes that had donor-specific PLT activity. However, only 15 of the 27 populations with donor-specific PLT activity were taken from biopsies that were histologically consistent with rejection. Twenty of 36 propagated lymphocyte populations were tested for CML activity. Seven of the 20 tested yielded donor-specific cytotoxicity. One hundred percent (seven of seven) of the donor-specific cytotoxic T cells (CTLs) were propagated from biopsies taken during clinical episodes of rejection. None of 36 manifested activity against host-derived cells, consistent with the absence of graft-vs-host disease (GVHD) in these patients. Finally, CD4/CD8 ratios were different in the proximal graft as compared with the distal portion of the graft. Both were different from the peripheral blood CD4/CD8 ratios. There was no specific increase in any one population of T cells during rejection.

CONCLUSIONS

T cells demonstrating donor-specific PLT activity were propagated regardless of the patients' clinical status. These cells likely represent adequately suppressed immunocompetent lymphocytes infiltrating the graft lamina propria. Their potential capabilities are suppressed by local endogenous factors and/or therapeutic FK 506 concentrations. CTLs were absent when the patients were free of rejection; however, when the patients rejected, donor-specific CTLs were propagated. The presence of both donor-specific PLT and CML activity was significantly associated with rejection ($P < .05$). It is also important to note that no host-specific PLT activity was manifested by any of the propagated T cells. This correlates with the absence of clinical GVHD episodes in all of the patients. This finding also substantiates previous reports that these grafts became repopulated with a lymphoreticular network of host origin[4] as these cells certainly would not manifest any host-specific PLT or CML activity. This study also suggests that the lymphoreticular network of the small bowel allograft maintains focally distinct populations of T cells. There was no correlation with the predominance of any one subpopulation and the occurrence of rejection.

REFERENCES

1. Todo S, Tzakis A, Abu-Elmagd K, et al: Transplantation 53:369, 1992
2. Monchik GJ, Russell PS: Surgery 70:693, 1971
3. Brousse N, Canioni D, Rambaud C, et al: Transplant Proc 22:2495, 1990
4. Iwaki Y, Starzl TE, Yagihashi A, et al: Lancet 337:818, 1991

From the Departments of Pathology (M.E.U., S.S., A.J.D., R.D., A.Z.), Surgery (K.A.-E., D.V.T., J.R., A.T., S.T., T.E.S.), and Medicine (G.B.), University Health Center of Pittsburgh, University of Pittsburgh, Pittsburgh, Pennsylvania.

Supported by project grant No. DK29961 from the National Institutes of Health, Bethesda, Maryland and the Pathology Education Research Fund (PERF).

Address reprint requests to Adriana Zeevi, PhD, Biomedical Science Tower, Rm. W1551, University of Pittsburgh, Pittsburgh, PA 15213-2582.

0041-1345/92/$3.00/+0