Published in final edited form as:
Clin Transplant. 1990 ; 4: 265–268.

Functional characteristics of lymphocytes propagated from a human multivisceral allograft

Jonathan D. K. Trager, **Adriana Zeevi**, **Ronald Jaffe**, **Marc I. Rowe**, **Satoru Todo**, **Thomas E. Starzl**, and **Rene J. Duquesnoy**
Division of Clinical Immunopathology, Department of Pathology, and Department of Surgery, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania, U.S.A.

Abstract

We investigated the characteristics of lymphocytes propagated from biopsies of the mesenteric lymph nodes, liver, and ileum of a human multivisceral allograft in order to provide functional evidence for the presence or absence of rejection and graft-versus-host disease (GVHD). The recipient was a 39-month-old girl with secretory diarrhea due to microvillus inclusion disease and end-stage liver disease secondary to prolonged parenteral nutrition. She developed a multifocal posttransplant lymphoproliferative disorder (PTLD) and died 37 days after transplantation. Four pairs of sequential mesenteric lymph node and liver biopsies (13, 17, 24, and 33 d posttransplant) and a single ileal biopsy (31 d posttransplant) were placed in culture with recombinant interleukin-2 (rIL-2) and phytohemagglutinin (PHA). T-cell phenotyping of cultured cells showed that CD8+ cells became dominant in all three tissues. The alloreactivity of biopsy-grown cells was determined using the primed lymphocyte test (PLT) and cell-mediated lympholysis test (CML). The proliferative and/or cytolytic responses of biopsy-grown cells to donor but not recipient or third party cells provided evidence for rejection and absence of GVHD. This donor-specific alloreactivity was detected before there was histologic evidence of rejection and during the period of active lymphoproliferation. This study suggests that the functional characterization of graft-infiltrating lymphocytes is useful in defining the immunologic events following multivisceral transplantation.

Keywords

multivisceral transplantation; lymphocytes; alloreactivity; rejection; graft-versus-host disease

Human multivisceral transplantation has been reported so far in only 5 children whose own small intestines were missing or irreversibly damaged and who had secondary liver disease due to prolonged intravenous feeding (1–3). Because of this limited experience, the clinical and pathologic criteria for the diagnosis of multivisceral graft dysfunction are not yet well established.

Several immunologic processes may potentially jeopardize the function of a multivisceral graft, including rejection, GVHD and lymphoproliferation. Morphologic monitoring remains the gold-standard for diagnosing these processes in heart, liver, and kidney recipients and was used to monitor the first 3 relatively long-surviving pediatric multivisceral recipients (192, 109, and 37 d) (1–3). However, such studies cannot determine the actual function of lymphoid cells detected in graft biopsies.

Rene J. Duquesnoy, Ph.D., Division of Immunopathology, Room 5725, One Children'S Place, Pittsburgh, PA 15213, U.S.A.

In vitro studies of cells propagated from transplant biopsies have provided important information about the function of cells involved in graft rejection. These cells can be expanded in the presence of IL-2, a lymphokine that induces proliferation of activated T cells, and tested for donor-specific alloreactivity in secondary proliferation and cell-mediated cytotoxicity assays (4–7). We have used these methods to analyze the characteristics of lymphocytes propagated from allograft biopsies of the mesenteric lymph nodes, liver, and ileum from a child with a multivisceral transplant. The studies were carried out to supplement the pathological analysis of graft biopsies which was complicated by the lack of prior relevant experience in human multivisceral transplantation (3). Specifically, we wanted to determine if the graft contained donor-reactive cells mediating rejection, host-reactive cells mediating GVHD, or both. We were also interested in correlating the functional studies of biopsy-grown cells with the pathologic findings in graft biopsies.

Methods

Patient

The patient (HLA A24,32; B27,-; Bw4,6; DR5,6; DRw52; DQw1,3) was a 39-month-old Navajo girl with secretory diarrhea since birth due to microvillus inclusion disease and end-stage liver disease secondary to prolonged parenteral feeding. On November 29, 1988, she received a liver, pancreas, partial stomach, small bowel, and colon transplant.

The donor (HLA A2,28; B27,35; Bw4,6; DRw6; DQw1) was an 18-month-old male who suffered a lethal head injury 7 1/2 hours before organ removal. The donor organs were pretreated with OKT3 to prevent GVHD. Preservation was by the “slush technique” after infusion with UW solution (1,8) and the cold ischemia time from removal of the organs was 6 h. The operative details have been reported elsewhere (3).

Immunosuppression consisted of cyclosporine, azathioprine, steroids, and rabbit antithymocyte globulin. In addition, a 2-wk course of OKT3 was started on postoperative d 18 for treatment of a clinical rejection episode which was confirmed by liver, stomach, and ileal biopsies taken on postoperative d 24 (3). On the 33rd postoperative d, a multifocal polyclonal Epstein-Barr virus-associated PTLD (9) was diagnosed (3). A capillary leak syndrome and progressive renal failure developed making fluid balance impossible. The patient died on 6 January 1989, 37 d after transplantation.

Biopsies and lymphocyte propagation

Four sequential pairs of mesenteric lymph node (LN1 through LN4) and liver (LBx1 through LBx4) biopsies were taken on postoperative d 13, 17, 24, and 33 during diagnostic laparotomies and an ileal biopsy was taken on postoperative d 31 through an ileostomy. As described previously (5), biopsies were placed into tissue culture medium (TCM) containing 30 U /ml rIL-2. Ileal biopsies were pretreated for 30 min with TCM containing 0.24 μg/ml Fungizone (Gibco Laboratories, Grand Island, NY). PHA (0.1 mg/ml) (Difco Laboratories, Detroit, MI) was initially added to all cultures. After 5–15 d of growth, cells were characterized by phenotypic and functional analysis.

Phenotyping

Phenotyping of liver- and ileum-derived lymphocytes was done by indirect immunoperoxidase staining of cytospins of cultured cells with anti-CD4 (helper/inducer T cell), anti-CD8 (suppressor/cytotoxic T cell) and anti-CD2 (pan T cell) monoclonal antibodies (Dako Corporation, Santa Barbara, CA). The mean of triplicate counts of 300 consecutive cells was used to calculate the percent positively staining cells. Phenotyping of

cultured lymph node-derived lymphocytes was performed on a flow cytometer (FACScan, Becton Dickinson, Mountain View, CA).

Functional testing

Functional testing of cultured lymphocytes was performed using the PLT and the CML, both previously described (5). Briefly, PLT reactivity of lymphocyte cultures was measured in 3-d proliferation assays. Stimulator cells were irradiated (2000 rad) donor lymph node lymphocytes and recipient and third party peripheral blood lymphocytes (PBL). In addition, proliferation to rIL-2 and spontaneous proliferation in 10% human AB serum (media) was assessed. Proliferation was measured by incorporation of ^{3}H-thymidine. CML reactivity of lymphocyte cultures was measured in 4-h ^{51}Cr release assays. Targets were PHA blasts derived from donor lymph node lymphocytes and recipient PBL. Effector: target ratios varied from 2: 1 to 20: 1 depending on the amount of effector cells available. The % CML was calculated from the formula:

$$\%\ \mathrm{CML} = \frac{\text{experimental release} - \text{background release}}{\text{total release} - \text{background release}} \times 100$$

Results

The phenotype and PLT and CML reactivity of lymphocyte cultures propagated from biopsy material is shown in Table 1. Also shown are the histologic diagnoses of nearby mesenteric lymph node, liver, and ileal biopsies taken during the same diagnostic procedures.

Cultured cells were 90–99% CD2+. Cells grown from the mesenteric lymph node biopsies were predominantly CD8+ and demonstrated donor PLT reactivity in early biopsies followed by a loss in reactivity with successive biopsies. In contrast, donor CML reactivity was positive with each biopsy. The complete loss of donor PLT reactivity in lymphocyte cultures propagated from LN3 and LN4 may have been related to the 2-wk administration of OKT3 beginning on postoperative d 18 for treatment of rejection. The most striking histologic feature of nearby lymph node biopsies taken at the same time as LNl, LN2, and LN3 was paracortical hyperplasia (3). Immunohistochemical staining demonstrated that the paracortical hyperplasia was due to a proliferation of T cells and monoclonal antibody staining for donor (HLA A2) and recipient (HLA A24) class I antigens showed that the proliferating T cells were of recipient origin (3). It is likely that this recipient T-cell hyperplasia reflected donor-specific proliferation as suggested by positive PLT and/or CML responses of lymphocytes propagated from LNl, LN2, and LN3. The specificity of the PLT responses was confirmed by the lack of proliferation to third party PBL.

A nearby mesenteric lymph node biopsy taken at the same time as LN4 did not show paracortical hyperplasia but a polyclonal B-cell lymphoproliferation of recipient origin (3). Cells grown from LN4 exhibited donor cytotoxicity. This indicates that LN4 initially contained donor-reactive cytotoxic cells within the population of proliferating B cells. The presence of B cells within LN4 itself was confirmed by flow cytometry of an early (3-d) culture of LN4 which showed 29% B cells and 51 % T cells. With time these B cells were lost from culture due to the lack of a stimulus for B-cell proliferation.

Liver biopsy-grown cells initially showed a high CD4:CD8 ratio, but this ratio decreased with each successive biopsy so that cells grown from the fourth biopsy were mainly CD8+. The donor PLT reactivity of cells grown from the sequential liver biopsies was similar to that of cells grown from the sequential lymph node biopsies, with high reactivity early on, followed by a decline in reactivity during OKT3 treatment. Third party PLT responses were

also negative. Donor CML reactivity was present in all liver biopsy cultures. It is important to note that, while donor-reactive cells were grown from LBx1 and LBx2, samples of nearby liver tissue taken at the same time as these biopsies showed infiltrating cells but no clear-cut evidence of rejection (3). While these differences may have been caused by sampling errors, it is more likely that the functional studies were able to detect rejection earlier than histologic examination. Indeed, histologic evaluation of a liver biopsy taken at the same time as LBx3 showed partially-treated acute cellular rejection (3), indicating that rejection had started before this biopsy was taken.

Cells grown from the ileal biopsy, taken at the end of OKT3 treatment, were mainly CD8+ and exhibited donor cytotoxicity. A nearby biopsy of the ileum taken at the same time showed a clear histologic picture of rejection (3).

An important clinical concern was the possible development of GVHD mediated by donor-derived lymphocytes reactive toward the recipient (1,10). As a measure of graft-versus-host reactivity of biopsy-grown cells, we used recipient PBL as stimulators in PLT assays and as targets in CML assays. Biopsy-grown cells were not able to proliferate in response to, or to kill, recipient PBL, indicating absence of sensitization to recipient PBL. This is consistent with the histologic evidence for absence of GVHD which included a determination of the donor/recipient origin of cells infiltrating the allo-graft mesenteric lymph nodes, ileum, and colon and the native colon and skin (3). The donor sites showed infiltrating cells only of recipient origin and the recipient sites showed no infiltrating cells of donor origin.

Discussion

Human multivisceral transplantation is a relatively new procedure; therefore, the clinical and pathologic spectrum of graft dysfunction has yet to be established. Although detailed pathologic studies of graft biopsies from the presently described patient indicated the occurrence of rejection and lymphoproliferation and absence of GVHD (3), the diagnosis of these processes was complicated by the lack of relevant experience in this area. It was thus important to incorporate other methods of investigating the pathologic events occurring within the graft.

We performed functional tests on lymphocytes propagated from mUltiple mesenteric lymph node and liver biopsies and a single ileal biopsy to determine if donor- and/or recipient-reactive cells were present in the graft. We were able to demonstrate donor, but not recipient, reactivity of the cultured cells in PLT and CML assays, a finding which reinforced the pathologic diagnosis of rejection and absence of GVHD. The specificity of the PLT responses was confirmed using third-party control lymphocytes.

In addition, the functional studies highlighted other important intragraft events. First, the studies indicated that a dynamic process of cellular infiltration was occurring. During OKT3 treatment, there was a shift in the population of cells infiltrating the mesenteric lymph nodes and liver from proliferative/cytolytic cells to cytolytic cells alone. What influence OKT3 had on this shift is not known. Second, the functional tests were able to detect donor-reactive cells within the liver *before* hepatic rejection was evident histologically (LBx 1 and LBx2). This is similar to our previous observation that growth of donor-reactive cells from heart allograft biopsies showing no histologic evidence of rejection was predictive of a subsequent rejection episode (11). Finally, the functional studies demonstrated that, histologically, *lymphoproliferation is able to mask rejection* (LN4). This is the first pediatric multivisceral recipient to undergo simultaneous lymphoproliferation and rejection.

Our studies suggest that the functional characterization of graft-infiltrating lymphocytes should prove useful in the future delineation of human multivisceral transplant pathology.

Acknowledgments

We gratefully acknowledge the expert assistance of Dr. Massimo Trucco and Ms. Marlyin Marrari in HLA typing of the donor.

This work was supported by Grants AI-23467 and HL-36416 from the National Institutes of Health and by the Pathology Education and Research Foundation.

References

1. Starzl TE, Rowe MI, Todo S, et al. Transplantation of multiple abdominal viscera. JAMA 1989;261:1449. [PubMed: 2918640]
2. Williams JW, Sankary HN, Foster PF, Lowe J, Goldman GM. Splanchnic transplantation: an approach to the infant dependent on parenteral nutrition who develops irreversible liver disease. JAMA 1989;261:1458. [PubMed: 2537436]
3. Jaffe R, Trager JDK, Zeevi A, et al. Multivisceral intestinal transplantation: surgical pathology. Pediatr Pathol 1989;9:633. [PubMed: 2557597]
4. Zeevi A, Fung J, Zerbe TR, et al. Allospecificity of activated T cells grown from endomyocardial biopsies from heart transplant patients. Transplantation 1986;41:620. [PubMed: 3085298]
5. Fung JJ, Zeevi A, Starzl TE, Demetris J, Iwatsuki S, Duquesnoy RJ. Functional characterization of infiltrating T lymphocytes in human hepatic allografts. Hum Immunol 1986;16:182. [PubMed: 3087922]
6. Markus BH, Demetris AJ, Saidman S, et al. Alloreactive T lymphocytes cultured from liver transplant biopsies: associations of HLA specificity with clinicopathologic findings. Clin Transplantation 1988;2:70.
7. Mayer TG, Fuller AA, Fuller TC, Lazarovits AI, Boy-Le LA, Kurnick JT. Characterization of in vivo-activated allospecific T lymphocytes propagated from human renal allograft biopsies undergoing rejection. J Immunol 1985;134:258. [PubMed: 3155462]
8. Todo S, Nery J, Yanaga K, Podesta L, Gordon RD, Starzl TE. Extended preservation of human liver grafts with UW solution. JAMA 1989;261:711. [PubMed: 2642982]
9. Nalesnik MA, Makowka L, Starzl TE. The diagnosis and treatment of posttransplant lymphoproliferative disorders. Curr Probl Surg 1988;25:367. [PubMed: 3063441]
10. Starzl TE, Kaupp HA, Brock DR, Butz GW, Linman JW. Homotransplantation of multiple visceral organs. Am J Surg 1962;103:219. [PubMed: 13916395]
11. Weber T, Kaufman C, Zeevi A, et al. Lymphocyte growth from cardiac allograft biopsy specimens with no or minimal cellular infiltrates: association with subsequent rejection episode. J Heart Transplant 1989;8:233. [PubMed: 2661773]

Table 1

Phenotype and reactivity of lymphocyte cultures propagated from allograft mesenteric lymph node (LN), liver (LBx) and ileal biopsies and histologic diagnoses of nearby biopsies

		Phenotype			PLT[a]					CML[a]		
Biopsy	**Post op day**	**%CD2 +**	**%CD4 +**	**%CD8 +**	**Media**	**Patient**	**Third party**	**Donor**	**IL-2**	**Patient (E:T[b])**	**Donor (E:T[c])**	**Histologic Diagnosis**
LN1	13	99	22	69	192	154	1986	18352	39768	1 (16:1)	16 (10:1)	Paracortical hyperplasia
LN2	17	99	3	91	440	233	1283	2879	60904	0 (16:1)	54 (2.5:1)	Paracortical hyperplasia
LN3	24	95	32	63	204	1913	1480	266	35124	0 (8:1)	16 (8:1)	Paracortical hyperplasia
LN4	33	90	5	83	16071[d]	n.t.[e]	17041	17965	46509	1 (20:1)	23 (10:1)	Lymphoproliferation
LBx1	13	97	82	15	131	358	986	17857	30059	0 (5:1)	30 (2:1)	Cholangitis
LBx2	17	92	41	52	175	172	1286	25268	45455	0 (10:1)	27 (4:1)	Pericholangitis
LBx3	24	97	25	58	280	473	1003	1802	26945	0 (5:1)	54 (5:1)	Partially-treated rejection
LBx4	33	96	9	89	1375[d]	n.t.[e]	1613	2218	21662	0 (5:1)	15 (5:1)	Lymphoproliferation
Ileum	31	97	27	62	4054	1548	4757	7628	47845	0 (5:1)	26 (5:1)	Rejection

[a] PLT = primed lymphocyte test; CML = cell-mediated lympholysis test; PLT results expressed as mean counts per minute of triplicate wells; CML results expressed as percent cytotoxicity of triplicate wells; S.D. < 15%.

[b] Highest effect: target ratio used.

[c] Lowest effect: target ratio yielding a positive CML.

[d] High background due to short-term culture (5 days).

[e] n.t. = not tested.