Brief Definitive Report

HUMAN T CELL CLONES EXPRESS FUNCTIONAL HOMING RECEPTORS REQUIRED FOR NORMAL LYMPHOCYTE TRAFFICKING

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Mature lymphocytes recirculate throughout the lymphatic system, a process thought to be essential to the dissemination of immune responses. Lymphocytes in both human and murine species migrate from the bloodstream into lymphoid tissues and inflamed sites via a mechanism requiring specific adherence to specialized postcapillary high endothelial venules (HEV) (1). Circulating lymphocytes adhere to HEV, and the lymphocytes then migrate through the endothelium into the surrounding tissue. In the mouse, we have shown (2) that a specific cell surface molecule on lymphocytes interacts with determinants on HEV of peripheral lymph nodes. A related but distinct set of surface receptor molecules are thought to be responsible for lymphocyte binding to HEV in gut-associated or Peyer's patch lymphoid tissue. Expression of functional lymphocyte surface receptors for endothelial cells is thus required for effective dissemination of lymphoid cells and immune responses.

The discovery of interleukin-2 (IL-2), or T cell growth factor, has made it possible to maintain clones of functional murine and human T cells in culture indefinitely (3, 4). This new technology gives rise to optimism for development of novel therapies based on the use of cloned effector cells to regulate or mediate in vivo immune responses in man. However, in vivo results using murine T cell clones have been somewhat discouraging (5–7). Cloned murine T cells have been shown to function in several in vivo systems, including transfer of experimental allergic encephalitis (8), mediation of delayed-type hypersensitivity (5), protection against influenza virus (9, 10), allogeneic tumor cell rejection (11), and even protection against syngeneic lymphomas (12). However, these effects have usually required either infusion of very large numbers of cloned cells, direct presentation

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of the cells to the tissue site where their function is to be assessed, or both (5–12). In one study (7, 11) of tumor cell rejection, allospecific cytotoxic T lymphocyte clones, even when preselected for the ability to eliminate allogeneic tumor cells in vivo, were found to be 5–20 times less effective than equally cytotoxic Lyt-2^+ cell populations from day 5 mixed leukocyte cultures. Although a variety of mechanisms, such as active suppression of the desired immune response, blocking of antigenic sites with antibodies, and more complex immune cell interactions might explain the surprising inefficiency of murine T cell clones in vivo, a simpler explanation is possible: the common observation that in vivo responses are demonstrable after local but not systemic presentation of effector cells suggests a deficiency in the capacity of cells maintained in vitro to localize effectively to in vivo tissue sites. In support of this, we reported (13, 14) that cloned murine T cells lack functional or antigenically-defined surface receptors for HEV. They are unable to bind HEV in vitro, lack the lymphocyte surface homing receptor for HEV defined by the monoclonal antibody MEL-14, and are unable to localize effectively in vivo to lymphoid tissues.

This lack of functional homing capacity could be a major barrier to the use of functional T cell clones for manipulation and analysis of immune responses in vivo. This problem could be especially important in the human system because of the potential of cloned effector T cells for antitumor therapy, or for immune regulation in allergic or autoimmune diseases. We wished to determine whether cloned human T cells, which are similar to their murine counterparts, also lack surface receptors for HEV. Expression of functional HEV-binding ability was assayed in an in vitro model, recently adopted from rodent systems, in which mature lymphocytes bind to HEV in frozen sections of human lymph nodes or appendix (15). Human T cell clones of both cytotoxic/suppressor and helper phenotypes were examined, and the results are reported here.

Materials and Methods

T Cell Clones. Human T cell clones were obtained from three independent sources. Several antigen-reactive and alloreactive T cells of both cytotoxic/suppressor and helper phenotypes were tested. Alloreactive IL-2-producing (helper) T cell clones T4-31, CWN, C2-2, and 22-27 (from J. Goronzy and C. Weyand) were grown in minimal amounts of recombinant IL-2 (CETUS, Emeryville, CA). Allospecific cytotoxic T cell clones AH7 and F1 (from C. Clayberger and A. Krensky) (16) were grown in IL-2 obtained from the supernatant of phytohemagglutinin (PHA)-stimulated lymphocytes, and purified by ammonium sulfate precipitation (17). Purified protein derivative–specific helper T cell clones I15, I5, III3, and the suppressor clone III27 were also grown in IL-2 obtained from supernatant of PHA-stimulated lymphocytes. These cells have been described previously (18): I5 is from cell line 5; III3 is from cell line 13; III27 is from cell line 27; and I15 was not described, but was maintained in a similar fashion.

In Vitro Assay of Lymphocyte Binding to HEV. This technique, recently described in humans (15), has been adapted from previous murine studies (19, 20). Briefly, lymphocytes in RPMI 1640 containing 25 mM Hepes, pH 7.3, and 5% fetal calf serum, were incubated with mild agitation for 30 min at 7°C on freshly cut frozen sections of human lymph nodes (normal or reactive cervical or axillary nodes from biopsies) or appendix (from elective appendectomy during abdominal surgery). The sections were then fixed by placing slides in cold phosphate-buffered saline containing 1% glutaraldehyde, and nonadherent cells were gently rinsed off. Quantitative determination of the degree of binding of sample lymphocytes was made by using an internal standard population of fluorescein isothiocyanate–labeled normal lymphocytes mixed with each sample. Data reduction and statistical

analyses have been described (15, 20). The relative adherence ratio (RAR) is a linear measure of the efficiency of cell binding to HEV (15), and is normalized so that the RAR of the reference population (ficoll-separated peripheral blood lymphocytes [PBL], included as an unlabeled sample population in each experiment) is unity. Individual clones were tested 1–2 times, with similar results in duplicate experiments.

Results

As shown in Table I, all human T cell clones examined bind efficiently to human peripheral lymph node HEV in frozen sections. The data are presented

TABLE I

HEV Binding Ability of Human T Cell Clones

Cell	Type*	Phenotype	Specificity	Relative adherence ratios‡	
				Lymph node HEV	Appendix HEV
15	Th	Leu-3$^+$	PPD	1.1 ± 0.3	—§
115	Th	Leu-3$^+$	PPD	0.7 ± 0.1	1.6 ± 0.5
1113	Th	Leu-3$^+$	PPD	0.8 ± 0.2	—
T4-31	Th	—	DW14	3.9 ± 0.8	—
CWN	Th	—	DR4	3.1 ± 0.6	—
C2-2	Th	—	DR4	1.5 ± 0.3	2.8 ± 0.6
22-27	Th	—	DW14	2.7 ± 0.5	—
1127	Tc/s	Leu-2$^+$	PPD	1.4 ± 0.4	—
AH7	Tc	Leu-2$^+$	HLA-A2	3.0 ± 0.7	—
F1	Tc	Leu-3$^+$	DR6	2.6 ± 0.6	2.5 ± 0.5

* Th, T helper cell; Tc, cytotoxic T cell; Ts, suppressor T cell.

‡ The relative adherence ratio is the calculated number of sample cells that would bind to HEV per PBL binding if an equal number of each were incubated on the sections under the same conditions. Values are reported ± SE.

§ Not determined.

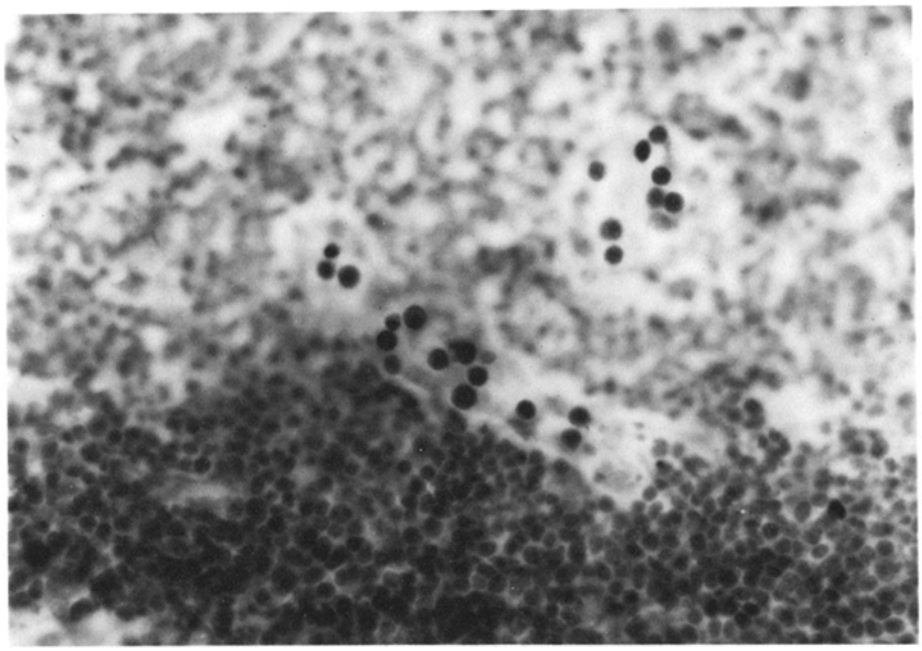

FIGURE 1. T cells of the C2-2 clone binding to two HEV in frozen section of appendix. The adherent cells are apparent as dark round circles above the plane of the section. The HEV are vaguely outlined by their lightly stained basement membranes. Thionine stain, × 250.

as RAR, relating the binding ability of the T cell clones or lines to that of normal PBL (defined as unity). The results show that the T cells tested adhere to lymph node HEV about the same as (RAR ~1), or even better than PBL (RAR >1). Also, three selected clones were assayed for binding to human appendix HEV, as indicated in the last column. As in the case of peripheral node HEV adherence, binding of these clones to appendix HEV was comparable to or better than that of normal PBL (see also Fig. 1).

Discussion

The results show that human T cell clones are able to specifically recognize and bind to HEV, a function that is essential for normal lymphocyte homing in vivo. The capacity of human T cell clones to interact with HEV appears to be independent of their antigenic specificity, their major histocompatibility complex subregion restriction or Leu-3/Leu-2 phenotype, and of their functional (helper/suppressor/cytotoxic) properties. Furthermore, the T cell clones examined were obtained from three independent sources, each using somewhat different methods of cell cultivation (see Materials and Methods). Thus it seems unlikely that expression of functional HEV-binding capacity is influenced by minor differences in tissue culture protocols. Taken together, the uniform expression of HEV-binding ability by the 10 clones examined suggests that the expression of functional receptors for HEV may well be characteristic of most, if not all IL-2-responsive human T cells maintained in vitro.

In murine systems, the capacity of lymphocytes to bind to HEV in vitro has proven an accurate reflection of their ability to interact with HEV in vivo, and to migrate from the blood into lymphoid tissues (19–22). The ability of normal human lymphocyte populations to bind in vitro to HEV also parallels their presumed migratory status in vivo (15). Thus, the binding of human T cell clones to HEV strongly suggests that they may be able to migrate effectively in vivo, as well. However, expression of receptors for HEV, while certainly a prerequisite for normal homing, may not in itself be sufficient for in vivo localization; clearly other requirements must also be met for normal recirculation and diapedesis. Critical studies of T cell clone migration in primate model systems, as well as in human systems using gamma imaging of cell localization to lymph nodes and other tissues, will be required to demonstrate finally the in vivo migratory competence of human T cell clones.

These findings stand in marked contrast to previous studies (13, 14) showing that murine T cell clones lack surface receptors for HEV, and are unable to localize to either peripheral or gut-associated lymphoid tissues in vivo. This functional difference between human and murine T cell clones may be a reflection of technical differences in procedures for derivation and in vitro propagation of T cells in the two species. For example, all the human T cell clones examined were derived from stimulated PBL, whereas murine clones are generally derived from stimulated spleen or lymph node cells. Experiments are in progress to rule out tissue source as a major determinant of receptor expression.

Another possibility is that there is a real difference between the two species in the regulation of expression of homing receptors during T cell differentiation, or in the stage of differentiation at which human and murine activated T cells express receptors for IL-2. Based on studies in the mouse, we have proposed

(21) that most virgin lymphocytes, before antigenic stimulation, express functional homing receptors for both lymph node and mucosal HEV; that antigenic stimulation leads to suppression of expression of both of these functional homing receptor classes; and that following a period of local differentiation, surviving cells are induced or selected by local microenvironmental factors to express homing receptors specific for the type of HEV associated with tissues similar to the site of antigenic stimulation (reviewed in 21). We have recently (S. Jalkanen, N. Wu, and E. C. Butcher, unpublished observations) identified transformed human B lymphoblastoid cell lines capable of discriminating between mucosal (appendix) and peripheral lymph node HEV, thus confirming the existence of organ-specific lymphocyte-endothelial cell recognition mechanisms in humans as in mice. In this study, three representative T cell clones were tested and found to bind well to appendix as well as lymph node HEV. This expression of dual recognition function, within the context of our model, may suggest either that human IL-2-stimulated T cell clones represent a somewhat earlier stage in the T cell differentiation sequence, before loss of homing receptors, or possibly that the period of suppression of homing receptor expression observed (21) during murine T cell proliferation is not a characteristic of proliferating human T cells.

Regardless of the reason for the species differences in homing receptor expression, it is clear that the murine T cell clone is an imperfect model for defining the in vivo immunoregulatory or therapeutic potential of human T cell clones. These findings suggest that human T cell clones may well be able to traffic normally in vivo. This ability may allow human T cell clones to perform more effectively, in both research and therapeutic settings, than their murine counterparts.

Summary

To function efficiently in vivo, lymphocytes must circulate from the blood into lymphoid tissues and other sites of immune reaction. Herein, we show that human cytotoxic and helper T cell clones and lines, maintained in vitro with IL-2, express the functional capacity to recognize and bind to high endothelial venules (HEV), a capacity essential for lymphocyte exit from the blood, and hence for normal lymphocyte trafficking. The expression of functional homing receptors distinguishes human T cell clones from their murine counterparts, which uniformly lack receptors for HEV and are unable to migrate normally from the blood in vivo. The results raise the possibility that human T cell clones may be more effective in mediating in vivo immune responses than is suggested by murine models.

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