Ref. 6325

In press in

Immunobiology of HLA. Vol #2 Immunogenetics and histocompatibility.
B. Dupont, editor. Springer-Verlag, New York.

20

Coordinate-Enhanced Expression of Three Adhesion Molecules (LFA-3, CD2, and LFA-1) and Three Other Molecules (4B4, UCHL1, and Pgp-1) Defines a Human T-Cell Subset Containing Memory Cells and Characterized by Enhanced γ Interferon Production

Martin E. Sanders, Malegapuru W. Makgoba, Susan O. Sharrow, David Stephany, Timothy A. Springer, Howard A. Young, and Stephen Shaw

Abstract: The present report summarizes our experimental findings regarding phenotypic and functional differences between two reciprocal subsets of human peripheral blood T cells: memory cells versus naive cells. Human memory T cells are phenotypically distinguishable from naive T cells by increased expression of six cell-surface molecules (LFA-3, CD2, LFA-1, 4B4, UCHL1, and Pgp-1). Neonatal cord blood consists almost exclusively of naive cells expressing lower levels of these markers, which increase in expression following phytohemagglutin (PHA) activation. As expected, only the high-expressing T cells in adults proliferate in response to recall antigens. Memory cells produce greater than five times more γ interferon with PHA activation than naive cells, although they produce equivalent amount of IL2. Our results integrate findings reported from multiple laboratories regarding different markers (e.g., 4B4, 2H4, HB-11, UCHL1, and Pgp-1),

Immunobiology of HLA
Volume II: Immunogenetics and Histocompatability

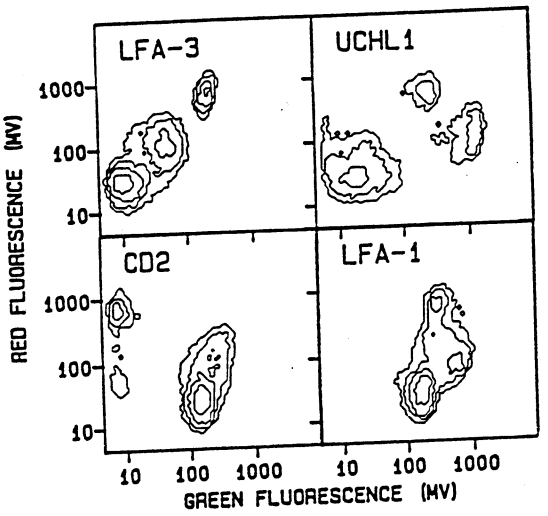

Figure 1. Two-color flow cytometric analysis of adult peripheral blood mononuclear cells for expression of various markers (x axis) vs. 4B4 (axis). Cells were stained with the x-axis MAb, followed by FITC-conjugated goat anti-mouse IgG, followed by blocking with mouse IgG, and subsequent staining with phycoerythrin-(PE) conjugated 4B4. In each panel, the cell population with the highest level of 4b4 represents monocytes.

Table 1. Proliferative Response and Lymphokine Production of LFA-3 Subsets of T Cells

	Proliferation (CPM)		Lymphokine Production with PHA (Units)	
	PHA	Tetanus Toxoid	IL2	IFN-γ
Donor 1				
LFA-3+	15483	2398	67	>50
LFA-3−	39500	0	119	8
Donor 2				
LFA-3+	7977	3401	9	>50
LFA-3−	59616	163	7	8
Donor 3				
LFA-3+	22351	5370	ND	ND
LFA-3−	66124	280	ND	ND

Data previously published in part in reference 10. Cells were electronically sorted after staining with the LFA-3 MAb TS2/9 followed by fluorescein isothiocynate- (FITC) conjugated goat anti-mouse IgG, with >95% purity for each population. Proliferation data are from 3-day cultures for PHA and 6-day cultures for tetanus toxoid. Irradiated autologous monocytes not treated with LFA-3 MAb were used as antigen-presenting cells. Cultures were pulsed with tritiated thymidine overnight prior to harvest. Data represent geometric means of counts per minute minus media control. Lymphokine data are from supernatants collected at 48 hours from PHA-activated cultures of T cells electronically sorted for LFA-3 expression. IL2 was determined using the CTLL-2 asay and IFNγ was measured using a radioimmunoassay (Centocor, Malvern, PA). ND indicates not done.

and demonstrate that these subsets represent different maturational stages of T cells rather than different T-cell lineages.

Antigen-independent adhesion is an important event in T-cell recognition (1,2). We have shown that such adhesion is mediated by at least two independent pathways; one pathway involving T-cell CD2 interaction with target cell LFA-3, and a second pathway involving T-cell LFA-1 interaction with target cell ICAM-1 and other as yet unidentified ligands (2–4). In addition, three of the molecules involved in antigen-independent adhesion, LFA-3, CD2, and LFA-1, each have been demonstrated to have signaling functions potentially of relevance to T-cell activation. LFA-3 interaction with CD2 augments PHA activation of T cells, and MAb to LFA-3 can induce IL1 secretion by monocytes and thymic epithelium (5–7). Certain pairs of MAb to CD2 activate T cells (8), and at least one MAb to LFA-1 has been reported to activate murine T cells (9). Thus, increased expression of these molecules on a subset of T cells could contribute to functional differences of that subset through a variety of mechanisms including enhanced adhesion properties and enhanced transmembrane signaling functions.

We have found enhanced expression of LFA-3, CD2, and LFA-1 on a single subset of T lymphocytes (10,11) and showed that this same subset of T lymphocytes also expresses enhanced levels of three molecules (4B4 or CDw29, UCHL1, and Pgp-1) previously shown by others to identify T-cell subsets that have memory function (12–15). Results of two-color flow cytometric analysis for five of these markers are shown in Figure 1, in which peripheral blood mononuclear cells from a normal donor are stained in each panel with 4B4 on the red axis and one of the other coordinately expressed markers on the green axis. In each panel, the population with highest expression of 4B4 is made up of monocytes, as this population stains with the Leu M3 MAb (10). However, each panel clearly demonstrates two major subsets of lymphoid cells differing in quantity of expression of 4B4. The lymphoid subset with higher expression of 4B4 also shows an enhanced mean expression of LFA-3 (approximately 8-fold), UCHL1 (approximately 30fold), CD2 (approximately 2.8-fold), and LFA-1 (approximately 2.4-fold). Other experiments have shown this same subset has enhanced expression of the human analogue of Pgp-1 and low expression of another molecule, 2H4 or CD45R (10).

If the subset with enhanced expression of LFA-3 and the other coordinately expressed molecules represents memory cells, there should be very few cells of this phenotype in neonatal blood because the fetus has limited antigenic exposure. Flow cytometric analysis of human cord blood showed that essentially all of the T cells were of the low-expression phenotype (10). Furthermore, if the low-expression subset represents naive cells, activation of that subset should lead to phenotypic

Immunobiology of HLA
Volume II: Immunogenetics and Histocompatability

conversion into the subset with high expression. Activation of cord blood T cells with PHA led to nearly uniform enhancement of expression of LFA-3, CD2, LFA-1, 4B4, and UCHL1, and a slight decrease in CD45R expression in 3 days (10). Likewise, activation of adult T cells with PHA led to uniform expression of LFA-3 and enhanced expression of CD2 on T cells by 3 days. This expression persisted at levels equivalent to that seen on the high expression subset in peripheral blood for at least 12 days, after the T cells had lost >85% of the peak expression of IL2 receptor (data not shown).

Proliferation studies performed with adult T cells electronically sorted for LFA-3 expression showed that proliferative response to the memory antigen tetanus toxoid was restricted to the high-expression subset (Table 1). Proliferative response to PHA was consistently better in the low-expression subset. However, despite more vigorous proliferation to PHA, the low-expression subset produced five-fold less γ interferon than the high-expression subset, whereas IL2 production was nearly equivalent or slightly greater in the low-expressing subset (Table 1). These proliferation data are consistent with those reported for cells sorted for expression of 4B4 (12), or UCHL1 (13), or reciprocally sorted for CD45R (16,17). The lymphokine data extend to the human species the finding of enhanced γ interferon production by memory cells, as previously reported for murine T cells sorted for Pgp-1 expression (18).

Acknowledgments. The authors thank Dr. Yoji Shimizu and Dr. Gene Shearer for helpful discussion of the manuscript, and the clinical testing laboratory of Program Resources Inc., NCI/FCRF, Frederick, MD, for assistance with the lymphokine assays.

References

1. Spits H, Van Schooten W, Keizer H, Van Seventer G, Van de Rijn M, Terhorst C, De Vries JE. Alloantigen recognition is preceded by nonspecific adhesion of cytotoxic T cells and target cells. Science 1986;232:403.
2. Shaw S, Luce GEG, Quinones R, Gress RE, Springer TA, Sanders ME. Two antigen-independent adhesion pathways used by human cytotoxic T cell clones. Nature 1986; 323:262.
3. Selvaraj P, Plunkett ML, Dustin M, Sanders ME, Shaw S, Springer TA. The T lymphocyte glycoprotein CD2 (LFA-2/T11/E-rosette receptor) binds the cell surface ligand LFA-3. Nature 1987;326:400.
4. Dustin ML, Sanders ME, Shaw S, Springer TA. Purified lymphocyte function associated antigen-3 (LFA-3) binds to CD2 and mediates T lymphocyte adhesion. J Exp Med 1987;165:677 .
5. Hunig T, Tiefenthaler G, Meyer zum Buschenfelde KH, Meuer SC. Alternative pathway activation of T cells by binding of CD2 to its cell-surface ligand. Nature 1987; 326:298.
6. Bierer BE, Peterson A, Takai Y, Greenstein J, Herrmann S, Seed B, et al. Evidence that LFA-3 activates T cells via the CD2 receptor. Fed Proc 1987;46:1498.
7. Le P, Denning S, Springer T, Haynes B, Singer K. Anti-LFA-3 monoclonal antibody induces interleukin 1 (IL1) release by thymic epithelial (TE) cells and monocytes. Fed Proc 1987;46:447A.
8. Meuer SC, Hussey RE, Fabbi M, Fox D, Acuto O, Fitzgerald KA, et al. An alternative pathway of T-cell activation: A functional role for the 50 kd T11 sheep erythrocyte receptor protein. Cell 1984;36:897.
9. Pircher H, Groscurth P, Baumhutter S, Aguet M, Zinkernagel RM, Hengartner H. A monoclonal antibody against altered LFA-1 induces proliferation and lymphokine release of cloned T cells. Eur J Immunol 1986;16:172.
10. Sanders ME, Makgoba MW, Sharrow SO, Stephany D, Springer TA, Young HA, et al. Three adhesion molecules (LFA-3, CD2, LFA-1) and three other molecules (UCHL1, 4B4, Pgp-1) show increased expression on a human T cell subset containing memory cells and characterized by enhanced gamma interferon production. J Immunol (in press). 1988 140 : 1401
11. Sanders ME, Makgoba MW, Luce GE, Shaw S. T cell adhesion receptors LFA-1 and CD2 and their ligands ICAM-1 and LFA-3: Analysis in adhesion, cell mediated lysis and as markers of T cell subsets. In: Kappler J, Davis M (eds): UCLA Symposia on Molecular and Cellular Biology. New Series. vol. 73 New York, Alan R. Liss, Inc., 1987.
12. Morimoto C, Letvin NL, Boyd AW, Hagan M, Brown HM, Kornacki MM, et al. The isolation and characterization of the human helper inducer T cell subset. J Immunol 1985; 134:3762.
13. Smith SH, Brown MH, Rowe D, Callard RE, Beverley PC. Functional subsets of human helper-inducer cells defined by a new monoclonal antibody, UCHL1. Immunology 1986; 58:63.
14. Budd RC, Cerottini JC, MacDonald HR. Phenotypic identification of memory cytolytic T lymphocytes in a subset of Lyt-2+ cells. J Immunol 1987;138:1009.
15. Budd RC, Cerottini JC, Horvath C, Bron C, Pedrazzini T, Howe RC, et al. Distinction of virgin and memory T lymphocytes. Stable acquisition of the Pgp-1 glycoprotein concomitant with antigenic stimulation. J Immunol 1987; 138:3120.
16. Morimoto C, Letvin NL, Distaso JA, Aldrich WR, Schlossman SF. The isolation and characterization of the human suppressor inducer T cell subset. J Immunol 1985; 134:1508.
17. Tedder TF, Cooper MD, Clement LT. Human lymphocyte differentiation antigens HB-10 and HB-11. II. Differential production of B cell growth and differentiation factors by distinct helper T cell subpopulations. J Immunol 1985; 134:2989.
18. Budd RC, Cerottini JC, MacDonald HR. Selectively increased production of interferon-gamma by subsets of Lyt-2+ and L3T4+ T cells identified by expression of Pgp-1. J Immunol 1987;138:3583.

Author Affiliations

Martin E. Sanders, Malegapuru W. Makgoba, Susan O. Sharrow, David Stephany, Stephen Shaw, the Immunology Branch, National Cancer Institute, Bethesda, MD 20892; *Timothy A. Springer*, the Dana Farber Cancer Institute, Boston, MA 02115; *Howard A. Young*, the Biological Response Modifiers Program, NCI/Frederick Cancer Research Facility, Frederick, MD 21701,USA

Immunobiology of HLA
Volume II: Immunogenetics and Histocompatability