

Bench Notes

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Human Lymphocyte Function Associated Antigens¹

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Introduction

Cytolytic T lymphocytes (CTL) are important effectors in the cell-mediated-response to viruses [1], allografts [2], and some tumors [3]. The definition of cell surface molecules important in the CTL response may elucidate the general mechanisms of cellular recognition, cell interactions, and the 'lethal hit' of cytotoxicity. The use of monoclonal antibodies (mAbs) as probes to detect cell surface molecules important in the murine CTL response has repeatedly demonstrated a role for Lyt-2,3 and LFA-1 in the CTL-target interaction [4–9]. Monoclonal antibodies to OKT3, OKT4, and OKT8 have been shown to inhibit lysis by human CTLs [10–16]. We have generated mAbs by immunizing mice with an OKT4+ CTL line specific for HLA-DR antigens and screened for their ability to block HLA-DR-specific cytolysis. Binding of

mAbs to four types of molecules, LFA-1, LFA-2, LFA-3 and HLA-DR, inhibited killing, suggesting that these molecules participate in the CTL-target interaction [17].

Generation of mAbs Which Recognize Lymphocyte Functional Antigens

44 of 864 cultures were positive for inhibition of killing. Subclones were selected for greater than 30% inhibition of CTL-mediated killing and then tested in an ¹²⁵I-labeled antimouse Ig indirect binding assay [17], and for immunoprecipitation of novel molecules from the CTL line and the target cell line (JY) (fig. 1).

Human LFA-1: A Broadly Distributed Leukocyte Antigen Involved in CTL and NK Cell-Mediated Cytolysis

A strikingly high proportion of the blocking supernatants, 20 of 44, immunoprecipitated polypeptide chains of Mr 177,000 and 95,000 from both the CTL line and JY (fig. 1, lanes 5, 12, 15, 18–21, 23–27, 20, 32, 34, 36, 39 and 42–44). 7 subcloned independent hy-

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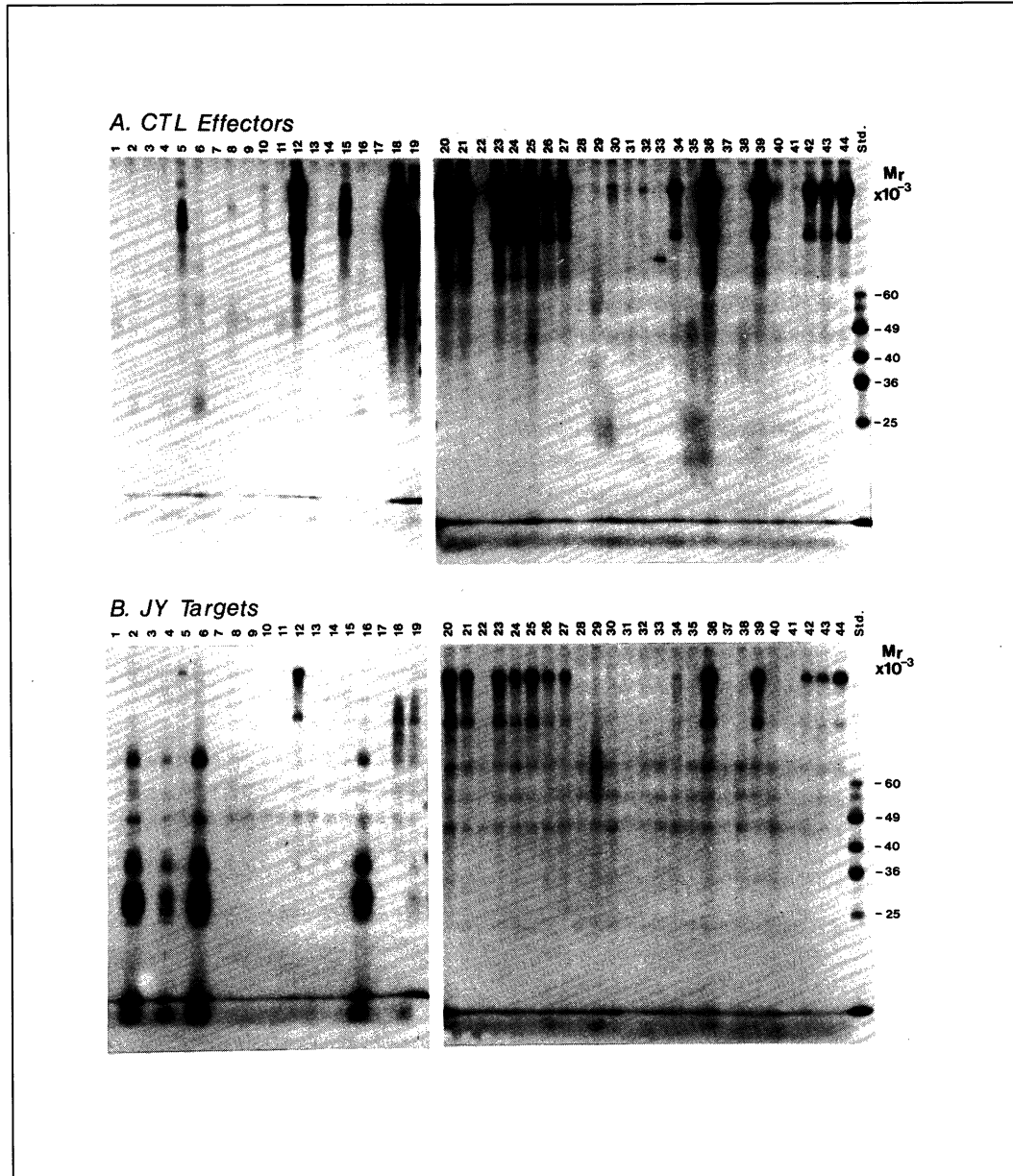


Fig. 1. NaDodSO₄/polyacrylamide gel electrophoresis of ¹²⁵I-labeled antigens immunoprecipitated by CTL-blocking hybridoma culture supernatants. Human anti-HLA-DR CTL (A) or JY target (B) cell lines were surface-labeled with ¹²⁵I by using IODO-GEN. Cell lysates were immunoprecipitated with 50 μ l of hybridoma culture supernatant from 44 selected culture lines: TS1/1-TS1/18, lanes 1-18, respectively; TS1/21 and TS1/22, lanes 19 and 20; TS2/1-TS2/24, lanes 21-44, respectively. Reduced samples were subjected to NaDodSO₄/10% polyacrylamide gel electrophoresis and autoradiography.

Table I. Cell distribution of LFA antigens

	LFA-1	LFA-2	LFA-3
PBL, %	> 95	55	38
Bone marrow, %	37	10	43
Thymes, %	> 82	86	7
Monocytes, %	> 95	0	> 82
Granulocytes, %	> 97	2	96
PHA blasts, %	> 79	84	65
CTL, %	> 91	> 96	95

Table II. Inhibition of function by LFA-monoclonal antibodies

	LFA-1	LFA-2	LFA-3
Inhibition of OKT4+, HLA-DR-specific CTL	+	+	+
Inhibition of OKT8+, HLA-A,B-specific CTL	+	+	+
Inhibition of NK-mediated cytotoxicity	+	-	-
Inhibition of proliferation to PHA	+	+	+
Inhibition of proliferation to MLR	+	+	+

bridomas have been isolated that immunoprecipitate this antigen. One mAb, TS1/18, recognizes the β -chain ($M_r = 95,000$) of LFA-1, while all the other mAbs recognize the α -chain ($M_r 177,000$). Three unique and three partially overlapping epitopes on human LFA-1 have been identified by competitive cross-inhibition binding assays using biosynthetically labelled anti-LFA-1 mAbs [18]. Titration of each of the anti-LFA-1 mAbs in a ^{51}Cr release cytolytic assay re-

vealed quantitative differences in the ability of the different anti-LFA-1 mAbs to block cytotoxicity suggesting distinct functional and antigenic epitopes on the human LFA-1 molecule [18].

The cell distribution of LFA-1 was investigated by FACS analysis (table I) in addition to immunoprecipitation [19]. LFA-1 is expressed on 95% of peripheral blood lymphocytes (PBL), including both T and B cells, as well as essentially all thymocytes, phytohemagglutinin (PHA)-activated blasts, CTL, granulocytes, monocytes, and 37% of bone marrow cells. Immunoprecipitation and FACS analyses show quantitative differences in the expression of LFA-1 on various cell types: CTL and PHA-activated T cells > PBL > thymocytes > B lymphoblastoid cells (JY).

The broad distribution of LFA-1 on lymphoid cells prompted us to assess the ability of LFA-1 mAbs to block a variety of immune responses, including CTL- and NK-mediated cytotoxicity and T cell proliferation to mitogen (PHA) and alloantigen (mixed lymphocyte responses). We found that anti-LFA-1 mAbs significantly inhibit cytotoxicity by OKT8+, HLA-A,B-specific CTL; OKT4+, HLA-DR-specific CTL, and NK cells, as well as the proliferative responses to alloantigens (MLR) and PHA (table II). Complete blocking was achieved with 1–5 $\mu\text{g}/\text{ml}$ of antibody.

Hildreth et al. [20] recently immunized mice with EBV-transformed human lymphoblastoid B cells and generated two mAbs, MHM23 and MHM24, which immunoprecipitate $M_r 180,000$ and $94,000$ polypeptide chains from both B and T cells. Both antibodies were shown to inhibit HLA-restricted lysis of influenza-infected and EBV-transformed target cells by CTL and to inhibit lysis at the level of the effector cell.

LFA-2: The Sheep Red Blood Cell Receptor Molecule Specifically Participates in T Cell Functions

2 of 44 inhibiting hybridoma cultures defined the LFA-2 antigen. These mAbs immunoprecipitate a diffuse band of Mr 49,000 (fig. 1, lanes 8, 35) present on CTL lines but not on B lymphoblastoid target cells. LFA-2 is present on all thymocytes, PHA blasts, and CTL but not granulocytes, monocytes, or B cells (table I). Both FACS analysis and immunoprecipitation demonstrate that LFA-2 is greatest on PHA blasts > CTL > thymocytes > PBL, whereas Leu 4 (OKT3) expression is similar on resting and activated T cells. Detailed studies have shown that LFA-2 is the sheep red blood cell (SRBC) receptor molecule. Anti-LFA-2 mAbs inhibit SRBC rosetting. Side by side immunoprecipitation on SDS-PAGE and preclearing experiments show that LFA-2 is identical to Leu 5 [19]. Anti-LFA-2 mAbs inhibit cytotoxicity by HLA-DR-specific CTL and HLA-A,B-specific CTL, but not NK cells, and block proliferative responses to PHA and the MLR (table II).

Martin et al. [21] recently demonstrated that two mAbs, 9.6 and 35.1, recognize epitopes of the SRBC receptor molecule in close

proximity, but that 9.6 inhibited E rosette formation and cell-mediated cytotoxicity while 35.1 did not. Of note, *Martin* et al. [21] found that 9.6 inhibits NK cell-mediated as well as CTL-mediated cytotoxicity.

LFA-3: A Novel, Broadly Distributed Antigen Associated with Lymphocyte Function

LFA-3 is expressed as 40–60% of PBLs, including both B and T lymphocytes. It is also present on essentially all monocytes, granulocytes, CTL, B lymphoblastoid cell lines, platelets, human vascular endothelial cells, smooth muscle, and fibroblasts [19]. Anti-LFA-3 mAbs block cytotoxicity by OKT4+ CTL and OKT8+ CTL, but by NK cells and inhibits PHA and MLR proliferative responses.

To establish the locus of inhibition by anti-LFA mAbs, effectors of targets were pretreated with LFA-1, LFA-2, or LFA-3 mAbs, washed, and inhibition of cytotoxicity was assessed in a standard ^{51}Cr release assay. Both anti-LFA-1 and LFA-2 mAbs block cytotoxicity by binding to effector cells while anti-LFA-3 mAb blocks cytotoxicity by binding to target cells (table III).

Table III. Locus of blocking by LFA mAbs

	LFA-1	LFA-2	LFA-3	HLA-DR
mAb added to assay	+	+	+	+
Effector cell-pretreated	+	+	–	–
Target cell-pretreated	–	–	+	+

Summary

Three cell surface molecules, designated LFA-1, LFA-2, and LFA-3 were identified by mAbs selected for their ability to block cytotoxicity by an OKT4+, HLA-DR-specific CTL line. The LFA mAbs block all CTL and proliferative functions studied. In addition, anti-LFA-1 mAbs inhibit NK-mediated cytotoxicity. By analogy with murine LFA-1, human LFA-1 may be involved in the adhesion stage of cellular interactions. LFA-2, the SRBC receptor molecule, appears to be a T cell function-specific molecule. We have not yet estab-

lished whether LFA-2 participates in antigen recognition or whether it is involved in antigen-non-specific interactions. The anti-LFA-3 mAb specifically blocks function by binding to the target cells, implying that LFA-3 may be a target ligand for an effector-specific receptor.

The CTL-target interaction involves a number of steps, including antigen recognition, cell adhesion, and delivery of the lethal hit [22]. The LFA antigens show the complexity of this process at the molecular level. The anti-LFA monoclonal antibodies will be useful probes into the T cell immune response and may prove clinically relevant, both diagnostically and therapeutically.

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