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A NOVEL LYMPHOCYTE FUNCTION-ASSOCIATED ANTIGEN (LFA-1): CELLULAR DISTRIBUTION, QUANTITATIVE EXPRESSION, AND STRUCTURE[1]

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We have previously described a monoclonal antibody (MAb), M7/14, which blocks a variety of T cell functions, including CTL-mediated killing, the mixed lymphocyte response, and antigen-specific proliferation. The antigen defined by M7/14 has been designated lymphocyte function-associated antigen one (LFA-1). In this report, LFA-1 has been studied as to cell distribution, surface abundance, structure, and in comparison to other CTL surface antigens. LFA-1 is expressed on lymphoid cells of both the T and the B lineages and on a large fraction of bone marrow cells, but not on exudate macrophages or non-lymphoid tissues. T cells express more LFA-1 than B cells, both in the unstimulated and stimulated states. Compared with unstimulated spleen cells, cytolytic T lymphocyte cell preparations (CTLP) and Con A blasts, but not LPS blasts, show increased LFA-1 expression relative to H-2, and for T cell-containing populations, Lyt-2. M7/14 MAb binds to about 1.5 × 10^4 and 7 × 10^4 LFA-1 sites per average spleen cell or CTLP cell, respectively.

M7/14 MAb binds to CTLP in quantities of 2.5-fold and ies10.4-fold less than H-2 and Thy-1 MAb, respectively; since the latter have little or no effect on CTL function, inhibition of killing by M7/14 MAb is specific for the LFA-1 surface site. M7/14 MAb and a blocking Lyt-2 MAb are bound in similar quantities of CTLP.

LFA-1 is a glycoprotein and consists of 2 noncovalently linked polypeptide chains of 180,000 and 95,000 M_r. The same molecular species as on CTL is present on other T cells and on B cells. The molecular structure and cell distribution of LFA-1 clearly distinguishes it from Lyt-2,3, Ly-5, T145, and T11, which were previously suggested to be either associated with the function of and/or present on the surface of CTL.

In previous reports (1, 2), we described the use of a doubly xenogeneic system for obtaining rat anti-mouse cytolytic T lymphocyte (CTL)[5] monoclonal antibodies (MAb) and screening them for blocking of killing by mouse CTL of rat tumor target cells. One of these MAb, M7/14, gave consistent, nearly complete inhibition of CTL function. Inhibition by trivial means such as binding to the target cell, toxicity, or agglutination of CTL were ruled out. M7/14 also inhibits allogeneic CTL in a number of strain combinations and anti-modified-self CTL. MAb to at least 12 other defined antigens, including Thy-1, H-2, and Ly-5, had little or no effect on CTL-mediated killing. The antigen recognized by M7/14 has therefore been designated a lymphocyte function-associated antigen (LFA-1). M7/14 was also shown to block the mixed lymphocyte response and antigen-specific T cell proliferation, but not B cell mitogenesis or T cell-independent plaque-forming cell responses. In these studies, we also confirmed previous reports (3–7) that antibodies to Lyt-2 antigens block CTL-mediated killing.

Although many anti-CTL antisera have previously been reported to inhibit CTL function (3–12), few antigens characterized for structure and cell distribution have been defined by these studies. The best-defined target molecule for CTL blocking is the Lyt-2,3 antigen complex (3–7). Lyt-2 and Lyt-3 antisera define products of 2 closely linked genetic loci and precipitate polypeptides of 30,000 and 35,000 relative molecular mass (M_r) (13, 14). Lyt-2,3 antigens are expressed on 80% of thymocytes and on a subpopulation of about 30% of peripheral T lymphocytes that includes suppressor cells and CTL (15, and reviewed in 16). Ly-5 (Lyt-4) antisera have also been reported to block T cell-mediated killing (4), but MAb defining a similar or identical antigen failed to block (2). The Ly-5 antigen is associated with polypeptide chains of about 220,000 M_r on B cells and 180,000 and 200,000 M_r on T cells (17–20). Preliminary characterization of LFA-1 antigen showed α- and β-chains of 180,000 and 95,000 M_r, respectively (2).

In this report, the M7/14 MAb has been used to define the cell distribution and surface abundance of LFA-1 relative to other antigens, and the structure of LFA-1 on different lymphoid subpopulations. The results show that LFA-1 is present on B cells, but in greater amounts on T cells, and is increased during T cell activation. In contrast to Ly-5, LFA-1 α- and β-chains have the same M_r, whether isolated from B or T lymphoid sources. The chains are not disulfide-linked.

MATERIALS AND METHODS

Cell preparation. Cells used for ^{125}I and fluorescent labeling were obtained from spleens of C57BL/6J mice according to standard procedures (21, 22). Purified T cells were prepared by depletion of Ig^+ cells on anti-Ig-coated plates, repeating twice the process described by Weinberger *et al.* (23). These cells gave no plaque-forming response *in vitro* to SRBC. For purification of B cells, spleen cells (10^8/ml, RBC-free) in modified minimal essential medium plus 5% fetal calf serum (FCS) were treated with 200 μg/ml of purified anti-Thy-1.2 MAb HO 13.4 (24) for 30 min on ice. After centrifugation at 300 × G for 10 min, cells were resuspended in 1:6 Low-Tox rabbit complement (C; Accurate Scientific, Hicksville, NY) to 10^8 cells/ml and incubated for 45 min at 37°C. After centrifugation, cells were resuspended in additional 1:6 C (1 ml/10^8 cells) containing 200 μg/ml of anti-Thy-1.2, incubated for 45 min at 37°C, and washed 3 times. These cells gave no Con A mitogenic response.

Mitogen stimulation was performed as follows: 1.0 to 1.3 × 10^6 viable normal or purified spleen cells/ml were inoculated in RPMI medium supple-

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[5] Abbreviations used in this paper: BN, brown Norway rat strain; B6, C57BL/6J; CLA, common leukocyte antigen; CTL, cytolytic T lymphocyte; CTLP, specifically sensitized CTL preparations; FACS, fluorescence-activated cell sorter; FI, fluorescence intensity; FITC, fluorescein isothiocyanate; GF SRBC, glutaraldehyde-fixed sheep red blood cells; LFA, lymphocyte function-associated antigen; MAb, monoclonal antibody; MAbCS, MAb-containing culture supernatant; M_r, relative molecular mass (molecular weight); NSISC, P3-NSI/Ag-4-1 culture Supernatant Control containing 50 μg/ml normal rat IgG; PBEBS, phosphate-buffered Earle's balanced saline; PMV, photomultiplier voltage; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

mented with 0.02 mg/ml gentamicin, 5% FCS, 5×10^{-5} M 2-mercaptoethanol, and either 2 μg/ml Con A (for Con A- or T-blasts) (25) or 10 μg/ml LPS-W (*Salmonella typhosis* 0901, Difco) (for LPS- or B-blasts). After 1 day the cells were diluted 1:2 with the same media, and unless otherwise indicated were allowed to grow for a total of 4 days in culture. Before iodination, both unstimulated and blast cells were purified by Ficoll-Isopaque sedimentation in order to deplete red and dead cells (26). Ficoll-Isopaque was not used before fluorescent studies, except for blood lymphocytes and CTL preparations (CTLP). CTLP were prepared as previously described (1). Peritoneal exudate cells were obtained 4 days after injection of 1.5 ml Brewer's thioglycollate medium (Difco).

Fluorescence analysis. For labeling, cells (50 μl, in RPMI 1640, 5% FCS, 20 mM HEPES,[5] 0.01 M NaN_3) were mixed with an equal volume of MAb-containing culture supernatant (MAbCS) in microtiter plates for 30 min and washed 3 times, and the pellets were suspended in 100 μl (blasts and CTL) or 50 μl (other cells) of fluorescein isothiocyanate (FITC) rabbit $F(ab')_2$ anti-rat IgG at 0.24 mg/ml (FITC:protein = 9 μg/mg) for saturation labeling. This reagent had been purified on rat IgG-Sepharose and absorbed with mouse IgG-Sepharose before coupling with FITC. After a further 30 min, cells were washed 3 times, and suspended in 1.5 ml, and held on ice until analysis. Fluorescence did not detectably change during at least 4 hr storage on ice.

Analysis of 50,000 cells/sample was on a Becton-Dickinson fluorescence-activated cell sorter (FACS II) equipped with a Nuclear Data log amplifier. The log amplifier was calibrated by determining the channel of the glutaraldehyde-fixed SRBC (GF SRBC) fluorescence peak at gains 1, 2, 4, 8, and 16 for photomultiplier voltages (PMV) 400, 500, and 600. The amplifier gave close to a logarithmic response above channel 80, but below 80 the number of channels per 2-fold step in gain gradually decreased. Fluorescence intensity (FI) was determined with the calibration curve and is given in units such that GF SRBC at PMV = 600, gain = 1 have FI = 1. Previously, it has been shown that fluorescence as measured on the FACS is proportional to the number of fluorescein molecules/cell and is linear with channel numbers using a linear amplifier (27). We therefore checked the channel number of the GF SRBC fluorescence peak on the linear amplifier and found it linearly related to the gain setting. Fluorescence histograms were stored on magnetic tape for later computer analysis or multiple-exposure photography. Since homogeneous peaks were roughly Gaussian on the log scale, their average FI was determined as the geometric mean (i.e., the arithmetic mean channel number of log-amplified intensities). If distributions were not homogeneous, as for spleen, they were divided into up to 3 regions corresponding to subpopulations of different FI, their means were converted to GF SRBC units, and means of the combined subpopulations were calculated. Analyses were done at gain and PMV settings, which placed fluorescent cells in the truly logarithmic channels. The same cells were often analyzed at several different PMV and gave FI in excellent (within 5%) agreement.

Indirect binding assay. Indirect binding assay was done as previously described (22). Tissues were minced and homogenized in PBS with a Teflon pestle homogenizer, washed twice in PBS at 1000 × G for 5 min, and suspended in PBS for protein assay or PBS + 10% BSA for binding assay. Lymphoid cell suspensions were prepared and similarly suspended at 10^8 cells/ml. Protein was determined according to Lowry *et al.* (28). Binding was determined with M7/14 MAbCS, and as control, the M1/69.16.11 HK inactive specific light (L) chain loss variant (29) MAbCS.

Iodination, precipitation, and electrophoresis of cell surface proteins. After 3 washes with cold phosphate-buffered Earle's balanced saline, pH 7.2 (PBEBS), the cells were vectorially iodinated by a modification of the chloroglycoluril method (30): 3×10^7 cells (viability >90%) in 1 ml of PBEBS at room temperature were added to a glass scintillation vial (25 x 45 mm) previously coated with 200 μg of 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (IODO-GEN, Pierce). $Na^{125}I$ (1 mCi carrier-free, New England Nuclear) was added, and after 10 min of gentle agitation at room temperature, the sample was transferred to a tube containing 250 μl of 0.4 mg tyrosine/ml and then washed 3 times with 8 ml of ice-cold PBEBS. For solubilization, cells were resuspended in 2 ml of 10 mM Tris-HCl, pH 7.5 at 20°C, 1% Triton X-100 (Sigma), 1% hemoglobin, and 1 mM phenylmethylsulfonylfluoride and allowed to stand at 4°C for 30 min. The supernatant, centrifuged at 100,000 × G for 1 hr, was dialyzed *vs* 0.14 M NaCl, 0.01 M Tris-HCl, pH 7.3 at 20°C, 0.05% NaN_3. Yields were 2 to 3% of input ^{125}I. Samples were precleared as previously described (22) and either used immediately or stored at −70°C after addition of 20% (v/v) glycerol. Immunoprecipitation and gel electrophoresis of the solubilized immunoprecipitates were carried out as described (22). Autoradiography on hypersensitized Kodak XR film was performed according to Laskey and Mills (31).

Coupling of M7/14 IgG to Sepharose. Purified M7/14 IgG (1) was coupled to CNBr-activated Sepharose CL-4B (Pharmacia) according to Cuatrecasas (32).

RESULTS

Cell distribution. The cellular distribution of LFA-1 was studied using M7/14 MAb, FITC anti-rat IgG 2nd antibody, and the FACS. Essentially all spleen nucleated cells, blood lymphocytes, thymocytes (Fig. 1), and lymph node cells (data not shown) were M7/14$^+$. Thus, LFA-1 appeared to be present on lymphocytes of both T and B lineages. Furthermore, nucleated bone marrow cells were 79% M7/14$^+$ (Fig. 1*e*). Since lymphocytes constitute only 25% (33) of bone marrow, this suggests that cells of the myeloid series were also M7/14$^+$. However, not all leukocytes were M7/14$^+$; only 6% of thioglycollate-induced peritoneal exudate macrophage-sized cells bore LFA-1 (Fig. 1*f*). In contrast, the same cells were 94% labeled by the granulocyte + macrophage-specific M1/70 antibody (22, data not shown).

The distribution of LFA-1 in nonlymphoid tissues was investigated using tissue homogenates in the indirect binding assay (Fig. 2). Thymus, spleen, and bone marrow gave strong binding, whereas lung, liver, brain, and kidney gave no significant binding (at a 10- to 100-fold higher protein concentration than that at which lymphoid tissues gave significant binding). In contrast, the M5/49 anti-Thy-1 MAb gave strong binding to brain homogenates (data not shown). LFA-1 was not expressed on erythrocytes, as determined both in indirect binding assays and FACS analysis

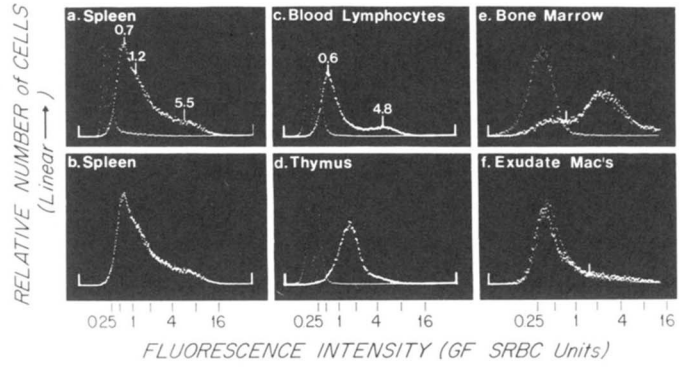

Figure 1. M7/14 labeling of different cell populations. Cells were labeled with an equal volume of either 94 μg/ml M7/14 MAbCS (bright curves) or NSISC as control (dim curves) *a*, *c* to *f*), then with FITC anti-rat IgG absorbed with mouse IgG as described in *Materials and Methods*. In *b*, the curves after labeling with 1 mg/ml M7/14 pure IgG (bright) and 94 μg/ml M7/14 MAbCS (dim) are superimposed. *a*, *b*, spleen cells (scatter-gated to exclude erythrocytes); *c*, Ficoll-Hypaque purified blood lymphocytes (no monocytes present); *d*, thymocytes; *e*, bone marrow cells (scatter-gated to exclude erythrocytes); *f*, 4 day thioglycollate-induced peritoneal exudate cells, scatter-gated to include primarily macrophages (large cells). Note the change in fluorescence scale between *a* to *d* and *e* and *f*. Markers in *a* and *c* show fluorescent intensities, those in *e* and *f* indicate the thresholds for positive cells.

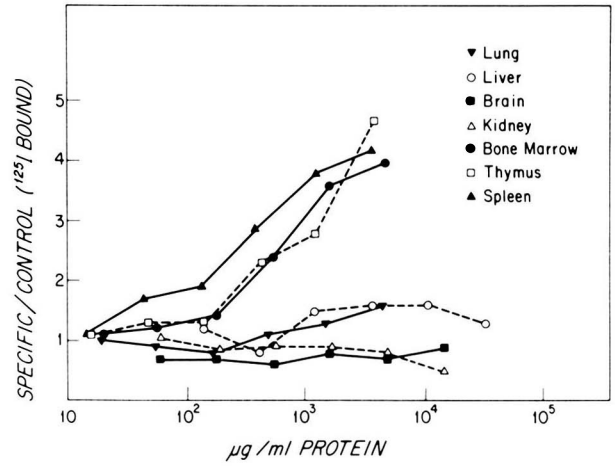

Figure 2. M7/14 tissue distribution measured by the ^{125}I-anti-rat IgG indirect binding assay. Washed tissue homogenates or cell suspensions were used as targets in the indirect binding assay as described in *Materials and Methods*. Specific/control (^{125}I bound) = the ratio of binding of M7/14 (*specific*):M1/69.16.11 HK (*control*). Background binding was >8,000 and <17,000 for all concentrations of testes, lung, spleen, bone marrow, and thymus, and at the highest concentrations of liver, kidney, and brain was 25,000, 37,000, and 32,000, respectively, and decreased to 10,000 to 8,000 cpm at the lowest concentrations.

(data not shown). Thus, LFA-1 does not appear to be expressed in nonleukon tissues.

The expression of LFA-1 on different cell types and compared with other surface markers has been studied in quantitative fluorescence experiments. All experiments reported here have been carried out under conditions that have been shown to be saturating for the 1st antibody (for M7/14, see Fig. 1*b*) and for the 2nd antibody (see below, Table II, footnote c). Response characteristics of the FACS log amplifier have been calibrated with GF SRBC, and average FI were calculated by integration (see *Materials and Methods*). Thus all FI reported here, although in arbitrary GF SRBC units, are directly proportional to the number of MAb bound per cell. The average FI of M7/14-labeled cells in spleen, thymus, blood, and bone marrow was similar (Table I, Expt. 1). However, distinct subpopulations could be observed in spleen and blood lymphocytes (Fig. 1*a*, *c*), which differed up to 8-fold in the quantity of LFA-1 expressed. In spleen, 2 poorly resolved peaks of FI = 0.7 and 1.2, in addition to a shoulder of FI = 5.5, were noted (Fig. 1*a*), whereas blood lymphocytes showed major and minor peaks with FI = 0.6 and 4.8, respectively.

To analyze further the heterogeneity of spleen cell LFA-1 expression, B and T cells were purified as described in *Materials and Methods* and labeled with M7/14 (Fig. 3). Purified B cells (Fig. 3*c*) appear to correspond to the most weakly staining spleen cell peak at FI = 0.7 and constitute a single, homogeneous peak. Purified T cells are themselves heterogeneous and contain 2 subpopulations, a peak of about two-thirds of the total cells at FI = 1.2 and a peak of about one-third of the cells at FI = 4.7. These appear to correspond to the intermediate and bright subpopulations in whole spleen (Fig. 3*a*). The average LFA-1 FI of purified T cells is 3.5-fold greater than that of B cells (Table I, Expt. 2).

The expression of LFA-1 antigen on normal spleen cells, CTLP, and activated T and B cells was also investigated, with 2 aims. The first was to determine whether specific increases in LFA-1 relative to other markers occur. Second, by comparing the density of LFA-1 to other surface markers, antibodies bound to which do not inhibit CTL function, it should be possible to determine whether CTL inhibition is due to binding to a specific surface site, or might simply be related to the density of antibody bound to the surface. For saturation-labeling experiments, anti-LFA-1, H-2, Thy-1, and Lyt-2 rat MAb, all of the IgG2a subclass, were selected to ensure a constant ratio of 1st to 2nd antibodies.

Spleen cells expressed low amounts of LFA-1 relative to H-2 and Lyt-2 (Fig. 4*a*). In contrast, CTLP expressed much greater amounts of LFA-1, whereas H-2 and Lyt-2 were little increased (Fig. 4*b*). Two CTLP subpopulations were defined by M7/14 MAb. A dim subpopulation (15%) expressed amounts of LFA-1 similar to those on most spleen cells, whereas a bright subpopulation (85%) expressed about 6-fold more. Con A blasts, but not LPS blasts, also showed increased expression of LFA-1 relative to H-2 (Fig. 4*c*, *d*; note the change in fluorescence scale). Quantitation of fluorescence (Table II) showed that LFA-1 expression increased 5-fold on CTLP and Con A blasts compared with normal spleen cells, whereas H-2 increased only 2-fold. On CTLP and Con A blasts, LFA-1 expression also increased relative to Lyt-2, and was similar to the increase in Thy-1. The increase in LFA-1 expression on CTLP and Con A blasts paralleled an increase in the percentage of Thy-1$^+$, Lyt-2$^+$ cells (Table II).

Of all 4 markers compared here, LFA-1 is expressed in the lowest quantity on all cell populations examined. On C57BL/6J (B6) anti-P815 CTLP, LFA-1 was expressed in an order-of-magnitude quantity lower than Thy-1, 2.5-fold less than H-2, and 1.5-fold less than Lyt-2. When the bright LFA-1$^+$ CTLP subpopulation (marked in Fig. 4*b*) was studied rather than total LFA-1$^+$ CTLP, it was found to have an FI 1.3-fold lower than the Lyt-2$^+$ subpopulation (Fig. 4*b*). Another preparation of B6 anti-P815 CTLP (both large and small cells were studied separately by scatter gating), and large cells in B6 anti-BN rat lymphoma CTLP preparation

TABLE I

M7/14 fluorescent labeling of different tissues

Expt. No.		Positive Cells	Average Specific Fluorescence Intensity[a]
		%	*GF SRBC units*
1	Spleen	97	1.7
	Blood lymphocytes	95	1.6
	Thymocytes	97	1.5
	Bone marrow	79	1.9[b]
	Exudate macrophages	6	NA[c]
2	Spleen	>88	1.5
	Purified splenic T cells	98	2.5
	Purified splenic B cells	>86	0.7

[a] Cells were stained with a 1st step of M7/14 supernatant and a 2nd step of FITC-anti-rat IgG. The background fluorescence of controls receiving NSISC in the first step, which ranged from 0.15 to 0.3, has been subtracted.

[b] Average of positive cells.

[c] Not applicable.

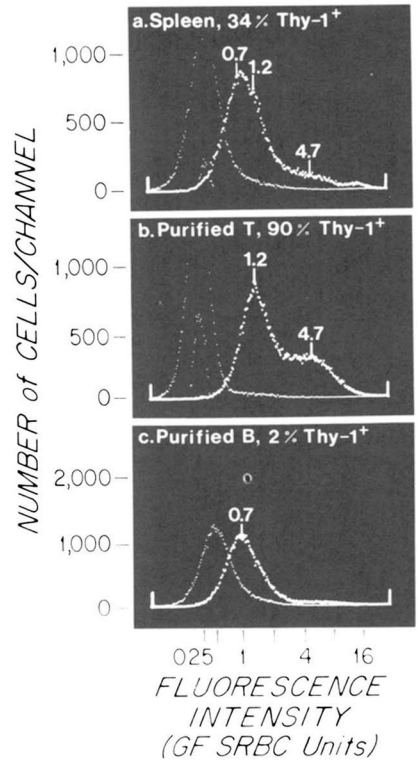

Figure 3. LFA-1 expression on spleen cells and purified splenic B and T cells. B and T cells were purified as described in *Materials and Methods*, labeling with M7/14 (bright) or NSISC (dim) and FITC anti-rat IgG. Fifty thousand cells were counted per analysis. The percentage of Thy-1$^+$ cells was determined with the M5/49 anti-Thy-1 MAb (1). Numbers assigned to the peaks are the specific fluorescence intensities (background fluorescence was subtracted).

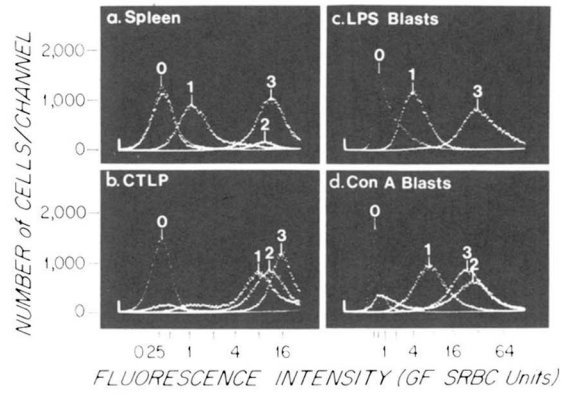

Figure 4. LFA-1 expression relative to H-2 is increased after T cell but not B cell activation. Cell populations were analyzed on the FACS after labeling with NSISC (control, curve 0), M7/14 MAbCS (LFA-1, curve 1), 53.6 MAbCS (Lyt-2, curve 2), or M1/42 MAbCS (H-2, curve 3). Peaks, *not* average specific FI of populations are marked. *a*, B6 spleen cells; *b*, B6 *in vitro* (7d) stimulated anti-P815 CTLP, $R^{2h}_{50\%}$ = 2.0 (1); *c*, 3 day LPS blasts; *d*, 3 day Con A blasts. Blasts were scatter-gated so that only blast (large) cells were counted.

showed a similar well-defined dull and bright LFA-1$^+$ subpopulations (data not shown). In all these cases where the bright LFA-1$^+$ subpopulation was defined, its FI was similar to that of the Lyt-2 subpopulation. The small cells in B6 anti-BN rat lymphoma CTLP did not show resolved dull and bright LFA-1$^+$ subpopulations, had a low percentage of Lyt-2$^+$ cells, and showed even smaller amounts of LFA-1 relative to Lyt-2.

Biochemical characterization. The M7/14 MAb has previously been shown to inhibit a number of T cell, but not B cell, functional responses (1, 2). Immunofluorescence demonstrated in the present report the expression of the LFA-1 determinant on cells of both T and B lineages, but it was possible that the structure of the antigen associated with the LFA-1 determinant might differ on these cells. Therefore, LFA-1 was isolated from different lymphocyte subpopulations, i.e., Con A-stimulated purified splenic T cells, thymocytes, LPS-stimulated purified splenic B cells, and unstimulated splenic B cells (Fig. 5*A*, *B*, *C*, and *D*, respectively, lane 2). Lysates of ^{125}I-surface-labeled cells were immunoprecipitated with M7/14 IgG and analyzed by SDS-PAGE and autoradiography (Fig. 5). In all cases, polypeptides of 180,000 and 95,000 M_r were precipitated. Lyt-2 and Thy-1 antigens could be immunoprecipitated from the purified T but not B lymphocyte populations (Fig. 5*A–D*), suggesting that the LFA-1 antigen precipitated from B cell preparations was not due to contaminating T lymphocytes. The same polypeptides of 180,000 and 95,000 M_r were also precipitated by M7/14 from normal spleen cells, pure normal splenic T cells, and Con A- and LPS-stimulated normal spleen cells (data not shown). However, nothing was precipitated by M7/14 from ^{125}I-labeled peritoneal exudate macrophages (data not shown), which were also negative by immunofluorescence (Fig. 1*f*). Direct comparison by immunoprecipitation showed that the molecular properties of LFA-1 antigen are clearly distinct from the Lyt-2, Thy-1, and H-2 antigens (Fig. 5*A*, *B*), which are also present on CTL. The LFA-1 polypeptides were also directly compared with and found to be distinct from the following antigens (Kürzinger *et al.*, unpublished): Lyt-1 antigen (70,000 M_r) and 2 antigens that are of high M_r and do not appear associated with CTL function (1), M7/83 antigen (95,000 M_r) and M7/7 antigen (140,000 and 250,000 M_r).

The relative abundance for different cell types of LFA-1 polypeptides was estimated by densitometer scanning of film blackening (31). The ratio of intensities of the 180,000 M_r band *vs* the 95,000 M_r band ranged between 2.5 and 4.5. The amount of LFA-1 on T blasts, as estimated from the intensity of the 180,000 M_r

TABLE II

Relative number of LFA-1 sites compared to H-2, Thy-1, and Lyt-2 on normal and activated cells[a]

	Average specific fluorescence intensity[b]				Relative number, other markers/LFA-1			T Cell Markers, Positive Cells	
	GF SRBC units							%	
Cell population	LFA-1	H-2	Thy-1	Lyt-2	H-2 / LFA-1	Thy-1 / LFA-1	Lyt-2 / LFA-1	Thy-1	Lyt-2
Spleen	1.5	10	21	6.7	6.7	14.0	4.5	34	11
CTLP[c]	7.2	18	75	10.5	2.5	10.4	1.5	86	84
Con A blasts	7.8	23	108[d]	23.0	2.9	13.8	2.9	87	78
LPS blasts	3.4	32	NA[e]	NA	9.4	NA	NA	3	ND[f]

[a] Antibodies used for labeling were all rat MAb of the IgG2a subclass: LFA-1, M7/14; H-2, M1/42; Thy-1, M5/49; Lyt-2, 53.6. FITC anti-rat IgG was used as the 2nd step reagent.

[b] Average intensity calculated for positive cells only (100% for LFA-1 and H-2, as indicated for Thy-1 and Lyt-2). Control fluorescence (NSISC) has been subtracted.

[c] C57BL/6 anti-P815 CTLP, primed *in vivo*, 7 days after restimulation *in vitro*, CTL activity: $R^{2h}_{50\%} = 2.0$ (1).

[d] Staining with 2-fold greater amount of FITC anti-rat IgG reagent increased the intensity by <5% establishing that the FITC reagent was in excess.

[e] Not applicable.

[f] Not done.

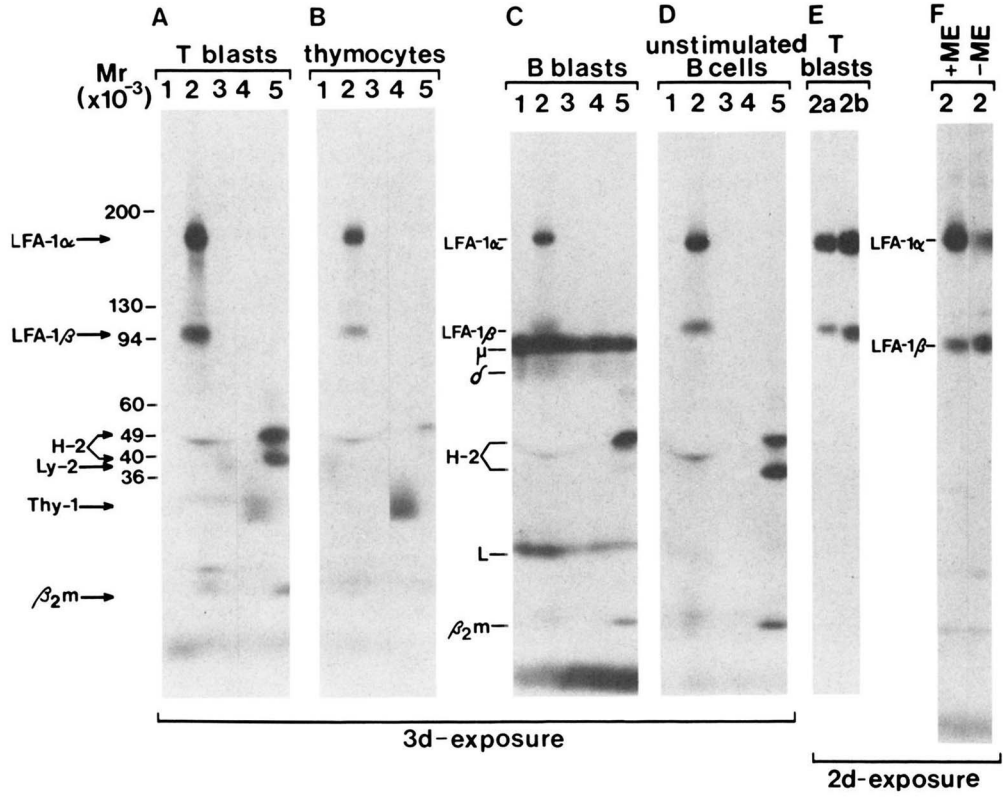

Figure 5. Immunoprecipitation and SDS-PAGE of LFA-1 and other antigens from different lymphoid cell types. Equal quantities (200,000 cpm) of solubilized antigens from ^{125}I-surface-labeled cells were indirectly precipitated with 50 μl of NSISC as control (*lane 1*), 20 μl of 1 mg/ml purified M7/14 IgG (*lane 2*), 40 μl of anti-Lyt-2 53-6.72 MAbCS (14) (*lane 3*; 4 μg of IgG), 50 μl of anti-Thy-1 M5/49 MAbCS (*lane 4*; 5 μg of IgG), or 30 μl of anti-H-2 M1/42 MAbCS (*lane 5*; 2 μg of IgG). As precipitating antibody, saturating amounts of rabbit anti-rat IgG were used. For *lanes 2a* and *2b* direct immunoprecipitation was done with 2.5 and 5 μg, respectively, of M7/14 IgG coupled to Sepharose. Cells were Con A-stimulated purified splenic T cells (T blasts) (*A*, E, F), thymocytes (*B*), LPS-stimulated purified splenic B cells (B blasts) (*C*), and unstimulated purified splenic B cells (*D*). Samples were dissociated with SDS buffer containing 5% 2-mercaptoethanol and subjected to SDS 5–15% gradient PAGE and autoradiography, except in F, one sample was treated with 50 mM iodoacetamide instead of 2-mercaptoethanol and electrophoresed in a separated lane. In panel C, μ, δ, and L chains of surface Ig were precipitated by cross-reaction with rabbit anti-rat IgG. M_r standards are as in Figure 6. A–B, C–E, and F are from three different gels.

band, consistently exceeded that on B blasts, unstimulated spleen cells, and thymocytes by a factor of 2 to 3 (data not shown). The percentage of incorporated ^{125}I precipitable as LFA-1 gradually increases up to day 4 of Con A stimulation (data not shown). T blasts also express little or none of the μ- and δ-chains, which cross-react with the rabbit anti-rat IgG 2nd antibody and migrate near the LFA-1 β-chain. Thus, T blasts are the cells of choice for studying LFA-1.

In addition to the prominent bands of 180,000 and 95,000 M_r, immunoprecipitation with M7/14 and 2nd antibody usually brought down minor bands running near the cold IgG heavy chain front and near the dye front. These minor species did not show up, however, when immunoprecipitation was carried out with Sepharose-coupled M7/14 IgG (Fig. 5*E*), demonstrating that the 180,000 and 95,000 M_r polypeptides are the only ones specifically recognized by M7/14.

M7/14 also precipitated the 2 polypeptides of 180,000 M_r and 95,000 M_r from lysates of Con A-stimulated spleen cells biosynthetically labeled with [^{35}S] methionine (data not shown).

Electrophoresis under nonreducing conditions (Fig. 5*F*) showed that the 180,000 and 95,000 M_r polypeptides are not linked by interchain disulfides.

To determine more reliably the M_r of the LFA-1 polypeptides, the immunoprecipitated antigen along with M_r markers in the same lanes were subjected to electrophoresis on gels of 5, 6.25, and 7.5% polyacrylamide (Fig. 6). On these 3 gels the M_r of the α-chain of LFA-1 varied between 170,000 and 185,000, mean = 177,000; the M_r of the β-chain varied between 93,000 and 98,000, mean = 96,000. We therefore refer to the LFA-1 α and β polypeptides as 180,000 and 95,000 M_r, respectively.

Since LFA-1 is expressed on a variety of lymphoid cells and is of high M_r, it was of interest to compare it directly with other antigens of widespread cell distribution and high M_r, namely the common leukocyte antigen (CLA) recognized by the monoclonal antibodies M1/9.3 and M1/89.18 (34–36), and T-200, recognized by the monoclonal antibody I3/2 (37) (Fig. 7). This comparison was particularly important because antiserum to Ly-5 (Lyt-4) has recently been reported both to detect a polymorphism of the T200 glycoprotein (17, 19) and to inhibit CTL function (4). M1/9.3, M1/89.18, and I3/2 all appear to recognize the same antigen, expressed as a polypeptide of 230,000 M_r on B blasts and as polypeptides of 200,000 and 180,000 M_r on T blasts. The 180,000 and 95,000 M_r polypeptides of LFA-1 are clearly distinct from those of Ly-5 (= T200 = CLA). Furthermore, in contrast to the CLA (T200) polypeptides, the M_r of LFA-1 polypeptides is invariant on T and B blasts as seen by side-by-side comparison.

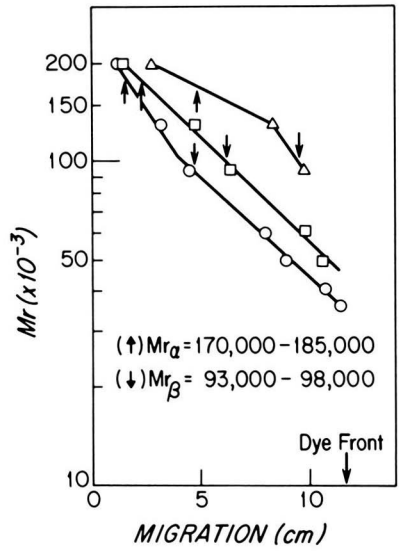

Figure 6. M_r determination of LFA-1 polypeptides. LFA-1 was immunoprecipitated from Con A-stimulated, ^{125}I-labeled spleen cells by incubation with Sepharose-coupled M7/14 IgG. M_r markers and M7/14 immunoprecipitates were both run in the same lane and also separately in adjacent lanes. The distance of migration of the specific bands was the same in both cases. SDS-PAGE after reduction was in 5% (△), 6.25% (□), and 7.5% polyacrylamide (○) gels. (↑) and (↓) indicate the position of LFA-1α and LFA-1β chains, respectively. The following [^{14}C]-carboxymethylated (34) proteins were used as M_r markers: myosin (200,000), β-galactosidase (130,000), phosphorylase-a (94,000), catalase (60,000), fumarase (49,000), creatine kinase (40,000), and glyceraldehyde-3-phosphate-dehydrogenase (36,000).

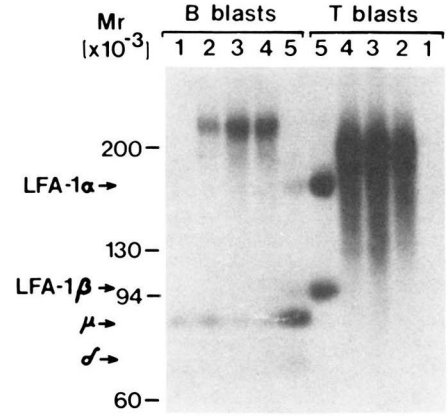

Figure 7. SDS-PAGE comparison of LFA-1, CLA, and T200 precipitated from B and T blasts. Material was immunoprecipitated with NSISC as control (*lane 1*): M1/9.3 anti-CLA MAb (*lane 2*); M1/89.18 anti-CLA MAb (*lane 3*); I3/2 anti-T200 MAb (37) (*lane 4*); or M7/14 anti-LFA-1 MAb (*lane 5*). SDS-PAGE in 5 to 15% polyacrylamide gradient gels was after reduction, and only the relevant upper half of the gel is shown. The amount of lysate subjected to immunoprecipitation was 200,000 cpm for M7/14 MAb, and 72,000 cpm for the control, M1/9.3, M1/89.18, and I3/2 MAb. Therefore, the μ and δ bands cross-reacting with the 2nd rabbit anti-rat IgG antibody are more intense in lane 5 of the B blasts. The M7/14 β chain in lane 5 of the B blasts is less intense than and directly above the μ-chain.

DISCUSSION

In previous reports it was shown that in the absence of complement, M7/14 MAb potently inhibits a number of T cell functional activities, including CTL-mediated killing, the mixed lymphocyte response, and antigen-specific T cell proliferation. Two B cell activities, the mitogenic response to LPS and the plaque-forming cell response to a T-independent antigen, TNP-Ficoll, were unaffected. Inhibition of CTL function was highly specific, since it was not produced by MAb to H-2, Thy-1, CLA (Ly-5), an antigen linked to Ly-6, and at least 7 other distinct antigens (1, 2).

In this report the cellular expression and biochemical properties of LFA-1 were studied. Immunofluorescence studies show that LFA-1 antigen is expressed not only on T cells in thymus, spleen, and after activation, but also on normal and activated B cells and on bone marrow cells. Expression of LFA-1 antigen on B cells, and its apparent identity to T cell LFA-1 were also confirmed in immunoprecipitation and SDS-PAGE experiments. Labeling of 79% of nucleated bone marrow cells implies expression on nonlymphoid cells, which probably include granulocytic precursors (33). Not all leukocytes express LFA-1; only 6% of peritoneal exudate macrophage-rich cells are M7/14 positive. LFA-1 does not appear to be expressed by nonlymphoid tissues such as lung, liver, kidney, and brain. It is interesting that the CTL-blocking activity of a xenoantiserum described by Rabinowitz *et al.* (10) could be removed by absorption with B cells. It is possible that LFA-1 was one of the surface components recognized by this complex antiserum, although no biochemical characterization was reported.

Splenic T cells express 3.5-fold more LFA-1 than B cells, and are themselves heterogenous in LFA-1 expression. About one-third of the splenic T cells express 4-fold more LFA-1 than the other two-thirds. Upon T cell activation by Con A for 3 days or *in vitro* stimulation of CTLP, LFA-1 expression is specifically increased 2.5-fold relative to H-2. This also coincides with an increase in the percentage of Lyt-2^+ cells. The possibilities that the splenic T cell subpopulation with high LFA-1 expression is identical to the Lyt-2^+ subpopulation, and that this subpopulation with a relatively high ratio of LFA-1 to H-2 is expanded by Con A blastogenesis or CTL generation will be tested in future experiments.

The relative surface abundance of LFA-1, H-2, Thy-1, and Lyt-2 antigens was determined by saturation labeling with MAb all of the same subclass and FITC anti-rat IgG and FACS analysis. Values

were expressed in arbitrary FI units (Table II), and may be standardized by comparison with published values for H-2. Liberti *et al.* (38) found 2×10^5 to 5×10^5 H-2 sites per cell for LPS blasts, whereas we found an FI = 32. This suggests that the values in Table II could be multiplied by 10^4 to estimate the number of MAb bound per cell. The values for Thy-1 and Lyt-2 antigens on spleen cells thus obtained, 21×10^4 and 6.7×10^4, are in good agreement with the values of 37×10^4 and 6.5×10^4, respectively, recently published by Ledbetter *et al.* (15). Thus, the average spleen cell appears to express 1.5×10^4 LFA-1 sites/cell, and dim and bright splenic T cell subpopulations 1.2×10^4 and 5.5×10^4 sites/cell, respectively. CTLP and Con A blasts appear to express 7.2×10^4 and 7.8×10^4 sites/cell, respectively.

Comparisons between LFA-1, H-2, and Thy-1 clearly show that the inhibitory effect on CTL of M7/14 MAb must require binding to a specific cell surface site (LFA-1). Binding of M7/14 MAb gives 90% inhibition of CTL function, whereas binding of 2.5-fold or 10.4-fold more anti-H-2 or anti-Thy-1 MAb, respectively, has little or no effect on CTL function (2). A MAb to rat T helper cells (W3/25) has been described (39) that blocks the mixed lymphocyte response by binding to a similar number of sites (2.2×10^4) per cell as M7/14 does. This antigen is restricted to T helper cells and thus does not seem analogous to LFA-1.

Titration of M7/14 MAb in functional assays previously showed half-maximal inhibition of CTL-mediated killing and the mixed lymphocyte response at 4×10^{-9} and 1.5×10^{-9} M, respectively (1). This represents about a 17-fold excess of M7/14 MAb over antigenic sites in both cases, although the actual excess may be lower, because only approximately one-fourth of M7/14 HLK MAb would be in the active H_2L_2 form. This suggests a Ki (inhibition constant) for M7/14 $H_2L_2 \leq 10^{-9}$ in these assays.

The LFA-1 antigen contains polypeptide chains of 180,000 and 95,000 M_r. The 2 chains are not linked by disulfide bonds. Peptide mapping by limited proteolysis and SDS-PAGE (40) has revealed the α- and β-chains have distinct peptides (Kürzinger *et al.*, unpublished), and thus it appears unlikely that the β-chain is derived from the α-chain by proteolysis. Our working hypothesis is that these 2 chains are noncovalently associated on the cell surface. Synthesis of the chains in the presence of an inhibitor of glycosylation, tunicamycin, results in a lower M_r for both α- and β-chains and thus suggests they are glycoproteins (unpublished data).

CLA, T200, and LFA-1 antigens have been directly compared. Ly-5 has been shown to be identical to the T200 antigen defined by I3/2 MAb (17, 19). Side-by-side comparison of the distinct polypeptides precipitated from T and B blasts by this MAb and the M1/89.18 and M1/9.3 MAb defining CLA confirm the previous suggestion (36) that CLA and T200 (Ly-5) are identical. This was important to establish, because conventional antisera to Ly-5 (previously known as Lyt-4) have been reported to block CTL-mediated killing (4), whereas the M1/89.18 and M1/9.3 MAb defining CLA (T200, Ly-5) were found not to block CTL function (2). The reason for this discrepancy is not understood. Although Ly-5 (T200, CLA) and LFA-1 are both widely distributed antigens, they clearly differ in polypeptide M_r (Fig. 7). Ly-5 is expressed as 180,000 and 200,000 M_r polypeptides on T cells, but as a 220,000 M_r polypeptide on B cells (17–20). In distinction to Ly-5, LFA-1 displays the same M_r whether isolated from T or B cells. LFA-1 also does not resemble in M_r any other previously reported lymphocyte surface antigens, including T11 (41), Lgp100 (14), Lyt-1 (16), T145 (42), and a polypeptide complex of 140,000 and 250,000 M_r (1).

The antigen most similar to LFA-1 in M_r is Mac-1, identified by the M1/70 MAb, which contains chains of approximately 190,000 and 105,000 M_r (22). However, Mac-1 is expressed on peritoneal macrophages but not on T and B lymphocytes, whereas LFA-1 has the opposite distribution. Furthermore, immunoprecipitation studies with M1/70 and M7/14 MAb show no cross-reaction between LFA-1 and Mac-1 antigens (unpublished observations). Thus, the Mac-1 and LFA-1 antigens are distinct in both cell distribution and antigenic determinants.

Previously, the M7/14 MAb defining LFA-1 has been found to specifically block a number of T cell functional responses and T-dependent B cell responses, but not T-independent B cell responses or B cell mitogenesis. These studies that suggested LFA-1 participated in or was closely associated with T cell function, but did not bear on its cellular distribution. The present findings that LFA-1 is present on myeloid and B cells as well as T cells are thus of considerable interest. They suggest that LFA-1 is not the T cell antigen receptor.

M7/14 was previously shown to block conjugate formation between CTL and target cells, suggesting that LFA-1 may participate in adhesion. It has been speculated that M7/14 could block an adhesion step closely linked to major histocompatibility complex-restricted recognition by CTL of H-2 on target cells and by helper cells of Ia on macrophages. The finding that M7/14 MAb binds to B cells but has no effect on T-independent PFC responses and LPS mitogenesis lends further support to the ideas that M7/14 MAb is not toxic to functional cells and blocks crucial interactions with other cells.

REFERENCES

1. Davignon, D., E. Martz, T. Reynolds, K. Kürzinger, and T. A. Springer. 1981. Monoclonal antibody to a novel lymphocyte function-associated antigen (LFA-1): Mechanism of blocking of T lymphocyte-mediated killing and effects on other T and B lymphocyte functions. J. Immunol. 127:590.
2. Davignon, D., E. Martz, T. Reynolds, K. Kürzinger, and T. A. Springer. 1981. Lymphocyte function-associated antigen 1 (LFA-1): A surface antigen distinct from Lyt-2,3 that participates in T lymphocyte-mediated killing. Proc. Natl. Acad. Sci. In press.
3. Shinohara, N., and D. H. Sachs. 1979. Mouse alloantibodies capable of blocking cytotoxic T-cell function. I. Relationship between the antigen reactive with blocking antibodies and the Lyt-2 locus. J. Exp. Med. 150:432.
4. Nakayama, E., H. Shiku, E. Stockert, H. F. Oettgen, and L. J. Old. 1979. Cytotoxic T cells: Lyt phenotype and blocking of killing activity by Lyt antisera. Proc. Natl. Acad. Sci. 76:1977.
5. Fan, J., A. Ahmed, and B. Bonavida. 1980. Studies on the induction and expression of T cell-mediated immunity. X. Inhibition by Lyt-2,3 antisera of cytotoxic T lymphocyte-mediated antigen-specific and -nonspecific cytotoxicity: evidence for the blocking of the binding between T lymphocytes and target cells and not the post-binding cytolytic steps. J. Immunol. 125:2444.
6. Hollander, N., E. Pillemer, and I. L. Weissman. 1980. The blocking effect of Lyt-2 antibodies on T cell functions. J. Exp. Med. 152:674.
7. Shinohara, N., U. Hämmerling, and D. H. Sachs. 1980. Mouse alloantibodies capable of blocking cytotoxic T cell function. II. Further study on the relationship between the blocking antibodies and the products of the Lyt-2 locus. Eur. J. Immunol. 10:589.
8. Kimura, A. K. 1974. Inhibition of specific cell-mediated cytotoxicity by anti-T-cell receptor antibody. J. Exp. Med. 139:888.
9. Redelman, D., and P. E. Trefts. 1978. *In vitro* studies of the rabbit immune system. VI. Rabbit anti-mouse cytotoxic T effector cells are inhibited by anti-rabbit T cell serum in the absence of complement. J. Immunol. 121:1532.
10. Rabinowitz, R., R. Laskov, and M. Schlesinger. 1980. Inhibition of cell-mediated lysis by xenoantibodies reactive with effector T lymphocytes. Eur. J. Immunol. 10:219.
11. Rabinowitz, R., and M. Schlesinger. 1980. Inhibition of the activity of cytotoxic murine T lymphocytes by antibodies to idiotypic determinants. Immunology 39:93.
12. Huber, S. A., and Z. J. Lucas. 1978. Immune response to a mammary adenocarcinoma. VI. Blocking of tumor-specific cell-mediated cytotoxicity by antibodies to the cytotoxic lymphocyte. J. Immunol. 121:2490.
13. Durda, P. J., and P. D. Gottlieb. 1976. The Ly-3 antigens on mouse thymocytes: immune precipitation and molecular weight characterization. J. Exp. Med. 144:476.
14. Ledbetter, J. A., and L. A. Herzenberg. 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol. Rev. 47:63.
15. Ledbetter, J. A., R. V. Rouse, H. S. Micklem, and L. A. Herzenberg. 1980. T cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens: two-parameter immunofluorescence and cytotoxicity analysis with monoclonal antibodies modifies current views. J. Exp. Med. 152:280.
16. Ledbetter, J. A., J. W. Goding, T. Tokuhisa, and L. A. Herzenberg. 1980. Murine T-cell differentiation antigens detected by monoclonal antibodies. *In* Monoclonal Antibodies, Edited by R. H. Kennett, T. J. McKearn, and K. B. Bechtol. Plenum, New York. Pp. 235–249.
17. Omary, M. B., I. S. Trowbridge, and M. P. Scheid. 1980. T200 cell surface glycoprotein of the mouse. Polymorphism defined by the Ly-5 system of alloantigens. J. Exp. Med. 151:1311.
18. Scheid, M. P., and D. Triglia. 1979. Further description of the Ly-5 system. Immunogenetics 9:423.
19. Siadak, A. W., and R. C. Nowinski. 1980. Identification of Ly-5 and T200 antigens on identical cell surface proteins. J. Immunol. 125:1400.
20. Michaelson, J., M. Scheid, and E. A. Boyse. 1979. Biochemical features of Ly-5 alloantigen. Immunogenetics 9:193.
21. Ford, W. L. 1978. The preparation and labeling of lymphocytes. *In* Handbook of Experimental Immunology, Edited by D. M. Weir, Blackwell, Oxford. p. 23.7.
22. Springer, T., G. Galfre, D. S. Secher, and C. Milstein. 1979. Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. Eur. J. Immunol. 9:301.

23. Weinberger, J. Z., R. N. Germain, S-T. Ju, M. I. Greene, B. Benacerraf, and M. E. Dorf. 1979. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. II. Demonstration of idiotypic determinants on suppressor T cells. J. Exp. Med. 150:761.
24. Marshak-Rothstein, A., P. Fink, T. Gridley, D. H. Raulet, M. J. Bevan, and M. L. Gefter. 1979. Properties and applications of monoclonal antibodies directed against determinants of the Thy-1 locus. J. Immunol. 122:2491.
25. Andersson, J., A. Coutinho, F. Melchers, and T. Watanabe. 1977. Growth and maturation of single clones of normal murine T and B lymphocytes *in vitro*. Cold Spring Harbor Symp. Quant. Biol. 41:227.
26. Davidson, W. F., and C. R. Parish. 1975. A procedure for removing red cells and dead cells from lymphoid cell suspensions. J. Immunol. Methods 7:291.
27. Loken, M. R., and L. A. Herzenberg. 1975. Analysis of cell populations with a fluorescence-activated cell sorter. Ann. N.Y. Acad. Sci. 254:163.
28. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265.
29. Springer, T. A. 1980. Quantitation of light chain synthesis in myeloma x spleen cell hybrids and identification of myeloma chain loss variants using radioimmunoassay. J. Immunol. Methods. 37:139.
30. Markwell, M. A. K., and C. F. Fox. 1978. Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3,6-diphenylglycoluril. Biochemistry 17:4807.
31. Laskey, R. A., and A. D. Mills. 1977. Enhanced autoradiographic detection of ^{32}P and ^{125}I using intensifying screens and hypersensitized film. F.E.B.S. Lett. 82:314.
32. Cuatrecasas, P. 1970. Protein purification by affinity chromatography. J. Biol. Chem. 245:3059.
33. Endicott, K. M., and H. Gump. 1947. Hemograms and myelograms of healthy female mice of C-57 Brown and CFW strains. Blood, Special issue 1:60.
34. Springer, T., G. Galfre, D. S. Secher, and C. Milstein. 1978. Monoclonal xenogeneic antibodies to murine cell surface antigens: identification of novel leukocyte differentiation antigens. Eur. J. Immunol. 8:539.
35. Stern, P. L., K. R. Willison, E. Lennox, G. Galfre, C. Milstein, D. Secher, A. Ziegler, and T. Springer. 1978. Monoclonal antibodies as probes for differentiation and tumor-associated antigens: a Forssman specificity on teratocarcinoma stem cells. Cell 14:775.
36. Springer, T. A. 1980. Cell-surface differentiation in the mouse. Characterization of "jumping" and "lineage" antigens using xenogeneic rat monoclonal antibodies. *In* Monoclonal Antibodies, Edited by R. H. Kennett, T. J. McKearn, and K. B. Bechtol. Plenum, New York. Pp. 185–217.
37. Trowbridge, I. S. 1978. Interspecies spleen-myeloma hybrid-producing monoclonal antibodies against mouse lymphocyte surface glycoprotein, T200. J. Exp. Med. 148:313.
38. Liberti, P. A., C. J. Hackett, and B. A. Askonas. 1979. Influenza virus infection of mouse lymphoblasts alters the binding affinity of anti-H-2 antibody: requirement for viral neuraminidase. Eur. J. Immunol. 9:751.
39. Mason, D. W., R. J. Brideau, W. R. McMaster, M. Webb, R. A. H. White, and A. F. Williams. 1980. Monoclonal antibodies that define T-lymphocyte subsets in the rat. *In* Monoclonal Antibodies, Edited by R. H. Kennett, T. J. McKearn, and K. B. Bechtol. Plenum, New York. Pp. 251–273.
40. Cleveland, D. W., S. G. Fischer, M. W. Kirshner, and U. K. Laemmli. 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. 252:1102.
41. Gately, M. K., and E. Martz. 1981. T11: A new protein marker on activated murine T lymphocytes. J. Immunol. 126:709.
42. Kimura, A. K., and H. Wigzell. 1978. Cell surface glycoproteins of murine cytotoxic T lymphocytes I. T 145, a new surface glycoprotein selectively expressed on Ly 1^-2^+ cytotoxic T lymphocytes. J. Exp. Med. 147:1418.

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STUDIES OF THE PHYSICAL BIOCHEMISTRY AND COMPLEMENT-FIXING PROPERTIES OF DNA/ANTI-DNA IMMUNE COMPLEXES[1]

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We have examined the influence of the DNA m.w., the nature of the anti-dsDNA antibodies, and the antibody/DNA ratio on the physical properties of prepared antibody/DNA immune complexes and their ability to fix complement (C). The sizes of the complexes were studied by isokinetic sucrose gradient ultracentrifugation. The ability of the complexes to fix C was examined by measuring the amount of immune-complexed ^3H-dsDNA that bound to red blood cells in a C-mediated reaction (The RBC-CF assay, Pedersen et al., J. Immunol. Methods, 1980). The mode of binding of antibodies to dsDNA (i.e., monogamous *vs* a cross-linking mechanism) was determined in a double-label study in which we ascertained under what conditions the binding of antibody to high m.w. ^{14}C-dsPM2 DNA could *enhance* the binding of a smaller ^3H-dsDNA fragment as well.

Our results indicate that, depending upon the dsDNA m.w. and the nature of the anti-dsDNA antibodies, DNA/anti-DNA immune complexes of predictable sizes and C-fixing properties can be prepared. For example, as the m.w. of the dsDNA decreases, the maximum size and C-fixing potential of the immune complexes decreases as well. In fact, if the dsDNA is very small (ca. 200 base pairs or less) the immune complexes that it forms with the anti-dsDNA antibodies in most SLE sera do *not* fix C. Finally, our double-label studies provide direct evidence that the majority of anti-dsDNA antibodies bind monogamously to dsDNA, although 1 serum has been identified that forms soluble, cross-linked antibody/DNA immune complexes.

Considerable attention has been focused on the physical chemistry of the anti-dsDNA/dsDNA[4] (double stranded deoxyribonucleic acid) immune complex system (1–14) because of its important role in the pathogenesis of tissue destruction in the autoimmune disease systemic lupus erythematosus (SLE) (15). The avidity of the antibody for dsDNA (1–10), the mode of antibody binding to DNA (1,

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[4] Abbreviations used in this paper: dsDNA, double-stranded deoxyribonucleic acid; SLE, systemic lupus erythematosus; RBC-CF, red blood cell-linked complement-fixation assay; GVB^{++}, gelatin Veronal buffer; GPC, guinea pig complement; NHS, normal human serum; HI, heat inactivated; CF, complement fixing.