that the lipid is noncovalently associated with CD45, since the lipid attachment survives boiling in the presence of SDS followed by separation by electrophoresis, precipitation in trichloroacetic acid (TCA) and washing in ethanol:ether or acetone, and extraction with chloroform:methanol. At present, there are four forms of lipid linkages known to occur among proteins of eukaryotic origin: (i) fatty acylation through cysteine residues forming thioester linkage, as in the case of the transferrin receptor (*10*); (ii) polyisoprenoid attached to COOH-terminal cysteine forming thioether linkage as reported for p21Ras (*24*); (iii) phosphatidyl inositol (PI) as a part of PI-glycan membrane anchor attached to the COOH-terminus of numerous membrane molecules such as Thy-1 (*25*); and (iv) myristoylation on NH_2-terminal glycine forming amide linkage in such proteins as $p60^{src}$ (*26*). Earlier studies of CD45 detected no incorporated radioactive palmitic acid (*10*) and no sensitivity to PI-specific phospholipase C treatment (*27*). Thus, the first and the third types of the possible lipid linkages seem unlikely. This is confirmed by our data because the lipid attached to CD45 is resistant to mild alkaline treatment, to which thioester and the PI-glycan types of fatty acyl linkages should be labile. Moreover, none of the HCl hydrolysis products migrates as long-chain hydrocarbons such as the polyisoprenoids that form thioether linkages with certain proteins. The presence of a fatty acyl and a sphingosine-type material in a comparable ratio in the acid hydrolysates renders it likely that they are derived from a form of sphingolipids. It is possible that such lipids are covalently linked to proteins.

Sphingolipids and their breakdown products, sphingosine and lysosphingosine, have numerous biological effects, including modulation of cell proliferation and junction formation, and cell contact inhibition (*28*). Furthermore, sphingolipid breakdown products inhibit protein kinase C and signal transduction, implicating a role for them as "negative" messengers (*28, 29*). Because CD45 is already known to affect at least one kinase, $p56^{lck}$ (*5*), it is tempting to speculate a role for CD45 and its sphingolipid in regulating protein kinases. CD45 may partially exert its effect by adjusting the amount of free, kinase-inhibiting sphingosine through formation and breakdown of sphingolipid-peptide linkage.

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13. The lowest molecular weight moiety of CD45 seen with the methionine label (the band indicated by arrows in Fig. 1) was absent from those preparations labeled with the fatty acid. Hence, it provides evidence that peptide synthesis and incorporation of the fatty acid label did not always coincide. This fast-migrating band of CD45 tended to appear only after long-term methionine labeling. Furthermore, surface-radioiodinated preparations of CD45 also did not reveal the fast-migrating band (A. Takeda, unpublished data). These observations suggest that the fast-migrating form of CD45 may be a modified or degraded intracellular form or may represent a minor isotype.
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17. YAC-1 cells were labeled by [^{3}H]myristic acid or [^{35}S]methionine incorporation, or by surface-^{125}I labeling, and CD45 electroeluates were prepared as described in Fig. 2. The eluates were digested with V8 protease for partial proteolysis; then the digests were analyzed by SDS-PAGE. The fatty acid and the surface-iodine–labeled samples showed a remarkable resemblance, producing only a few dominant peptides among which the largest two bands comigrated. The methionine-labeled sample, on the other hand, exhibited a pattern quite different from the other two, producing many peptides of various sizes. The resemblance between the myristic acid and the surface-iodine patterns implies that the myristic acid label was incorporated either into or in the proximity of the extracellular portion of CD45.
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30. Single-letter abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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Expression of Murine CD1 on Gastrointestinal Epithelium

PAUL A. BLEICHER,* STEVEN P. BALK, SUSAN J. HAGEN, RICHARD S. BLUMBERG, THOMAS J. FLOTTE, COX TERHORST

Cluster of differentiation 1 (CD1) in humans is a family of major histocompatibility complex (MHC) class I–like molecules expressed on the surface of immature thymocytes, Langerhans cells, and a subpopulation of B cells. The only function identified for human CD1 is as a ligand recognized by a subpopulation of T lymphocytes. In order to study the distribution and function of these molecules in the mouse, a murine CD1 complementary DNA was expressed in mouse fibroblasts and used to produce monoclonal antibodies. These antibodies revealed prominent expression of murine CD1 only on gastrointestinal tract epithelium and in the cytoplasm of hepatocytes. Low levels of expression were also detected on thymocytes and peripheral lymphocytes. The gastrointestinal distribution of murine CD1 suggests that this molecule may be important in epithelial immunity.

THE INTRAEPITHELIAL COMPARTMENT of mouse skin, lung, vagina, uterus, and tongue each contain T cells bearing γδ receptors of limited diversity (*1–3*). Similarly, the intestinal epithelium is populated by γδ-bearing T cells, which use a limited number of V_γ genes, but do have extensive junctional diversity (*4*). These observations suggest that antigen recognition in epithelial immunity may differ from antigen recognition as it is understood in systemic immunity. The limited V_γ gene usage and restricted heterogeneity of the γδ receptors on intraepithelial T cells suggest that

these T cells may recognize nonclassical MHC-like molecules, with or without nominal antigen (*2, 3, 5*). CD1 is one such family of MHC-like proteins.

The human CD1 locus encodes a family of at least three serologically defined cell surface glycoproteins that bind to β_2-microglobulin and show structural similarity to MHC class I molecules. Five apparently functional CD1 genes are clustered on chromosome 1, distinct from the MHC complex on chromosome 6 (*6*). The CD1 proteins are found on the surface of immature cortical thymocytes, and members of the family are also found on a subset of B cells, Langerhans cells, and dermal dendritic cells (*7, 8*). CD1a and CD1c are recognized by some $CD4^-CD8^-$ T cells bearing either $\alpha\beta$ or $\gamma\delta$ receptors (*9, 10*), thus implicating CD1 in T cell recognition. The frequency, distribution, and function of such CD1-reactive T cells is unknown.

Two genes encoding the murine homolog of CD1 (mCD1.1 and mCD1.2) have been described (*11*), but the protein product of these genes has not been demonstrated. To study the structure and distribution of mCD1, we have used a cloned mCDC1 cDNA to generate monoclonal antibodies (MAbs) to mCD1.

A rat was primed with mouse thymocytes and then immunized with an L cell clone expressing the full-length mCD1.1 cDNA. Hybridoma cell lines were produced by fusion of spleen cells from the immunized rat with the murine myeloma line NS1/1-Ag4-1. Hybridoma supernatant fluids were screened by indirect immunofluorescence with mCD1.1-transfected L cells. Initially, 34 of 138 supernatant fluids contained antibodies that bound to mCD1.1 transfectants. Two MAbs, 3C11 and 1H1, stained mCD1.1-expressing L cells but not untransfected L cells; therefore, they were candidates for mCD1.1-specific antibodies (Fig. 1, A and C).

To confirm the specificity of the 3C11 and 1H1 antibodies, a murine T-T hybridoma (*12*) (BY155.16) was transfected with mCD1.1, selected for neomycin resistance, and randomly cloned. Indirect immunofluorescence with both 3C11 and 1H1 revealed that most of the 18 transfectant clones analyzed expressed the ligand for both antibodies, whereas nontransfected or mock-transfected cells did not. In each case, the fluorescence intensity of cells stained with 1H1 (Fig. 1, B, E, and F) matched that of the same cells stained with 3C11 (Fig. 1, D, G, and H). Thus, the two MAbs appeared to detect the same surface antigen, present only on cells transfected with mCD1.1.

To examine the structure of the mCD1.1 membrane protein, we used 3C11 and 1H1 to immunoprecipitate surface mCD1.1 from mCD1.1-expressing BY155.16 cells labeled with ^{125}I and lactoperoxidase. Both antibodies immunoprecipitated a 48-kD and a 12-kD protein, the latter identical in size to β_2-microglobulin as precipitated by the MHC class I–specific antibody (*13*), M142 (Fig. 2). A faint 48-kD protein band was also detectable on the nontransfected BY155.16, which suggests very low surface expression on this T-T hybridoma. Since the predicted molecular weight of the mCD1.1 protein was 36 kD, the measured molecular mass of 48 kD accommodated the five glycosylation sites predicted from the available amino acid sequence data (*11*). Although both 1H1 and 3C11 efficiently immunoprecipitate a 48-kD protein, the 12-kD protein was much more prominent in immunoprecipitates made with 1H1 than with 3C11. It is possible that the 3C11 antibody interferes with the association of the 12-kD protein and mCD1.1. A faint 37-kD band was also found in 3C11 and 1H1 immunoprecipitates at varying ratios to the 48-kD protein. This most likely represents a partial proteolytic product of mCD1.1 similar to that seen with human CD1c (*14*). Protein immunoblots of anti-murine β_2-microglobulin immunoprecipitates that were stained with the 1H1 antibody showed prominent staining of the 48-kD band and very weak staining of the 37-kD band (*15*). Thus, the target of 3C11 and 1H1 MAbs is a 48-kD mCD1 protein that is expressed on the cell surface in association with β_2-microglobulin.

When 3C11 and 1H1 were used to determine the localization of mCD1.1 in mouse

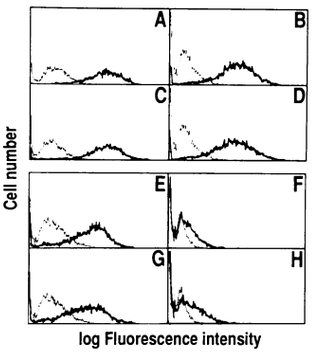

Fig. 1. Monoclonal antibodies 3C11 and 1H1 recognize mCD1.1 on the surface of transfected cells. (**A**) 1H1 antibody staining of murine L cells transfected with pSR-NEO-mCD1.1 (dark) and L cells mock-transfected with pSR-NEO (light). (**C**) 3C11 antibody staining of mCD1.1–L cell transfectant (dark) and mock transfectant (light). (**B, E,** and **F**) Three representative randomly cloned BY155.16 transfected with pSR-NEO-mCD1.1 and stained with 1H1 (dark) or normal rat serum (NRS) (light). (**D, G,** and **H**) Paired cells stained with 3C11 (dark) or NRS (light). mCD1.1 cDNA was ligated into pSR-NEO (*19*) and transfected into L cells by calcium phosphate–mediated transfer (*9*). Transfectants were selected in G418 at 0.5 mg/ml, expanded, and screened for mCD1.1 expression by Northern (RNA) hybridization (*20*). Monoclonal antibodies against mCD1.1 were produced by fusion of NS/1-Ag4-1 with spleen cells from Lewis rats immunized first with 10^7 BALB/c thymocytes, then in 3 weeks with 2×10^6 mCD1.1-transfected L cells. Hybridoma supernatants (3C11 and 1H1), which bound to transfected but not untransfected L cells, were cloned by limiting dilution. BY155.16 was transfected with pSR-NEO-mCD1.1 by electroporation (*9*), plated in 96-well plates (Costar), and G418-resistant cells (4 mg/ml) were selected. mCD1.1 expression in transfected cells was assessed by indirect immunofluorescence with undiluted 3C11 or 1H1 supernatant followed by fluorescein isothiocyanate–conjugated affinity-purified goat antibody to rat immunoglobulin G (IgG) (TAGO, 1:50) with the use of standard techniques (*21*). Cells were fixed with 1% paraformaldehyde and analyzed on an Epics IV cell sorter (Coulter) with gating for live cells. Histograms represent analysis of 5000 cells.

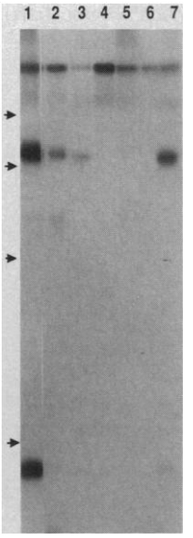

Fig. 2. Murine CD1.1 consists of a 48-kD and a 12-kD glycoprotein. Target cells are mCD1.1-transfected BY155.16 cells immunoprecipitated with 1H1, 3C11, and M142 (lanes 1, 2, and 3), and untransfected BY155.16 cells precipitated with 1H1, 3C11, and M142 (lanes 5, 6, and 7). Lane 4 represents the final nonspecific incubation with NRS-coated beads. Arrows indicate molecular weight markers at 66, 45, 31, and 14 kD. The high molecular weight band was nonspecific and was present in all immunoprecipitations. Immunoprecipitation of cells surface-labeled with ^{125}I was performed as previously reported (*22*) with Sepharose 4B–protein A beads (Pharmacia). Immunoprecipitates were resuspended in Laemmli sample buffer without reducing reagents and run on a 12.5% SDS-polyacrylamide gel under nonreducing conditions. The gel was dried and exposed to XAR-5 film (Kodak) with an intensifying screen at −70°C.

P. A. Bleicher, Laboratory of Molecular Immunology, Dana-Farber Cancer Institute, Boston, MA, 02115, Cutaneous Biology Research Center and Wellman Laboratories, Department of Dermatology, Massachusetts General Hospital, Boston, MA 02114.
S. P. Balk, Laboratory of Molecular Immunology, Dana-Farber Cancer Institute, and Department of Hematology and Oncology, Beth Israel Hospital, Boston, MA 02115.
S. J. Hagen, Department of Gastroenterology, Brigham and Women's Hospital, Boston, MA 02115.
R. S. Blumberg, Laboratory of Molecular Immunology, Dana-Farber Cancer Institute, and Department of Gastroenterology, Brigham and Women's Hospital, Boston, MA 02115.
T. J. Flotte, Wellman Laboratories, Department of Dermatology, Massachusetts General Hospital, Boston, MA 02114.
C. Terhorst, Laboratory of Molecular Immunology, Dana-Farber Cancer Institute, Boston, MA 02115.

*To whom correspondence should be addressed at the Wellman Laboratories.

tissues by immunoperoxidase techniques, prominent surface labeling was found only on the luminal surface of epithelium in the stomach, small intestine, and colon (Fig. 3, A to D). Low levels of membrane labeling of all lymphoid cells in the thymus and lymph node could be seen (Fig. 3, E to G). This was confirmed by flow cytometry of thymus and spleen, which demonstrated dim staining of most thymocytes and low to undetectable staining of spleen cells (*15*). The liver demonstrated prominent labeling, but the pattern of staining was confined to the cytoplasm (Fig. 3H). Control sections of liver show that endogenous peroxidase activity was not evident (Fig. 3I). Finally, no mCD1.1-specific staining was detectable in brain, skin, heart, skeletal muscle, kidney, lung, or esophagus.

The pattern of mCD1 distribution in the small intestine was studied in more detail by immunofluorescence of longitudinal sections of villi from the terminal ileum (Fig. 4). Intestinal villi revealed prominent staining of the apical surface of cells along the extrusion zone, whereas no staining was found on the surface of less mature cells along the villus, in the crypts, or on the dome epithelium overlying Peyer's patches. Interestingly, mCD1 was also present on basolateral membranes and portions of the epithelial cells that protruded into the lamina propria (Fig. 4).

Although the function of mCD1.1 is unknown, analogy with the known function of other non–class I β_2-microglobulin–associated proteins and its prominent distribution on intestinal epithelium suggest that mCD1.1 may be important in mucosal immunity. FcRn is a class I–like β_2-microglobulin–associated glycoprotein found in rat neonatal enterocytes, which acts as an Fc receptor for immunoglobulins from maternal milk. However, the limited sequence homology of FcRn with human and murine mCD1.1 (*16*) (12 to 22% amino acid homology in the α1 to α3 domains) and the expression of mCD1.1 on adult enterocytes are less suggestive of such a function for mCD1.

We favor a role for mCD1 in epithelium-specific T cell recognition, in particular by $\gamma\delta$-bearing T cells. The limited receptor usage and limited junctional diversity of intraepithelial $\gamma\delta$ cells suggest that they may recognize MHC-like molecules of limited polymorphism (*5*). In fact, some mouse $\gamma\delta$ cells are known to recognize other MHC class I–like molecules, such as Tla and Qa antigens (*17*). Similarly, two $\gamma\delta$ T cell clones that recognize CD1c have been identified (*9, 10*). The antigen-specific receptors of these T cells may recognize CD1 as either a target ligand or an antigen-presenting structure. Mouse CD1 is an MHC-like molecule whose structure and unique distribution on gastrointestinal epithelium suggest that it may be a target for intraepithelial $\gamma\delta$ cells in the mouse.

In support of this hypothesis, it is known that the same gastrointestinal epithelial cells that express mCD1.1 can process and present antigen in association with cell surface MHC class II molecules (*18*). Interestingly, mCD1 was distributed on the basolateral membranes of intestinal epithelium, adjacent to the known localization of intraepithelial lymphocytes, and on the pseudopodial extensions of the gastrointestinal epithelium into the lamina propria at a site where they might encounter lymphocytes. Mouse

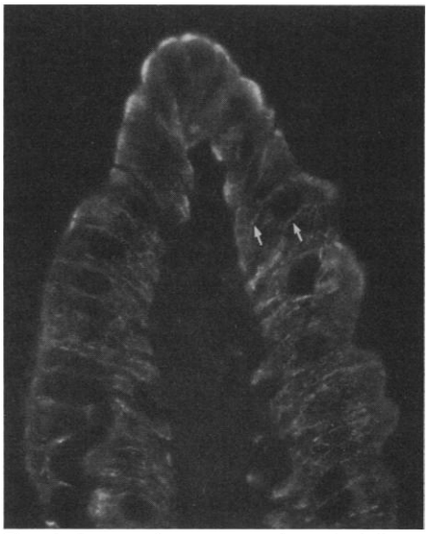

Fig. 4. Immunofluorescence of terminal ileum with 1H1 antibody. Arrows indicate staining at the basolateral membranes. Magnification, ×700. Control tissues stained with rat IgG showed no fluorescence staining. Indirect immunofluorescence was performed on unfixed segments of mouse distal ileum from an adult BALB/c mouse. Frozen sections were sectioned and fixed in acetone at −20°C. Sections were preincubated in 2% NRS in gelatin and phosphate-buffered saline (*23*) and then stained in rat IgG at 10 μg/ml (control) or undiluted 1H1 culture supernatant, followed by rhodamine-conjugated goat antibody to rat IgG (Pel-Freeze, 1:200). Sections were mounted in glycerol containing *p*-phenylenediamine and photographed as described (*24*).

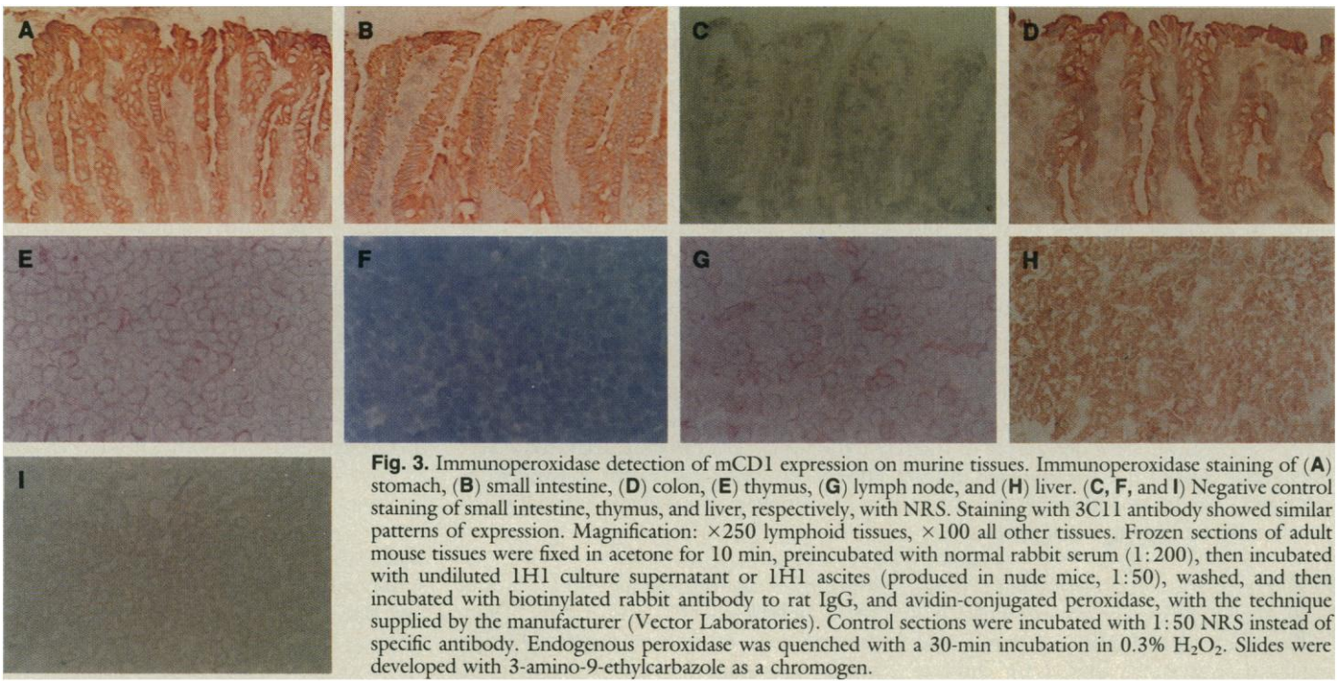

Fig. 3. Immunoperoxidase detection of mCD1 expression on murine tissues. Immunoperoxidase staining of (**A**) stomach, (**B**) small intestine, (**D**) colon, (**E**) thymus, (**G**) lymph node, and (**H**) liver. (**C, F,** and **I**) Negative control staining of small intestine, thymus, and liver, respectively, with NRS. Staining with 3C11 antibody showed similar patterns of expression. Magnification: ×250 lymphoid tissues, ×100 all other tissues. Frozen sections of adult mouse tissues were fixed in acetone for 10 min, preincubated with normal rabbit serum (1:200), then incubated with undiluted 1H1 culture supernatant or 1H1 ascites (produced in nude mice, 1:50), washed, and then incubated with biotinylated rabbit antibody to rat IgG, and avidin-conjugated peroxidase, with the technique supplied by the manufacturer (Vector Laboratories). Control sections were incubated with 1:50 NRS instead of specific antibody. Endogenous peroxidase was quenched with a 30-min incubation in 0.3% H_2O_2. Slides were developed with 3-amino-9-ethylcarbazole as a chromogen.

CD1 in this location could act either as a target for the T cell receptor or as an antigen-presenting molecule for bacterial pathogens and toxins to regional T cells. Alternatively, mCD1 could be involved in the gastrointestinal localization of intraepithelial lymphocytes. Functional experiments elucidating the role of mCD1 in gastrointestinal epithelium should lead to a broader understanding of epithelial immunity.

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Down-Regulation of LFA-1 Adhesion Receptors by C-*myc* Oncogene in Human B Lymphoblastoid Cells

GIORGIO INGHIRAMI, FRANCESCO GRIGNANI, LARS STERNAS, LUIGIA LOMBARDI,* DANIEL M. KNOWLES, RICCARDO DALLA-FAVERA†

The function of the c-*myc* gene and its role in tumorigenesis are poorly understood. In order to elucidate the role of c-*myc* oncogene activation in B cell malignancy, the phenotypic changes caused by the expression of c-*myc* oncogenes in human B lymphoblastoid cells immortalized by Epstein-Barr virus were analyzed. C-*myc* oncogenes caused the down-regulation of lymphocyte function–associated antigen–1 (LFA-1) adhesion molecules (α_L/β_2 integrin) and loss of homotypic B cell adhesion in vitro. Down-regulation of LFA-1 occurred by (i) posttranscriptional modulation of LFA-1 α_L-chain RNA soon after acute c-*myc* induction, and (ii) transcriptional modulation in cells that chronically express c-*myc* oncogenes. Analogous reductions in LFA-1 expression were detectable in Burkitt lymphoma cells carrying activated c-*myc* oncogenes. Since LFA-1 is involved in B cell adhesion to cytotoxic T cells, natural killer cells, and vascular endothelium, these results imply functions for c-*myc* in normal B cell development and lymphomagenesis.

THE C-*myc* PROTO-ONCOGENE IS involved in the control of cellular proliferation and differentiation; its deregulated expression, caused by chromosomal translocation, amplification, or retroviral insertion, is associated with tumorigenesis in different species (*1*). The precise function of the c-Myc protein in normal cells as well as in tumorigenesis is unknown. Consistent with the predicted structure of c-Myc (*2*), this function is presumably accomplished through the modulation of specific gene expression programs. However, only a few genes have been identified that may be physiologically regulated by c-Myc, either transcriptionally (*3*) or posttranscriptionally (*4*).

As an approach to elucidating the role of c-*myc* oncogene activation in Burkitt lymphoma (BL) (*5*), we studied the phenotypic changes induced by c-*myc* oncogenes in Epstein Barr virus (EBV)–immortalized B lymphoblastoid cell lines (LCLs), which may represent the natural target for c-*myc* activation during lymphomagenesis in vivo. The constitutive expression of c-*myc* oncogenes under the control of heterologous enhancer-promoter elements causes (i) the in vitro transformation of LCLs, which acquire the ability to be cloned in semisolid media, and (ii) tumors in immunodeficient mice (*6*). To identify further changes that are consistently associated with c-*myc* expression, we transfected several LCLs with a vector (pHEBoSVmyc2.3) that constitutively expresses a normal c-Myc protein (*7*) and studied the expression of molecules related to histocompatibility, stage of differentiation, and B cell function by cytofluorometric analysis with a panel of B cell–specific monoclonal antibodies (MAbs) (*8*).

Comparison of c-*myc*–transfected and control [pHEBoSV (SV)–transfected] LCLs (Table 1) shows that, in general, expression of most cell surface molecules is unchanged after c-*myc*–induced transformation, except for a slight increase in two activation markers (CD71 and CD38), consistent with the increased proliferative rate of c-*myc*–transformed LCLs. A consistent decrease in the expression of BA-2 (CD9) antigen (*9*) was also observed. The expression of transfected c-*myc* oncogenes consistently caused the down-regulation of the leukocyte-specific cell-adhesion molecule LFA-1 (lymphocyte function–associated antigen–1) (*10*), a member of the integrin superfamily of adhesion receptors (*11*) involved in homotypic B cell adhesion, cell conjugate formation between B cells and cytotoxic T cells or NK (natural killer) cells, and adhesion to vascular endothelium (*10, 12*). The reduction or absence of the cell surface expression of both LFA-1 chains in all c-*myc*–transfected LCLs was confirmed by immunoprecipitation of ^{125}I-labeled cell surface proteins with MAbs to α_L and β_2 chains (Fig. 1A). Analogous changes in surface LFA-1 expression were observed in nine distinct LCLs transfected with vectors carrying c-*myc* oncogenes. In all cases, the degree of LFA-1 down-regulation was pro-