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Impaired Immune Responses Toward Alloantigens and Tumor Cells but Normal Thymic Selection in Mice Deficient in the β_2 Integrin Leukocyte Function-Associated Antigen-1

Peter Shier,*† Gail Otulakowski,† Karen Ngo,*† Julie Panakos,† Erika Chourmouzis,† Lisa Christjansen,† Catherine Y. Lau,† and Wai-Ping Fung-Leung[1]*†

We have generated mice deficient in the β_2 integrin LFA-1 by targeted disruption of the CD11a gene in embryonic stem cells. In vitro LFA-1 −/− cells exhibit a delayed proliferative response toward alloantigens in the MLR. In vivo the host-vs-graft reaction toward injected allogeneic cells is also reduced. Alloantigen-specific CTLs generated from LFA-1 −/− mice are impaired in their cytotoxic activity toward allogeneic spleen cells as well as cell line targets. The proliferative response of LFA-1 −/− splenocytes following stimulation by LPS, PMA plus ionomycin, or immobilized anti-CD3ε mAb is normal, but Con A-stimulated proliferation is greatly diminished. We observe typical edema formation in a delayed type hypersensitivity reaction to SRBC with normal extravasation of leukocytes and demonstrate recruitment of neutrophils to an LPS-induced inflammatory site in these mice, suggesting that LFA-1 does not play an essential role in lymphocyte homing and leukocyte extravasation. We further show that LFA-1 −/− mice are susceptible to metastasis of B16 melanoma tumors, although their in vitro NK cell activity appears normal. A study of LFA-1 −/− mice expressing transgenic TCRs indicates that thymic maturation and selection of T cells are unaffected by the loss of LFA-1. Our results indicate that LFA-1 is important for alloantigen-triggered T cell proliferation and cytotoxicity, for Con A stimulation of T cells, and in tumor rejection. It does not appear to play an essential role in lymphocyte homing and leukocyte extravasation or in T cell maturation and selection in the thymus. *The Journal of Immunology,* **1996, 157: 5375–5386.**

Activation of naive T cells and their differentiation into Ag-specific effector cells is a multistep process. It involves migration of naive cells to the lymph node, activation by foreign Ags presented by APCs, differentiation into effector cells, and finally migration to peripheral tissues, where T cells mediate their effector functions on target cells. While recognition of the MHC-bound Ag peptide by the TCR is central to the immune response (1), various adhesion molecules on T cells are also required for these events to provide costimulatory signals and enhance cell-cell interactions. Three families of adhesion molecules have been identified in the interaction of T cells with other cells: integrins, selectins, and members of the Ig superfamily. One of the integrins proposed to play an important role is the β_2 integrin LFA-1. It is present as an integral membrane protein on virtually all cells of myeloid and lymphoid lineages and has been implicated in a wide range of lymphocyte, monocyte, NK cell, and granulocyte interactions with other cells during inflammation and immunity (2–4). Many of these effector functions, including T cell-mediated killing, T helper cell and B cell responses, NK cell activity, and monocyte-mediated Ab-dependent cytotoxicity, can be inhibited by mAbs directed against LFA-1 (5–7). LFA-1 has also been implicated as playing an important role in leukocyte adherence to endothelial vasculature and extravasation (8). This process involves selectin-mediated tethering and rolling along blood vessel walls, chemoattractant-serpentine receptor interaction, and activation of LFA-1 and several other integrins, leading to increased adhesiveness and finally arrest of the rolling leukocyte. Extravasation into surrounding tissue is believed to depend on traction provided by activated integrins.

Like all integrins, LFA-1 is a dimer, consisting of a unique 180-kDa α subunit, αL (CD11a), and a noncovalently associated 95-kDa β subunit, β_2 (CD18) (9). This particular β subunit can also associate with at least two other closely related α subunits, αM (CD11b) and αX (CD11c), giving rise to the leukocyte-specific integrins, Mac-1 and p150,95 (10). Unlike LFA-1, these latter β_2 integrins are largely restricted to monocytes and granulocytes and, in addition to cell-cell interactions, are involved in cell-substratum adhesion (11). The human genetic disease leukocyte adhesion deficiency I (LAD I)[2] is characterized by the defective expression or complete absence of all β_2 integrins due to one of several mutations identified in the gene encoding the common β subunit (12). As a consequence, affected individuals suffer severe recurrent bacterial infections, leukocytosis, progressive periodontitis, and hypoplasia of lymphoid tissue. Three counter-receptors have been identified for LFA-1: intercellular adhesion molecules (ICAMs)-1, -2, and -3. All are membrane proteins with multiple Ig-like domains, that are differentially expressed and regulated on various leukocyte subpopulations, cells of the endothelial vasculature, keratinocytes, and fibroblasts (13–15). LFA-1 is not constitutively avid, but rapidly attains a high avidity binding state

*The R. W. Johnson Pharmaceutical Research Institute, San Diego, CA 92121; and †The R. W. Johnson Pharmaceutical Research Institute, Don Mills, Ontario Canada

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[1] Address correspondence and reprint requests to Dr. Wai-Ping Fung-Leung, The R. W. Johnson Pharmaceutical Research Institute, 3535 General Atomics Court, Suite 100, San Diego, CA 92121.

[2] Abbreviations used in this paper: LAD I, leukocyte adhesion deficiency I; ICAM, intercellular adhesion molecule; ES, embryonic stem; HVG, host-vs-graft; DTH, delayed type hypersensitivity; H&E, hematoxylin and eosin; DNFB, 2,4 dinitro-fluorobenzene; VLA-4, very late antigen-4.

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upon activation (16, 17). In addition to its function as an adhesion receptor, it has been postulated that LFA-1 engagement itself results in the transduction of signals into the cells. For example, cross-linking of LFA-1 by Abs, which mimics the receptor-ligand interaction, causes the release of intracellular Ca^{2+} and an increase in intracellular pH, while co-immobilizing anti-LFA-1α and anti-CD3 Abs enhance lymphocyte proliferation (18–21).

To address the role of LFA-1 in immune responses and inflammation and its potential role in thymic maturation of T cells, we have generated a mutant mouse strain devoid of cell surface LFA-1 expression by disrupting the CD11a gene using methods of homologous recombination in embryonic stem (ES) cells (22).

Materials and Methods

Strategy for the generation of LFA-1 −/− mice

A recombinant EMBL4 λ phage containing CD11a gene sequences was isolated from a BALB/c mouse genomic library (kindly provided by Dr. K. Kishihara), and a 3.3-kb *Xba*I fragment encompassing exons 3 to 6 was subcloned. The targeting construct was obtained by inserting a 1.2-kb *neo*^{r} cassette (blunted *Bam*HI-*Xho*I fragment of plasmid pMC1NeoPoly(A); Stratagene, La Jolla, CA) into the *Sal*I site of exon 6 sequences that code for the so-called I domain. Two separate constructs, differing only in the orientation of the *neo*^{r} cassette, were restricted with *Xba*I to detach the vector sequences and electroporated into ES cells, as described previously (23). Two different ES lines were used: D3, derived from the 129/Sv mouse strain (24), and E14, derived from the 129/Ola strain (25), both of the H-2^b haplotype. Transfected cells were selected by G418, and homologous recombination events were detected by PCR using primers specific for the *neo*^{r} gene and for an intronic sequence 3′ to the targeting construct. Genomic DNAs of three homologous recombination PCR-positive ES clones were subjected to Southern blot hybridization with probes NEO and F (*Xba*I-*Bam*HI genomic fragment). Results were consistent with disruption of the CD11a wild-type gene (data not shown). The three positive clones arose at a frequency of approximately 1 per 250 G418-resistant colonies despite the fact that the genomic construct and the ES genome were not isogenic. All three clones gave rise to germ line-transmitting chimeric male mice that were bred with C57BL/6J females (The Jackson Laboratories, Bar Harbor, ME). F_1 mice heterozygous for the mutation (CD11a +/−) were crossbred to obtain mice homozygous for the mutation (CD11a −/−). Loss of the wild-type CD11a gene copies in mice was monitored by PCR using genomic tail DNA and by flow cytometry. The three knockout mouse lines were maintained by sibling-sibling breeding.

Flow cytometric analysis of leukocytes

Peripheral blood leukocytes (20 μl) or 10^6 resuspended cells prepared from various lymphoid organs were stained with mAb(s) for 30 min at 4°C in 100 μl of PBS containing 2% FCS and 0.1% sodium azide. Cells were then washed and analyzed for single, double, and triple color flow cytometry on a FACScan (Becton Dickinson Co., Mountain View, CA). mAbs used were specific for CD3, CD4, CD8, CD11a, CD11b, CD18, CD45R/B220, and Gr-1 (PharMingen, San Diego, CA) or for the transgenic 2C TCR-$\alpha\beta$ (kindly provided by Dr. D. Y. Loh).

In vitro culture conditions for assays involving leukocytes

Except for the NK cell assay, all proliferation and cytotoxic assays involving splenocytes or mesenteric lymph node cells were performed using RPMI 1640 (Life Technologies, Gaithersburg, MD) with 20% heat-inactivated FCS supplemented with L-glutamine, penicillin, streptomycin, and 50 μM 2-ME at 37°C in the presence of 5% CO_2. The above medium with 5% FCS was used to assess NK cell activity.

Alloantigen-stimulated proliferation in MLR

Responding spleen cells from LFA-1 +/+, +/−, or −/− mice (H-2^b), maximally numbering 2.5×10^5 cells/well or twofold serially diluted, were mixed with 5×10^5 irradiated (2000 rad) allogeneic BALB/cJ (H-2^d; The Jackson Laboratories) or syngeneic C57BL/6J splenocytes in round-bottom 96-well plates at 200 μl/well. Three days later, [^{3}H]thymidine at 1 μCi/50 μl of medium was added per well for a 6-h incubation. For the MLR kinetics experiment, 1.5×10^5 responders were mixed with 3×10^5 stimulators, and their proliferation was assessed as described above on days 3 to 7. Proliferation was measured by [^{3}H]thymidine incorporation using a Wallac 1205 Betaplate scintillation counter (Wallac, Inc., Gaithersburg, MD) or a Topcount Microplate Scintillation Counter (Packard, Downers Grove, IL).

Host-versus-graft (HVG) reaction

LFA-1 +/− and −/− mice (H-2^b) were injected s.c. in one hind footpad with 8×10^6 splenocytes (in 50 μl of PBS) from B6D2F$_1$ donor mice (H-2$^{b/d}$; The Jackson Laboratories) and with an equivalent volume of PBS in the other footpad. Some mice received identical treatment in both footpads. Seven days later, the draining popliteal lymph nodes were removed from mice and weighed. For those mice that received identical treatment for both footpads, the combined weight of both popliteal lymph nodes was halved to represent the weight of one.

^{51}Chromium release assays following MLR

Effector splenocytes from LFA-1 +/+, +/−, and −/− mice (H-2^b) and irradiated stimulator spleen cells (2000 rad) from BALB/cJ mice (H-2^d) were resuspended at 5×10^6 cells/ml, mixed at a 1:1 ratio, and incubated for 6 days. Spleen target cells were subjected to a hypotonic shock to eliminate RBCs, resuspended at 5×10^6 cells/ml, and stimulated for 3 days with 2.5 μg/ml Con A (Sigma Chemical Co., St. Louis, MO). EL-4 thymoma cells (H-2^b), p815 mastocytoma cells (H-2^d), and YAC-1 lymphoma cells (H-2^a) were maintained in exponential growth phase (all from American Type Culture Collection, Rockville, MD). On the day of the cytotoxicity assay, spleen and cell line targets were incubated in the presence of ^{51}Cr for 1 h, washed, plated onto serially diluted effector cells in round-bottom 96-well plates (total volume, 200 μl/well), and centrifuged for 5 min at 150 × *g*. Targets totaled 10^4 or 5×10^3/well, and the E:T ratios were maximally 100:1 with threefold serial dilutions. In some experiments, CD8$^+$ effector cells were enriched by depletion of CD4$^+$ T cells and B cells with complement and Abs from hybridoma RL172 (anti-CD4), J11d (anti-HSA), and 28-16-8s (anti-I-A^b) essentially as previously described (26) (provided by Dr. Z. Cai). In these experiments, the E:T ratios were maximally 33:1. Following a 4-h incubation, plates were centrifuged for 5 min at 300 × *g*, and the radioactivity in the supernatants was counted using a gamma counter. Maximum lysis was obtained by counting detergent-lysed target cells; spontaneous release was obtained by incubating target cells in the absence of effector cells. The percent specific lysis was calculated as: (measured ^{51}Cr release − spontaneous ^{51}Cr release)/(maximum ^{51}Cr release − spontaneous ^{51}Cr release) × 100. In one case, human IL-2 was supplemented for a final concentration of 20 U/ml, and in this case the ^{51}Cr release assay was performed on day 5.

NK cell function using a ^{51}Cr release assay

NK cell activity was induced in vivo by i.p. injection of 100 μg of poly(I):poly(C) (Sigma Chemical Co.) on 2 consecutive days. Two of the four mice also received a single 0.5-ml i.p. dose of anti-asialo-GM1 antiserum (Wako Chemicals, Richmond, VA), diluted 1/10 in PBS, 2 h before the first poly(I):poly(C) injection. On the third day, mice were killed, and their spleens were processed for single cell suspensions. Splenocytes were plated in round-bottom 96-well plates with threefold serial dilutions, starting at 10^6/well. ^{51}Cr-labeled YAC-1 targets (5×10^3/well) were added (total volume, 200 μl), and the plates were centrifuged for 5 min at 150 × *g*. Following a 4-h incubation, plates were centrifuged for 5 min at 300 × *g*, and the radioactivity in the supernatants was counted using a gamma counter. The percent specific lysis was calculated as described above.

B16 pulmonary metastasis

B16 melanoma cells (American Type Culture Collection) were maintained in culture in DMEM (Life Technologies) with 10% heat-inactivated FCS supplemented with L-glutamine, penicillin, and streptomycin. Just before inoculation, cells were removed from culture vessels by trypsinization, washed, and resuspended in PBS at 10^5 cells/ml. Mice were injected i.v. with 0.5 ml of tumor cells. Fourteen days later, the mice were killed, their lungs were removed, and the lobes were separated and examined under a dissecting microscope. The total number of pulmonary tumors visible on the surface was counted. Some mice received a single 0.5-ml i.p. dose of anti-asialo-GM1 antiserum, diluted 1/50 in PBS, 1 day before inoculation. LFA-1 +/+ mice in one experiment (Fig. 6*B*) were from the C57BL/6J strain. In earlier experiments, mice of this background were found to be equivalent to LFA-1 +/− siblings of LFA-1 −/− mice in their susceptibility to B16 tumor metastasis.

Delayed type hypersensitivity (DTH)

LFA-1 +/− and −/− mice were sensitized by i.v. injection of SRBC (0.3 ml of 0.01% in PBS). Four days later, they were challenged (50 μl of 20% SRBC in PBS) in one hind footpad and with an equivalent volume of PBS

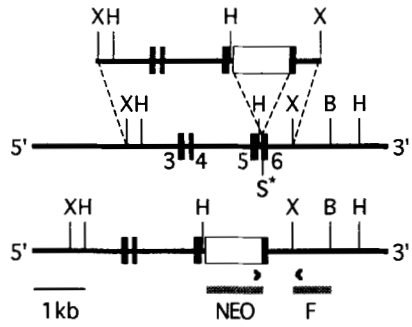

FIGURE 1. Disruption of the CD11a gene in mouse embryonic stem cells by homologous recombination. The targeting construct, consisting of a 3.3-kb *Xba*I fragment from the CD11a gene and containing exons 3 to 6, the intervening and parts of the flanking introns, and the neomycin phosphotransferase (*neo*^{r}) gene disrupting exon 6, is depicted at the *top*. A map of a portion of the murine CD11a gene and alignment with the construct are depicted in the *middle*. The *Sal*I site of *neo*^{r} insertion is indicated by S and an asterisk. Only the exons with mapped positions are shown. The disrupted CD11a genomic region, the positions of the *neo*^{r} (NEO) and flanking (F) genomic probes, and sites of primers (arrowheads) used for PCR screening are depicted at *bottom*. Partial restriction mapping of the genomic region is indicated with the following enzymes: *Bam*HI (B), *Hin*dIII (H), and *Xba*I (X).

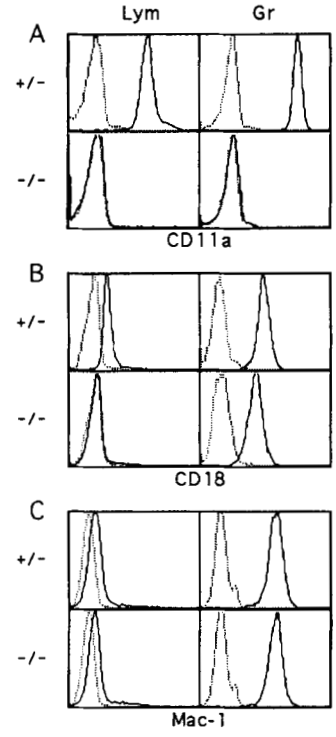

FIGURE 2. Absence of LFA-1 expression without compensation by other members of the β_2 integrin subfamily on leukocytes from LFA-1 −/− mice. Representative flow cytometric analysis of peripheral blood leukocytes from LFA-1 +/− and −/− mice stained with mAb to CD11a (*A*), CD18 (*B*), and Mac-1 (*C*). The dotted line represents isotype control staining in *A* and *B*, and fluorescence of unstained control cells in *C*. The populations gated are lymphocytes (Lym) and granulocytes (Gr). Note the lack of compensatory expression of other β_2 integrins on LFA-1 −/− lymphocytes and granulocytes.

in the other. Footpads were measured at 16, 19, and 23 h postchallenge. Subsequently, three representative mice per group were killed, their spleen cells were resuspended at 2×10^6 cells/ml and plated at 200 μl/well in a round-bottom 96-well plate. Three days later, [^{3}H]thymidine uptake by proliferating cells was measured as described above. In addition, feet were removed immediately after death, fixed in formalin, and decalcified. The fixed tissue was subjected to routine paraffin processing, with sections cut and stained with hematoxylin and eosin (H&E).

LPS-induced inflammation

Backs of LFA-1 +/− or −/− mice were shaved before intradermal injection of 5 μg of LPS (*Escherichia coli*; Sigma Chemical Co.) in 10 μl of PBS. Twenty-four hours later dorsal skin was excised and fixed in formalin. The fixed tissue was subjected to routine paraffin processing, with sections cut and stained with H&E.

Mitogen-stimulated splenocyte proliferation assays

Splenocytes from LFA-1 +/+, +/−, and −/− mice were resuspended at 1×10^6 cells/ml and plated at 200 μl/well in round-bottom, 96-well plates. To each well, with the exception of the anti-CD3ε assays, 50 μl of straight medium or medium containing the appropriate mitogens was added, such that the final concentrations were: PMA, 50 ng/ml; ionomycin, 0.5 μM; LPS, 10.0 μg/ml; and Con A, 4.0, 2.0, or 1.0 μg/ml (all from Sigma Chemical Co.). For anti-CD3ε assays, the wells were preincubated with 5.0 μg/ml of mAb in PBS (145–2C11; PharMingen) or with PBS alone for >90 min and washed three times with PBS before the addition of splenocytes. The three blocking Abs, FD441.8, M17/4.4.11.9, and 2E6, were produced from hybridoma lines obtained from American Type Culture Collection. Following 2 days of stimulation, [^{3}H]thymidine uptake by proliferating cells was measured as described above.

Results

Generation of CD11a −/− mice

To ablate the function of LFA-1 without affecting other β_2 integrins, the CD11a gene that encodes the α-chain unique to LFA-1 was disrupted by insertion of a *neo*^{r} cassette into exon 6 (Fig. 1). Three LFA-1 −/− mouse lines were generated from independently isolated ES clones that underwent individual homologous recombination events. The three independent mouse lines showed the same phenotypic characteristics and the same defects in functional assays as those described in the following sections.

Heterozygous mutant mice produced wild-type, heterozygous, and homozygous offspring at the expected Mendelian ratios, and cross-breeding of LFA-1 −/− mice resulted in normal litter sizes. Therefore, LFA-1 is not essential for embryogenesis, viability, or fertility. Homozygous mutant mice, 2 to 4 mo of age, were used in phenotypic and functional analyses. These mice did not exhibit any obvious gross histopathologic abnormalities, and the sizes of their lymphoid organs were within the normal range, albeit near the lower end for peripheral lymph nodes. Flow cytometric analysis and differential cell count studies showed a normal distribution of different leukocyte subsets, such as T cells, B cells, and granulocytes, in the thymus, spleen, lymph nodes, as well as peripheral blood of these mice (data not shown). Therefore, distribution of different leukocyte subsets does not seem to be compromised by the absence of LFA-1.

Lack of LFA-1 expression and LFA-1-mediated cell aggregation in LFA-1 −/− mice

Expression of LFA-1 on leukocytes was assessed by flow cytometry using Abs recognizing the LFA-1α subunit. LFA-1 was no longer detectable on lymphocytes and granulocytes from LFA-1 −/− mice (Fig. 2*A*). Furthermore, expression of the β-chain, CD18, was not detected on LFA-1 −/− lymphocytes and was partially decreased on LFA-1 −/− granulocytes (Fig. 2*B*). Expression of the β_2 integrin Mac-1 on granulocytes from LFA-1 −/− mice remained unchanged (Fig. 2*C*). These results suggest that LFA-1 is the only β_2 integrin expressed on lymphocytes, and that the absence of LFA-1 on granulocytes is not compensated by

an elevated level of Mac-1. Activation of lymphocytes by some mitogens, including phorbol esters, has been reported to induce the avidity of LFA-1 in cell-cell interactions and can be demonstrated by the aggregation of lymphocytes shortly after PMA treatment (27). Leukocytes isolated from the mesenteric lymph nodes of either wild-type or heterozygous mutant mice exhibited PMA-induced aggregation that was noticeable within 60 min of treatment and resulted in massive clusters within 3 to 4 h. In contrast, LFA-1 −/− leukocytes completely failed to aggregate (data not shown). Furthermore, in all the proliferation studies described below, LFA-1 −/− leukocytes could be easily identified by their characteristic absence of tight colonies and clustering seen in LFA-1 +/+ or +/− proliferating leukocytes.

Alloantigen-specific proliferation in MLR and HVG response are impaired in LFA-1 −/− mice

The proliferative capacity of splenocytes to alloantigens was assessed in vitro by MLR. LFA-1 −/− spleen cells (H-2^b), stimulated with BALB/cJ splenocytes (H-2^d), consistently showed a marked decrease in proliferation on day 3, as measured by [^{3}H]thymidine uptake, compared with cells from LFA-1 +/+ or +/− mice (Fig. 3*A*). The decrease was observed at different ratios of responder to stimulator cells. This proliferation is alloantigen specific, since stimulation of any of the three responder populations with irradiated syngeneic C57BL/6J splenocytes resulted in minimal levels of proliferation. To determine whether the reduction in proliferation is an absolute decrease in the proliferative capacity of LFA-1 −/− cells or the consequence of a shift in the kinetics of the response, [^{3}H]thymidine uptake was assessed for 5 consecutive days (Fig. 3*B*). In these experiments, reduced numbers of responders and stimulators were used to ensure that the kinetics of cell proliferation are not affected by a limit in nutrients. Maximum proliferation of LFA-1 −/− splenocytes was shown to shift from day 4 to day 6 and was comparable in scope to that in control cells.

The response of LFA-1 −/− mice to alloantigens was also examined in vivo in the unidirectional HVG reaction, in which live spleen cells from B6D2F_1 mice (H-$2^{b/d}$) were injected into the footpads of LFA-1 −/− or control mice. Seven days later, the draining popliteal lymph nodes were removed and weighed to assess the extent of hyperplasia resulting from lymphocyte proliferation and migration in response to alloantigens. LFA-1 −/− mice exhibited a significant decrease in lymph node hyperplasia compared with LFA-1 +/− control mice (Fig. 3*C*).

Alloantigen-specific cytotoxicity is compromised in LFA-1 −/− splenocytes

Alloantigen-specific CTL activity of LFA-1 −/− splenocytes was assessed using a ^{51}Cr release assay. CTLs were generated by coculturing splenocytes from LFA-1 −/− or +/− control mice with irradiated allogeneic BALB/cJ spleen cells. Following 6 days of stimulation, alloantigen-specific cytotoxicity was assessed. It was found to be consistently decreased in spleen cells isolated from LFA-1 −/− mice compared with that in cells isolated from LFA-1 +/− mice (Fig. 4*A*). There was no difference between CTL activity of wild-type and heterozygous splenocytes, and spleen cells obtained from each of the three independently derived LFA-1 −/− mouse lines showed a similar CTL defect (data not shown). In a separate experiment, when IL-2 was supplemented during a 5-day stimulation phase, LFA-1 −/− effectors attained an enhanced CTL activity toward allogeneic target cells relative to the level attained without cytokine addition, but did not fully recover to the level of the LFA-1 +/− control (Fig. 4*A*). A similar decrease in cytotoxic activity of LFA-1 −/− splenocytes toward YAC-1 cell targets was observed (Fig. 4*B*). This deficit could not be fully corrected by the addition of IL-2 during the stimulation period, a situation parallel to that seen with the generation of $CD8^+$ cytotoxic T cells.

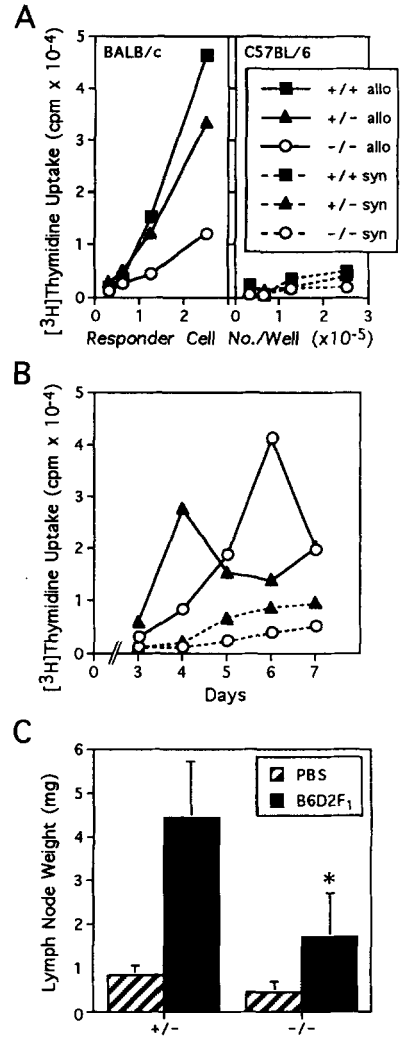

FIGURE 3. Alloantigen-specific proliferation of LFA-1 −/− lymphocytes in MLR and in HVG response is impaired. *A*, Proliferative responses of LFA-1 +/+, +/−, and −/− spleen cell responders to alloantigenic (BALB/c) or syngeneic (C57BL/6) stimulators in a 3-day MLR. All points are means of triplicate values of one representative experiment of three performed. *B*, Time course of proliferative responses of LFA-1 +/− and −/− spleen cell responders following MLR stimulation on days 3 to 7. All points are means of triplicate values. Decreased numbers of responders and stimulators were used for this experiment relative to the 3-day MLR to ensure that the kinetics of cell proliferation were not affected by limited nutrients. *C*, HVG response in LFA-1 +/− and −/− mice assessed by hyperplasia of the draining popliteal lymph nodes. Results are presented as the mean ± SD (n = 10/group). A statistically significant difference ($p < 0.001$) in the increase in lymph node weight between the two groups is indicated by an asterisk.

On the day of the ^{51}Cr release assay, alloantigen-stimulated splenocytes from both LFA-1 −/− and control mice exhibited a typical blasting appearance and expressed the CD25 and CD69 activation markers. To determine whether the observed decrease in CTL activity results from reduced numbers of effector CTLs generated or from decreased cytolytic efficiency of the CTLs generated, cells from 6-day MLR cultures were enriched for $CD8^+$ T cells before assaying for cytotoxic activity. The CTL activity of

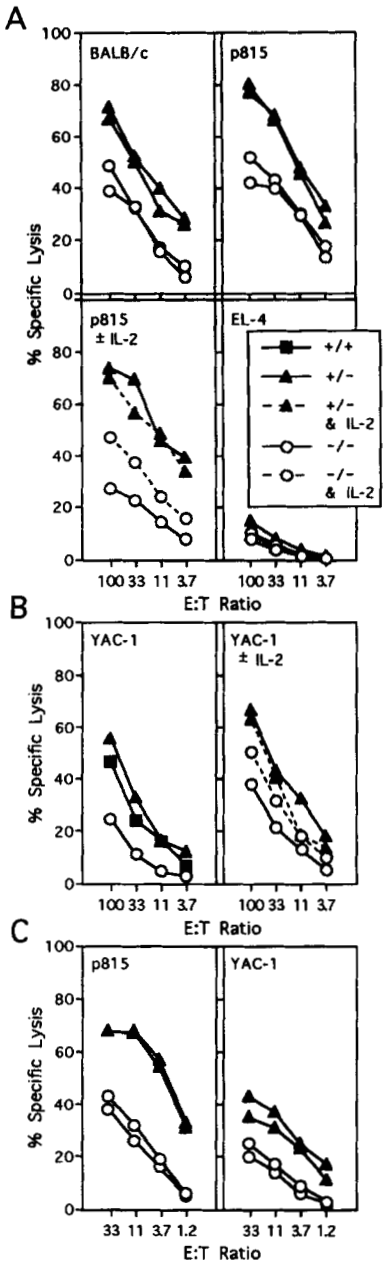

FIGURE 4. Cytolytic activity of LFA-1 −/− splenocytes is impaired following allogeneic stimulation. Cytolytic activity of alloantigen-stimulated splenocytes from LFA-1 −/− and control mice toward (*A*) allogeneic target spleen cells (BALB/c), allogeneic p815 mastocytoma cells, syngeneic EL-4 thymoma cells; and (*B*) YAC-1 lymphoma cells. The effects of supplementation of IL-2 during the in vitro culture on the cytolytic activity of effectors toward p815 and YAC-1 cells are shown. *C*, Cytolytic activity of $CD8^+$ T cells from LFA-1 +/− and −/− mice after alloantigen stimulation. $CD8^+$ effector cells were enriched by Ab and complement depletion of $CD4^+$ and B cells. The percent specific lysis of syngeneic EL-4 cell targets by $CD8^+$-enriched effectors was 3% or less for all E:T ratios (data not shown). All points are the means of triplicate values of representative experiments.

LFA-1 −/− effectors was still compromised relative to that of control effectors to an extent comparable in scope to what was seen with bulk cultures, indicating that LFA-1 −/− CTLs are inherently less efficient in their cytotoxicity toward allogeneic target cells (Fig. 4*C*). These cells also displayed a substantial decrease in cytotoxicity toward YAC-1 target.

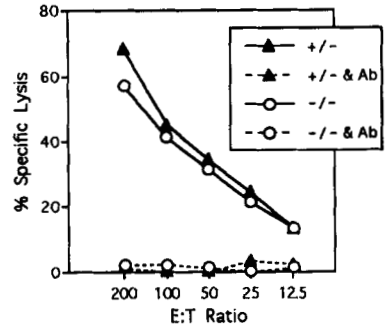

FIGURE 5. In vivo induction of NK activity generates normal LFA-1 −/− NK cytotoxicity toward YAC-1 cells. Cytolytic activity of freshly isolated splenocytes toward YAC-1 cell targets following in vivo NK induction with poly(I):poly(C), and the elimination of this cytotoxicity following prior administration of anti-asialo-GM1 antiserum, Ab. All points are the means of triplicate values of one representative experiment of three performed.

NK cell function is normal in LFA-1 −/− mice

Following in vivo induction of NK activity with poly(I):poly(C), the cytotoxicity of splenocytes toward YAC-1 cell targets was assessed using a ^{51}Cr release assay. LFA-1 −/− and control splenocytes lysed target cells equally well at all E:T ratios. In both cases, administration of Ab to asialo-GM1, which is expressed on NK cells as well as macrophages, before NK induction eliminated this cytotoxicity toward YAC-1 cells (Fig. 5). Other NK cell-specific Abs, such as those recognizing NK1.1, have been used in some mouse strains to similarly deplete NK cells. However, they could not be used with LFA-1 −/− mice because they are derived from a mouse strain that lacks the appropriate epitopes.

Tumor rejection is compromised in LFA-1 mice

An in vivo model for syngeneic tumor rejection, the B16 melanoma metastasis model, was used to assess the efficiency of tumor rejection mediated by innate immunity in LFA-1 −/− mice (28–30). LFA-1 −/− or control mice were inoculated with live B16 cells, and 14 days later their lungs were removed, the lobes were separated, and established B16 pulmonary tumor foci on their surfaces were counted. LFA-1 −/− mice were found to develop dramatically higher number of tumors than LFA-1 +/+ or +/− mice (Fig. 6, *A* and *B*). Administration of anti-asialo-GM1 antiserum to LFA-1 +/− mice before inoculation with tumor cells resulted in an increase in pulmonary tumors to approximately twice the number in untreated LFA-1 −/− mice (Fig. 6*A*). In a separate experiment, administration of the same Ab to LFA-1 −/− mice again increased the number of tumors in the lung approximately twofold above the level in untreated LFA-1 −/− mice (Fig. 6*B*). Our results indicate that innate immunity is partly impaired in mice lacking LFA-1, and that it can be further compromised by pretreatment with the anti-asialo-GM1 Ab. Protection from metastasis of B16 tumor cells has been shown to involve endogenous NK cells, since depletion of the NK cell population by administration of Abs recognizing NK cells, anti-NK1.1 or anti-asialo-GM1, has been shown to render mice susceptible to B16 tumor metastasis (29–31). NK-deficient Beige mice have also been shown to have a low endogenous resistance to such tumors (31–33). Activated macrophages, which, likewise, express the asialo-GM1 surface marker, have also been shown to play a role in anti-tumor immunity. We have demonstrated here that rejection of syngeneic tumors is impaired in LFA-1 −/− mice. The effector cells responsible for

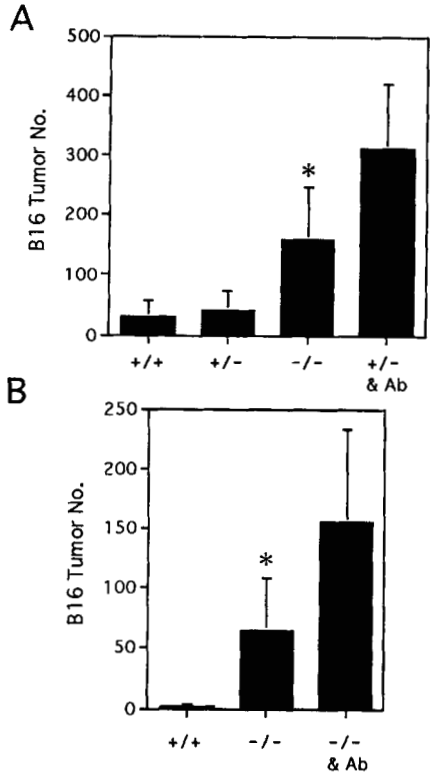

FIGURE 6. LFA-1 −/− mice exhibit an increased susceptibility to syngeneic tumor metastasis. *A* and *B*, Number of pulmonary tumors present on the lungs of LFA-1 +/+, +/−, and −/− mice 14 days after inoculation with live B16 melanoma tumor cells. Ab, anti-asialo-GM1 antiserum given to deplete the mice of NK cells. Data from two independent experiments are shown; results are presented as the mean ± SD (n = 5/group). Statistically significant differences ($p < 0.025$) in the numbers of pulmonary tumors between LFA-1 −/− and LFA +/+ or +/− control mice are indicated by an asterisk in each panel.

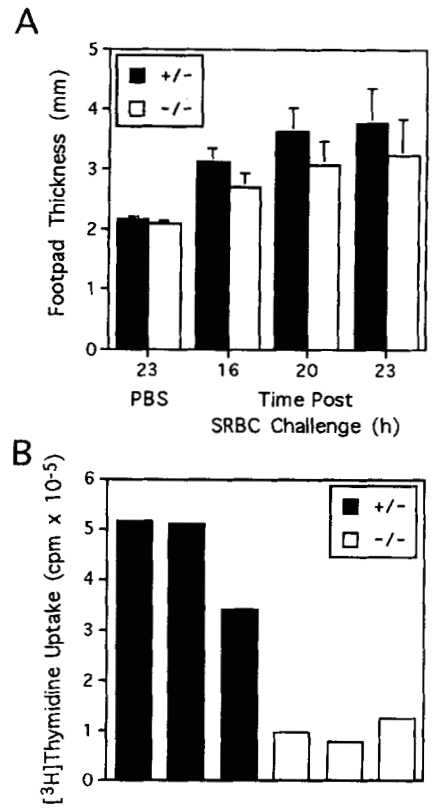

FIGURE 7. Normal edema formation but reduced ex vivo proliferation of LFA-1 −/− splenocytes in a DTH response to SRBC immunization. *A*, Footpad thickness of LFA-1 +/− and −/− mice 16, 20, and 23 h following SRBC challenge or 23 h post-vehicle only injection. Results are presented as the mean ± SD of data obtained from one of two similar experiments (n = 5/group). *B*, Ex vivo proliferation of splenocytes from LFA-1 +/− and −/− mice isolated 4 days following an in vivo DTH response. Results are presented as the means of triplicate values; the proliferation of spleen cells from three mice per group is shown.

the defective tumor immunity are to be elucidated in future studies.

DTH response is minimally affected in LFA-1 −/− mice

To assess the ability of LFA-1 −/− mice to mount a $CD4^+$ T cell-mediated DTH response, mice were sensitized to SRBC and subsequently injected with SRBC in the footpad 4 days later. The extent of ensuing edema at the site of the SRBC challenge was not significantly different between LFA-1 +/− and −/− mice (Fig. 7*A*). Furthermore, histologic examination showed extensive infiltration of leukocytes into the footpads of LFA-1 −/− mice, which was comparable to that of the control LFA-1 +/− mice (Fig. 8). However, when spleen cells from these challenged mice were isolated for proliferation assays ex vivo, the extent of LFA-1 −/− splenocyte proliferation was substantially less than that of LFA-1 +/− cells (Fig. 7*B*).

Neutrophil migration appears to be normal in LFA-1 −/− mice

Injection of LPS into the skin has been reported to result in a profound local inflammatory response characterized by an influx of neutrophils (34). To assess the extent of infiltration by neutrophils lacking LFA-1 but expressing normal levels of the β_2 integrin Mac-1, cell migration to LPS-induced skin inflammatory sites was assessed histologically 24 h following an intradermal injection of 5 μg of LPS. In both LFA-1 −/− and control +/− mice, a marked cell infiltration into the dermis was observed (Fig. 9). The comparable level of infiltrating neutrophils, characteristically displaying their multilobed nuclei, suggests that LFA-1 does not play a crucial role in neutrophil extravasation.

Con A-stimulated proliferation is drastically reduced in LFA-1 −/− splenocytes

Lymphocyte activation and proliferation through different signaling pathways were assessed by stimulating splenocytes with various mitogenic agents in in vitro proliferative assays. When LPS, PMA plus ionomycin, or immobilized anti-CD3ϵ Ab were used, equivalent proliferations were attained by spleen cells from LFA-1 +/+, +/−, and −/− mice (Fig. 10*A*). In contrast, the proliferative response of the LFA-1 −/− spleen cells was consistently and drastically curtailed when stimulated with the T cell lectin Con A at all concentrations tested (Fig. 10, *A* and *B*). To directly demonstrate the involvement of LFA-1 in Con A-stimulated proliferation, two blocking Abs recognizing the α and β subunits of LFA-1, FD441.8 and 2E6, respectively, were added during the stimulation phase. The presence of either blocking Ab reduced Con A-stimulated proliferation of LFA-1 +/+ and +/− splenocytes by >85% to approximately the same level as that attained by LFA-1 −/− splenocytes (Fig. 10*C*). The LFA-1 −/− cells were not substantially affected by addition of the Abs. Similar results were obtained with

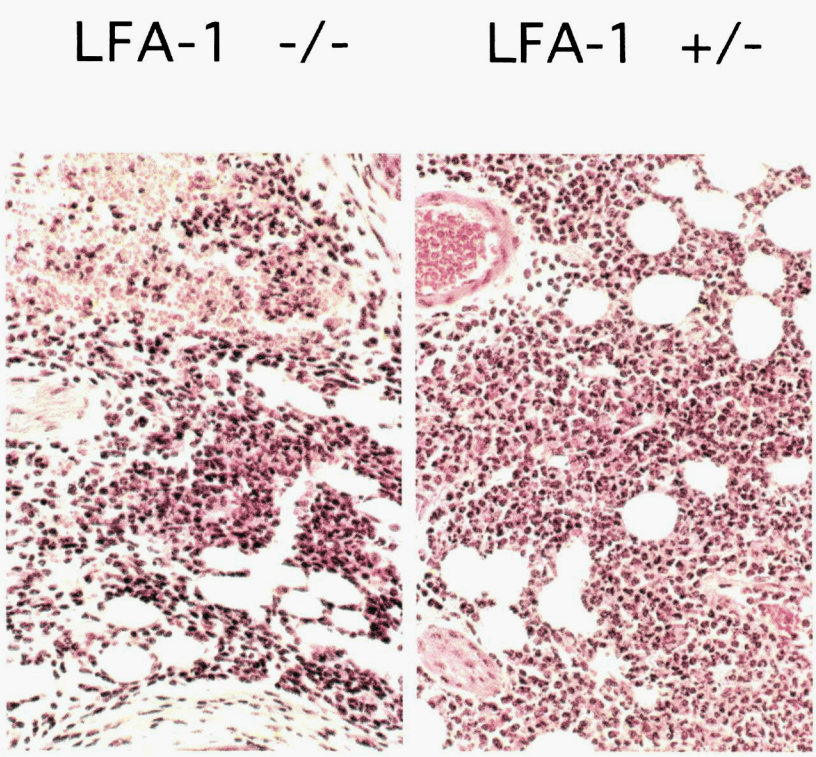

FIGURE 8. Leukocyte infiltration into a DTH site in response to SRBC challenge is normal in LFA-1 −/− mice. H&E staining of footpad sections obtained from LFA-1 +/− and −/− mice 24 h following SRBC challenge. Both LFA-1 +/− and −/− footpad sections reveal densely packed, infiltrating leukocytes, which exhibit the typically round morphology and contain heavily stained nuclei. Injected SRBC are visible at the *top* of the *left* micrograph; both sections contained similar amounts of residual SRBC.

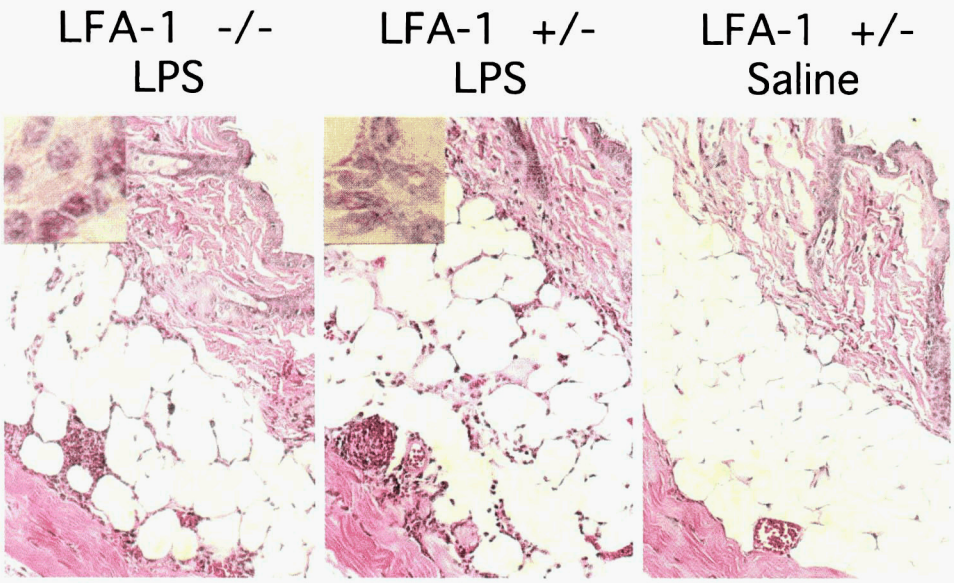

FIGURE 9. Leukocyte infiltration into an LPS-induced inflammatory site is normal in LFA-1 −/− mice. H&E staining of inflamed skin sections isolated from LFA-1 +/− and −/− mice 24 h following an intradermal injection of LPS. Heavily stained infiltrating leukocytes are visible within the dermis in the *middle* and *left* micrographs. Insets reveal neutrophils at higher magnification, with their characteristically multilobed nuclei, which make up the majority of these cells.

a third blocking Ab recognizing the α subunit, M17/4.4.11.9 (data not shown). These results indicate that Con A-stimulated proliferation is strongly dependent on the presence of LFA-1.

Positive and negative selection of thymocytes appear normal in LFA-1 −/− mice

During thymocyte maturation, T cells progress from an immature, double-negative phenotype, through a double-positive stage, and finally into mature, single-positive $CD4^+$ or $CD8^+$ cells that are exported to the periphery (35). This developmental process involves positive and negative selection and culminates in a self MHC-restricted, self tolerant T cell repertoire. Positive selection permits the survival of thymocytes bearing TCR with low affinity for Ags presented by self MHC. Negative selection eliminates autoreactive T cells, thereby contributing to self tolerance. LFA-1 is expressed on virtually all neonatal and adult thymocytes, while ICAM-1 is expressed on thymic epithelial cells as well as thymocytes (36, 37). Earlier studies showed that addition of Abs against either of these counter-receptors to a fetal thymic organ culture system resulted in impaired generation of double-positive thymocytes (37). This suggests that LFA-1/ICAM-1 interaction may be involved in the process of T cell maturation. Initial assessment by

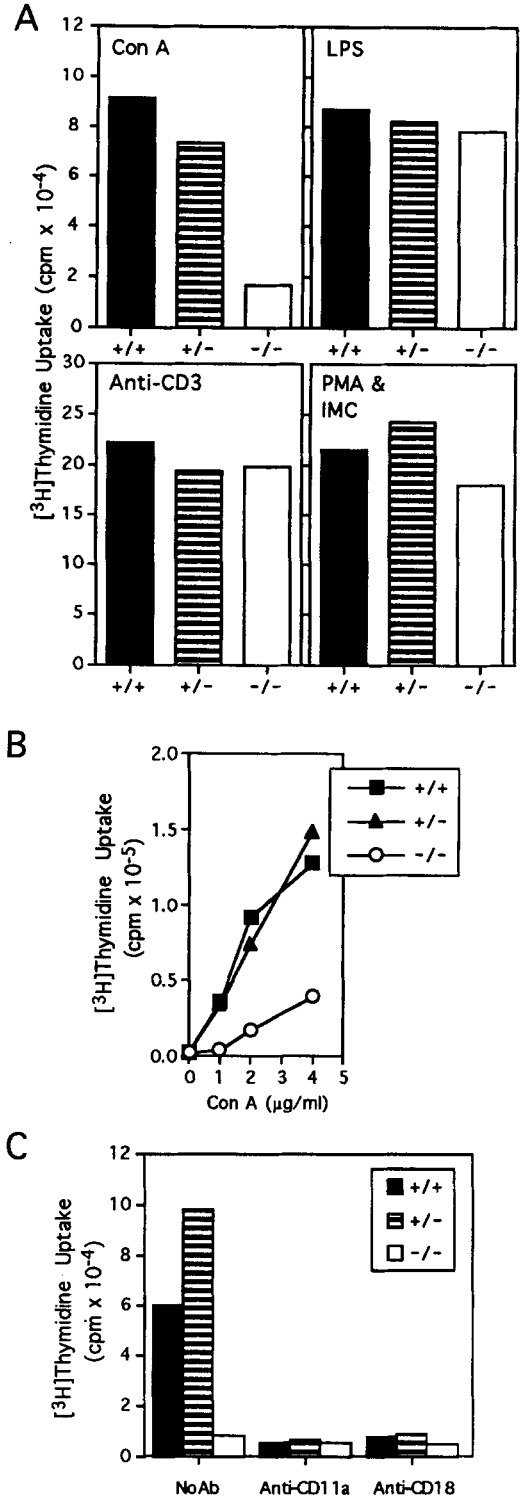

FIGURE 10. Con A-stimulated proliferation of LFA-1 −/− splenocytes is drastically reduced. *A*, Proliferative responses of LFA-1 +/+, +/−, and −/− splenocytes stimulated by 2 μg/ml Con A, 10 μg/ml LPS, immobilized anti-CD3ε Ab, or 50 ng/ml PMA plus 0.5 μM ionomycin. Results are presented as the means of triplicate values of one representative experiment of two performed. *B*, Proliferation of LFA-1 +/+, +/−, and −/− splenocytes stimulated by different concentrations of Con A. All points are the means of triplicate values of one representative experiment of two performed. *C*, Inhibition of Con A-stimulated proliferation of LFA-1 +/+ and +/−, but not LFA-1 −/−, splenocytes by mAbs recognizing the α or β subunit of LFA-1 (anti-CD11a, FD441.8; anti-CD18, 2E6). Results are presented as the means of triplicate values of one representative experiment of two performed.

flow cytometry, using Abs specific for CD3, CD4, and CD8, showed normal size populations of different thymocyte subsets and mature $CD4^+$ and $CD8^+$ T cells in the periphery of LFA-1 −/− mice (data not shown). To further address the question of whether positive or negative selection is affected in the absence of LFA-1, transgenic 2C TCR was bred into LFA-1 −/− mice. The 2C TCR has been reported to undergo positive selection to MHC class I K^b molecules and to show strong alloreactivity to L^d molecules (38, 39). Consequently, expression of CD4, CD8, and the transgenic TCR (using the clonotypic Ab 1B2 specific for the 2C TCR) was assessed by flow cytometry on thymocyte and peripheral lymph node populations isolated from mice of selecting (H-2^b), nonselecting (H-2^k), or deleting (H-$2^{b/d}$) backgrounds.

Positive selection of 2C TCR in LFA-1 −/− thymocytes of the H-2^b background was evidenced by skewing toward $CD8^+$ mature T cells, the majority of which expressed the transgenic receptor (Fig. 11*A*). Similar phenomena were observed in the periphery of these mice, where the majority of $CD8^+$ T cells expressed the transgenic TCR. In contrast, total thymocyte numbers were dramatically reduced, and few LFA-1 −/− thymocytes of the H-$2^{b/d}$ background expressed both CD8 and 2C TCR. This was reflected in the periphery, which was virtually devoid of CD8 cells expressing the transgenic TCR. Transgenic TCR-positive cells were predominantly CD8 negative. The data suggest that LFA-1 is not critical for positive and negative selection of T cells during thymic maturation. While slight differences in the sizes of the various thymocyte subsets exist between LFA-1 −/− and +/− mice, they vary from individual to individual, and no consistent trend is apparent. Results similar to these were obtained when a transgenic receptor specific for the H-Y male Ag (40, 41) was bred into LFA-1 −/− mice, and their thymocytes and lymph node cells were analyzed (our unpublished observations).

Positive selection of thymocytes has been suggested to result in up-regulation of TCR levels (42). 2C TCR on double-positive thymocytes from LFA-1 −/− mice was up-regulated in the H-2^b selecting background compared with that in the H-2^k nonselecting background (Fig. 11*B*). This elevated 2C TCR level was comparable to that in control LFA-1 +/− mice. In contrast, only double-positive thymocytes with low levels of 2C TCR remained in LFA-1 −/− mice in the H-$2^{b/d}$ deleting background.

Discussion

LFA-1 has been implicated in a variety of cell-cell interactions among leukocytes as well as between leukocytes and endothelial cells, yet its specific role in these interactions has not been well defined. The possibility that it may play a critical role in some of these processes was heightened by the discovery that LAD I patients, who exhibit defects in defense against bacterial and fungal pathogens that may be so severe as to be fatal, are deficient in LFA-1 and other β_2 integrins. While this finding pointed to the importance of β_2 integrins in immunosurveillance, it did not lead to a rapid elucidation of the specific functions of each β_2 integrin. Because all of these integrins overlap in expression and adhesion function, it is difficult to assign a particular pathology to a specific integrin deficiency. The pathologic symptoms in LAD I patients may even arise as a composite of more than one integrin defect. Since all leukocytes are affected and contribute to the overall immune dysfunction, it is also difficult to correlate any of these pathologies with any one defective leukocyte subpopulation. Furthermore, because this is a human disease, certain issues, such as the role of LFA-1 in thymic development, have not been addressed. Consequently, we generated LFA-1 knockout mice to determine the role LFA-1 plays in immunologic and inflammatory responses

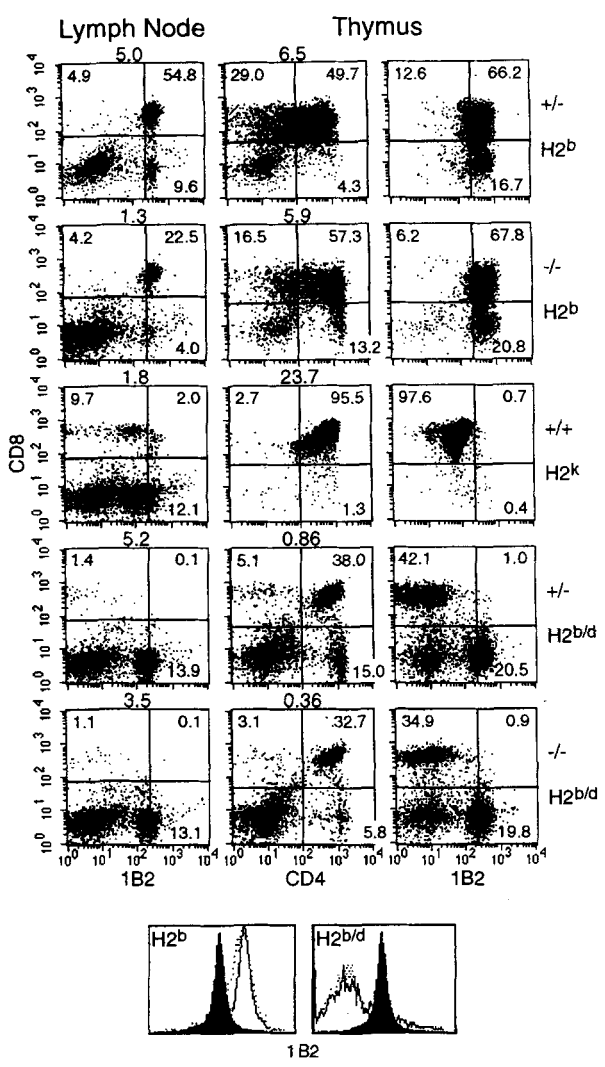

FIGURE 11. Normal thymic maturation and selection of T cells in LFA-1 −/− mice. *A*, Representative flow cytometric analysis of 2C transgenic TCR LFA-1 +/+, +/−, or −/− lymph node cells and thymocytes triple stained with mAbs to murine CD4, CD8, and the clonotypic mAb, 1B2, recognizing the transgenic TCR. Numbers within quadrants represent the percentage of cells in each subpopulation. Numbers above quadrants represent the total numbers of cells isolated from each organ × 10^{-7}. *B*, Histograms of 1B2 staining of gated double-positive thymocytes from 2C transgenic LFA-1 +/− (dotted line) or LFA-1 −/− (solid line) mice of selecting H-2^b or deleting H-$2^{b/d}$ backgrounds. The nonselecting H-2^k control is represented by a solid histogram.

and during thymic maturation. Recently, an independent group reported the generation and initial characterization of LFA-1-deficient mice and found them to exhibit defects in MLR, tumor rejection, and viral immunity (43). Our analysis of LFA-1−/− mice extends their findings and gives further insight into the biology of LFA-1 in several ways. We demonstrate that the reduction in proliferation of LFA-1 −/−splenocytes following MLR stimulation is the consequence of a shift in the kinetics of the response and not of an absolute decrease in the ability of the cells to proliferate. We show that LFA-1 −/− CTLs generated in vitro to alloantigens are defective in their cytotoxic activity, and that LFA-1 −/− splenocytes show defective proliferation in response to Con A stimulation. We demonstrate that LFA-1 −/− mice mount a reduced HVG response and are more susceptible to metastasis of B16 melanoma tumors. However, we detect normal lymphocyte homing and leukocyte extravasation and find the DTH response to SRBC to be minimally affected in these mice. Furthermore, we show that normal thymic selection can occur without LFA-1.

Ag-specific stimulation of naive T cells is strictly dependent on professional APCs, which present the Ag-MHC complex and provide costimulatory signals. We show that T lymphocytes from LFA-1 −/− mice are defective in their proliferative response to alloantigens following MLR stimulation and during an HVG response. This deficiency is unlikely to be an artifact because it is manifested both in vitro and in vivo, and suggests that LFA-1 on T cells is important for interaction with its counter-receptor on APCs to facilitate the alloresponse by mediating adhesion, providing a signaling function, or both. In the MLR, this requirement for LFA-1 is eventually overcome by the mutant splenocytes, resulting in a delayed peak of proliferation of a magnitude comparable to that in control cells. In agreement with our observation, Schmits et al. (43) showed similarly defective MLR proliferation with LFA-1 −/− T cells. Furthermore, spleen cells isolated from a knockout mouse model deficient in ICAM-1, a counter-receptor of LFA-1, failed to stimulate haplotype mismatched wild-type responder cells to proliferate (44, 45), and cells from LAD I patients have also been reported not to mount allogeneic mixed lymphocyte responses (46).

TCR engagement accompanied by a lack of adequate secondary costimulatory signaling may lead to tolerance or anergy of T cells to Ags (47). Dramatic results have been reported in inducing tolerance to cardiac allografts between fully incompatible mice by using a combined treatment of Abs against both LFA-1 and ICAM-1 (48). Our results as well as those of Schmits et al. (43), however, indicate that the lack of LFA-1 impairs, but does not completely abrogate, the alloantigen response. While LFA-1 −/− splenocytes exhibit a delayed proliferative response to Ags, cytotoxic function of the $CD8^+$ T cells could still be demonstrated in the alloantigen-specific CTL assay, albeit at lower efficiency. By day 6 of alloantigen stimulation, LFA-1 −/− CTLs exhibit the characteristic blasting appearance, express activation markers CD25 and CD69, and attain proliferation levels comparable to control cells (our unpublished observations). Following enrichment of $CD8^+$ effector cells, CTL activity of LFA-1 −/− effectors is still reduced compared with that of +/− cells, suggesting that a decreased efficiency of the CTLs generated rather than a decreased number of CTLs is responsible for the observed defect. Together, these results suggest that LFA-1-mediated cell-cell adhesion promotes efficient T cell-APC interaction, leading to Ag-specific T cell activation. However, the absence of LFA-1 does not abort Ag-specific T cell activation and effector function and, consequently, does not result in the induction of tolerance.

In our model of DTH, LFA-1 is found to be dispensable for edema formation and leukocyte infiltration. This is in contrast to the findings of Schmits et al. (43), who observed a dramatically reduced response in 2,4 dinitro-fluorobenzene (DNFB)-induced contact hypersensitivity. A possible explanation for the difference may be that while our sensitization with SRBC is systemic, sensitization with DNFB is local, implying that with a more potent stimulus, the presence of LFA-1 is less essential. However, although edema and leukocyte infiltration at the site of the DTH response to SRBC in LFA-1 −/− mice are not qualitatively different from those in controls, ex vivo proliferation of their splenocytes is compromised. The lack of LFA-1 may reduce the proliferative response of these splenocytes to SRBC in a manner analogous to that seen in the MLR. The same defect may explain the virtual absence of a DNFB-triggered DTH response, possibly due to an inefficient T cell activation, if the activating stimulus is

not adequately strong. Taken together, these results suggest that alloantigens may not be the only Ags displaying a reduced competence in stimulating naive LFA-1 knockout T cells.

Splenocytes from LFA-1 −/− cells also exhibit a profound reduction in Con A-stimulated proliferation, yet respond normally to LPS, PMA plus ionomycin, or immobilized anti-CD3ε mAb. While LFA-1 function is unnecessary for proliferation following stimulation with immobilized anti-CD3ε, treatment of LFA-1 −/− cells with soluble anti-CD3ε resulted in diminished proliferation (43). This again suggests that the strength of the activation signal may be the determining factor for the LFA-1 requirement. Interestingly, Con A-stimulated proliferation of T cells from CD28 −/− mice was dramatically reduced, similar in scope to the decrease in proliferation observed in Con A-stimulated LFA-1 −/− spleen cells (49). Thus, both LFA-1 and CD28 may be critical in this process, and disruption of either receptor/ligand interaction renders the cells only weakly responsive to the mitogen. CD28 has been proposed to provide costimulatory signaling during T cell activation, yet disruption of the CD28 gene results in impaired humoral, but not cell-mediated, immune responses (49, 50). This implies that alternative costimulatory pathways may exist. LFA-1 involvement has also been implicated in TCR signaling in several in vitro systems (51–55). Thus, the similar defects in Con A stimulation of T cells in the two knockout models suggest that both LFA-1 and CD28 make distinct contributions to T cell activation, or that one lies downstream of the other in the same signaling pathway.

Rejection of MHC disparate bone marrow transplants is believed to be mediated by both NK and CTL activities (56–58). Since LAD I patients show a high success rate in accepting bone marrow from haplotype-mismatched donors, they may have both defective T lymphocyte and defective NK activities (59). CTL-mediated lysis of allogeneic targets as well as NK activity have previously been shown to be inhibited by LFA-1-specific Abs (5, 60–63). Our results indicate that alloantigen-specific and nonspecific cytotoxicities (toward YAC-1 cell targets) are decreased following in vitro alloantigen stimulation of LFA-1 −/− splenocytes, yet in vivo induction of NK cells appears to consistently generate normal NK cytotoxicity in LFA-1 −/− mice. Surprisingly, using a similar protocol and assay, Schmits et al. (43) observed a defect in the NK cytotoxicity of LFA-1 −/− mice, a discrepancy that is difficult to reconcile. Nonetheless, following i.v. injection of NK-sensitive B16 melanoma tumor cells, mice lacking LFA-1 are more susceptible to syngeneic tumor growth and metastasis than are mice that express LFA-1, suggesting a possible defect in tumor immunity. Schmits et al. (43) describe a defect in the ability of LFA-1 mutant mice to reject a transplant with immunogenic fibrosarcoma tumor cells, which the authors attribute to defective CTL priming. Our study showing inefficient rejection of B16 tumors is consistent with a defect in innate immunity in this metastasis model, whether involving NK cells or macrophages, without excluding a possible contribution from defective CTLs.

Several members of the integrin family have been suggested to participate in lymphocyte homing and leukocyte extravasation, including LFA-1, Mac-1, very late Ag-4 (VLA-4; $\alpha_4\beta_1$), and lymphocyte Peyer's patch adhesion molecule −1 (LPAM-1; $\alpha_4\beta_7$) (8). The seemingly normal distribution of T cells, B cells, and granulocytes in different lymphoid compartments of LFA-1 −/− mice suggests that LFA-1 does not play a decisive role in homing of lymphocytes (43) (our unpublished observations). In the HVG response, hyperplasia of draining lymph nodes of LFA-1 −/− mice, while decreased relative to that of draining lymph nodes of control mice, is still considerable. This demonstrates that the role of LFA-1 is not critical for homing of lymphocytes to lymph nodes following Ag stimulation. In a DTH response, extravasation of LFA-1 −/− leukocytes to the site of Ag challenge is comparable to that in control mice. Presumably, $CD4^+$ T cells in the knockout mice were activated by SRBC-derived peptides during the sensitization stage, subsequently migrated to the site of Ag challenge, and recruited additional leukocytes. While our DTH data indicate that LFA-1 is dispensable in leukocyte extravasation, DNFB-induced contact hypersensitivity is greatly reduced in LFA-1 −/− mice (43). It may be that the observed defect resides in T cell priming rather than leukocyte extravasation in this model of DTH. Abs recognizing LFA-1 have been reported to partially block lymphocyte homing to peripheral lymph nodes and inhibit a DTH response (64, 65). This discrepancy may arise from differences in the approaches used to investigate leukocyte migration or may arise as artifacts resulting from inadvertent manipulation of the experimental systems, such as nonspecific inhibitory effects of Abs in vivo. The use of knockout mice abrogates the requirement for external intervention, and hence, such animals constitute the most rigorous models to study gene function in vivo. The dispensable role of LFA-1 in homing and extravasation indicated by our data can be explained by the overlapping function of other integrins expressed on leukocytes. It may be that in LFA-1 −/− mice, VLA-4, which has been implicated in directing cell migration of lymphocytes in the rat (66), is being used by extravasating lymphocytes during the DTH response to SRBC. Indeed, lymphocytes from LAD I patients are known to express VLA-4, are able to bind endothelial cells, and retain a diminished capacity to transmigrate an endothelial monolayer (67, 68). In LFA-1 −/− mice, granulocytes express a normal level of Mac-1. This integrin may permit essentially normal recruitment of neutrophils into inflamed tissues during a DTH response to SRBC or following an intradermal LPS injection. The differences between the effectively normal DTH response in the LFA-1 knockout mouse and the substantially reduced DTH response in ICAM-1 knockout mice (44, 45) or when anti-ICAM-1 mAb is used (65) probably stem from the fact that in the latter two cases neutrophils do not extravasate effectively because, in addition to a disruption of the LFA-1/ICAM-1 interaction, they also lack the Mac-1/ICAM-1 interaction. Taken together, these results imply that the migration of neutrophils is not critically affected by the loss of the LFA-1/ICAM-1 interaction, but is dramatically curtailed when the Mac-1/ICAM-1 interaction is also abolished.

The absence of LFA-1 expression does not have any noticeable effect on the sizes of the various thymocyte subsets or mature $CD4^+$ and $CD8^+$ cells in the periphery (43) (our unpublished observations). However, the presence of normal size populations does not exclude the possibility that LFA-1 is involved in either positive or negative selection during T cell maturation. While some evidence indicates that LFA-1 may have a role in thymic selection (37), no reports to date have used knockout mice to address this issue. To investigate both positive and negative selection, transgenic 2C TCR was bred into LFA-1 −/− mice of selecting and deleting haplotypes. Analysis of 2C TCR expression on thymocytes from these mice clearly demonstrates that for this transgenic model (as well as for H-Y TCR transgenic/LFA-1 −/− mice; our unpublished observations) normal T cell selection can occur without LFA-1.

Establishment of this LFA-1 −/− mutant mouse strain now provides an important model for analysis of the specific role of LFA-1 in immune responses and inflammation. Study of LFA-1-deficient mice may contribute to potential new therapeutic interventions in the treatment of diseases that have inflammatory or autoimmune

components, including rheumatoid arthritis, insulin-dependent diabetes mellitus, and multiple sclerosis or in the induction of tolerance and the prevention of transplant rejection.

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