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J Immunol 2008; 180:4656-4667; doi: 10.4049/jimmunol.180.7.4656
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Integrin CD18, a component of the LFA-1 complex that also includes CD11a, is essential for Th2, but not Th1, cell homing, but the explanation for this phenomenon remains obscure. In this study, we investigate the mechanism by which Th2 effector responses require the LFA-1 complex. CD11a-deficient T cells showed normal in vitro differentiation and function. However, Th2 cell-dependent allergic lung disease was markedly reduced in CD11a null mice and wild-type mice given LFA-1 inhibitors, whereas control of infection with Leishmania major, a Th1-dependent response, was enhanced. In both disease models, recruitment of IL-4-, but not IFN-γ-secreting cells to relevant organs was impaired, as was adhesion of Th2 cells in vitro. These diverse findings were explained by the markedly reduced expression of CD29, an alternate homing integrin, on Th2, but not Th1, cells, which precludes Th2 homing in the absence of CD11a. Thus, murine Th1 and Th2 cells use distinct integrins for homing, suggesting novel opportunities for integrin-based therapeutic intervention in diverse human ailments influenced by Th2 cells. The Journal of Immunology, 2008, 180: 4656–4667.

Helper T cells are recruited to sites of inflammation by the coordinated expression of several homing molecules, including integrins (1, 2). Integrins are a family of membrane glycoproteins that mediate cell adhesion to extracellular matrix components and to other cells (3, 4) and are composed of two noncovalently associated type I transmembrane glycoproteins (5). The CD18 (β2) integrin subfamily is comprised of four members sharing a common β subunit associated with the following four distinct but structurally homologous α subunits: LFA-1 (α1β2), CD11a/CD18, Mac-1 (α1β2, CD11b/CD18), p150,95 (α5β2, CD11c/CD18), and α6β2 (CD11d/CD18) (6, 7). Although LFA-1 is expressed on virtually all leukocytes and is the major, but not exclusive, CD18-related T cell integrin, other integrins expressed on T cells include VLA-4 (CD49d/CD29) and integrin β7 (8, 9).

CD11a is constitutively expressed on the surface of leukocytes in an inactive state. It is activated by high concentrations of divalent cations, chemokines, engagement of the TCR, and binding to its major endothelial counterreceptor CD54 (ICAM-1) (10, 11). CD11a is involved in lymphocyte recirculation and leukocyte extravasation at sites of inflammation (10, 12), but also participates in immunological synapse formation and may provide costimulatory signals for T cell activation (13, 14). Despite the apparently broad roles played by CD11a in T effector immunity, however, the major mechanisms by which it controls inflammatory responses remain uncertain.

CD18, the β integrin partner of CD11a, is selectively required for Th2, but not Th1, homing and is not required for Th effector development (15). In this study, we investigated the expression and function of CD11a in vitro and used in vivo models of Th2- and Th1-dependent inflammation to understand the role of the LFA-1 complex in Th cell development and tissue homing.

Materials and Methods

Mice

BALB/c and C57BL/6 mice were purchased from Harlan; Rag2−/− mice were purchased from Taconic Farms. CD11a-LFA-1 complex and congenic CD11a−/− mice (backcrossed to C57BL/6 and BALB/c backgrounds 13 and 8 generations, respectively) were originally obtained from C. M. Ballantyne (16). CD54−/− mice were obtained from A. L. Beaudet (17). DO11.10 mice (18) were obtained from D. Wofsy (University of California, San Francisco). Mice were bred in the Association for Assessment and Accreditation of Laboratory Animal Care-accredited transgenic animal facility at Baylor College of Medicine and used on accordance with all federal and institutional guidelines.

Abs and reagents

For ELISA and ELISPOT analyses, 11B11 and AN1819 were used for capturing and biotinylated polyclonal Ab (BAF413) for detection were used for capturing and biotinylated polyclonal Ab (BAF413) for detection were purchased from R&D Systems. For chicken egg OVA-specific Ab isotypes, biotinylated anti-mouse IgE, IgG1, and IgG2a Abs were purchased from Caltag Laboratories. For bronchoalveolar lavage (BAL) glycoprotein detection, biotinylated Jacalin was purchased from Vector Laboratories.

Allergens

OVA (Sigma-Aldrich) was precipitated in alum (OVA/alum) as previously described (19). Aspergillus fumigatus allergen was prepared from a clinical isolate of A. fumigatus and combined with OVA as previously described.
described (20). The combined allergen is referred to throughout as *A. fumigatus*/OVA.

**Allergen sensitization**

Mice were sensitized with OVA or *A. fumigatus*/OVA allergens as previously described (15).

**In vitro T cell stimulation**

T cells (2 × 10^5) positively purified from splenocytes using magnetic cell sorting (Miltenyi Biotec) were stimulated with 5 μg/ml plate-bound Ab against CD3 (clone 145-2C11) and 5 μg/ml soluble Ab against CD28 (clone 37.51; BD Biosciences) in flat-bottom 96-well culture plates (Corning). After 4 days of stimulation, cell pellets were collected for cell proliferation analysis (MTT assay; Roche Applied Bioscience) and supernatants were collected for IL-4, IL-13, and IFN-γ analysis (21).

For Th1 and Th2 differentiation, wild-type or CD11a (LFA-1)-deficient DO11.10 CD4 T cells were stimulated with OVA sub339 peptide (ISQAVHAAHAEINEAGR) and mitomycin C-treated APC in round-bottom 96-well cell culture plates. For the Th1 condition, IL-2 (20 U), IL-12 (2 ng), IFN-γ (100 U), and anti-IL-4 (11B11, 10 μg) and for the Th2 condition, IL-2 (20 U), IL-4 (200 U), IL-6 (100 U), anti-IFN-γ (AN18, 5 μg), and anti-IL-12 (C17.8, 2 μg) were added. Viability and polarization were confirmed by assessing proliferation and IL-4 and IFN-γ secretion following stimulation of resting cells with OVA sub339 peptide alone for 72 h.

**Parasites and infectious challenge**

Leishmania major strain MRHO/SU/59/P/LV39 was cultured and used to infect mice as described elsewhere (22, 23). Six weeks after infection, mice were sacrificed and footpads and spleens were harvested, minced, and homogenized using a tissue homogenizer in 3 ml of complete Schneider’s *Drosophila* medium. Parasite burdens were expressed as previously described (15).

**Analysis of the asthma phenotype**

For all allergen-challenged mice, airway hyperresponsiveness, BAL cytology, glycoproteins, OVA-specific Ab isotypes, and lung histopathology were determined as previously described (15, 21, 24).

**Adoptive transfer of DO11.10 Th2 cells and CD4 T cells**

In vitro-differentiated DO11.10 Th2 cells were injected i.p. (1.4 × 10^6) into Rag2-deficient mice. Twenty-four hours after reconstitution, mice were challenged with *A. fumigatus*/OVA allergen intranasally every other day for a total of four challenges. Data were collected 24 h after the final intranasal challenge.

Mice were challenged with *A. fumigatus*/OVA allergen and CD4+ T cells were purified from splenocytes and injected i.p. (1 × 10^6) into wild-type and CD54+/+ mice. Twenty-four hours after reconstitution, mice were challenged twice intranasally with allergen 4 days apart and data were collected 24 h later.

**CD11a blockade**

The blocking Ab against CD11a (KBA) was used as previously described (25) and control rat IgG Ab was purchased from Sigma-Aldrich. Wild-type C57BL/6 mice were primed four times i.p. with *A. fumigatus* OVA allergen and at least 2 h before intranasal challenge, Abs (0.5 mg/mouse) were injected i.p. and the asthma phenotype was determined 24 h later.

The CD11a-specific inhibitor LFA703 and vehicle were prepared as previously described (26). Wild-type BALB/c mice were primed three times i.p. with OVA allergen, and, 2 h before the intranasal challenge, the inhibitor (30 μg/kg) and vehicle only were injected i.p. and the asthma phenotype was determined 24 h later.

**Quantification of cytokine-producing cells**

Efficiency of homing was estimated by calculating the percentage of total cytokine+ cells represented in lung or lymph node (no. of lung cytokine+ cells/no. of spleen + lung cytokine+ cells or no. of lymph node cytokine+ cells/no. of spleen + lymph node cytokine+ cells) as described elsewhere (15).

**In vitro cell adhesion assay**

The interaction of Th1 and Th2 cells with CD54 and CD106 was assessed under static (27, 28) or continuous flow (29, 30) as previously described.
FIGURE 2. Attenuation of allergic lung disease in CD11a null mice. A–C, OVA- immunized BALB/c-background CD11a null mice and genotype -matched wild-type mice were challenged intranasally with OVA ( ■ ) or saline ( □ ) and airway hyperresponsiveness by PC200 ( A ), total eosinophils ( B ), and secreted airway glycoproteins ( C ) were quantitated from BAL. D, Representative bronchovascular bundles from whole lung are shown from saline- and OVA-challenged mice. Typical allergic inflammation (black arrows) accompanied by goblet cell transformation of the airway epithelium (red arrow; inset) is seen only in wild-type mice challenged with OVA (periodic acid-Schiff; relative size is indicated by the 200-μm bar). OVA-specific IgE ( E ), IgG1 ( F ), and IgG2a ( G ) titers as determined from the same mice. IL-4 ( H and I ) and IFN-γ ( J and K ) cells were enumerated by ELISPOT assay from spleens and lungs of the same mice, respectively. Data are representative of two independent experiments ( n = 5 for each experiment). * p < 0.05 compared with allergen-challenged wild-type mice.
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DO11.10 TCR; KJ1-26; R-PE-conjugated anti-CD11a: 2D7; FITC-conjugated anti-CD29: Ha2/5; for spleen and lung cells; all from BD Biosciences, except KJ1-26, which was from eBioscience). Data were collected using a Counter Epics flow cytometer and analyzed using FlowJo software (Tree Star).

Statistics
Data are presented as means ± SEM and are representative of at least two independent in vivo experiments that used at least four mice in each group. Significant differences (p < 0.05) are expressed using Student’s t test for logarithm-transformed PC200 data and the Kruskal-Wallis test for all other data.

Results

In vitro T effector development of CD11a−/− Th cells
Previously, we reported that in the absence of CD18, CD4 T cells develop into normal effector subsets (15). To address the effect of CD11a gene deletion on Th cell differentiation, we examined CD4 T cells isolated from wild-type and CD11a null mice. We found that naive, CD11a null Th cells proliferate and secrete Th1 and Th2 cytokines normally in response to in vitro stimulation with anti-CD3 and CD28 mAb (Fig. 1, A–D). Similarly, CD11a−/− T cells from DO11.10 OVA TCR-transgenic mice proliferated and secreted cytokines normally under Th2-polarizing conditions in response to OVA peptide (Fig. 1, E and F). These studies demonstrate intact general (anti-CD3/CD28) and Ag-specific (OVA-TCR) effector T cell differentiation and proliferation in the complete absence of CD11a. Thus, under diverse activation conditions, CD11a is not required for activation and cytokine secretion of either naive T cells or Th2 cells.

Reduced allergic lung disease in CD11a (LFA-1)-deficient mice
To determine the role of CD11a in Th2 effector function and homing in vivo, we next used a well-established model of allergic lung inflammation that is dependent on activation and recruitment of the Th2 subset of CD4 T cells to the lung (19, 31). Lack of CD11a

FIGURE 3. Enhanced resistance against L. major infection in CD11a null mice. A, Hind footpad thickness was measured weekly for 6 wk for fully inbred BALB/c, C57BL/6, and C57BL/6-background CD11a−/− mice following inoculation of L. major stationary phase promastigotes (*, p < 0.05 and †, p < 0.002 compared with CD11a null mice). B and C, L. major promastigotes were enumerated from footpads and spleens of the same mice, respectively (*, p < 0.005 and †, p < 0.05 compared with BALB/c mice). D, Hind footpad thickness of BALB/c-background CD11a null (B/c−/−), syngeneic littermate control mice type (B/c+/+), and fully inbred C57BL6 (B/6) and BALB/c (B/c) wild-type mice were measured weekly for 9 wk (*, p < 0.005 and †, p < 0.05 compared with CD11a−/− mice). E and F, L. major promastigotes were enumerated from footpads and spleens of the same mice (*, p < 0.05 compared with BALB/c mice). From the mice in D–F, total IFN-γ (G) and IL-4 (H)-secreting cells were enumerated from spleens and PLN and data are expressed as the percentage (fraction) of this total represented in PLN (PLN cytokine+ cells/PLN plus spleen cytokine+ cells). Data are representative of two independent and comparable experiments (n = 5 for each experiment).
FIGURE 4. Differential integrin expression and adherence of Th1 and Th2 cells to vascular addressins. A and B, OVA TCR-transgenic DO11.10 T cells were stimulated weekly in vitro with cognate peptide under Th1- and Th2-biasing conditions and the expression of CD11a and CD29 was assessed. Data are representative of three independent experiments. The adherence of in vitro- differentiated Th1 and Th2 cells to CD54 and CD106 was assessed using a dynamic (C) and static adhesion assay (D). C, Adherence to CD54 (left) and CD106 (right) over 10 min of continuous flow. D, Static cell adhesion assay showing the percentage of T cells adhering to CD54 (left) and CD106 (right) over 16 min. Data are representative of two or three independent experiments.
consistently resulted in reduction or abrogation of all allergic lung disease features as determined by reduced airway hyperresponsiveness, goblet cell metaplasia and reduced glycoprotein secretion, and reduced numbers of eosinophils in BAL fluid compared with wild-type mice (Fig. 2, A–C, and data not shown). Pathologic analysis demonstrated that lung inflammation and goblet cell metaplasia, highly characteristic features of the allergic lung disease phenotype, were almost entirely ablated in CD11a−/− mice (Fig. 2D). In contrast to the lung findings, but consistent with the normal cytokine and proliferative responses of CD11a−/− T cells (Fig. 1), Ag-specific Ab isotype titers were identical between allergen-challenged wild-type and CD11a−/− mice, indicating normal Th2 cell development in vivo (Fig. 2, E and F). ELISPOT analysis of lung and spleen cells showed that in the absence of CD11a, Th2 cells (IL-4+ cells) developed in the spleen (Fig. 2H), but that recruitment of these cells to the lung was significantly impaired (Fig. 2I). Furthermore, the homing defect apparent in the CD11a-deficient Th2 cell subset was specific because CD11a-deficient IFN-γ-secreting cells, representing predominantly Th1 cells, albeit at lower numbers, were of equal distribution in spleen and lung relative to wild-type littermate control mice (Fig. 2J and K). These findings indicate that lack of CD11a confers a homing defect that is specific to Th2 cells.

Enhanced protective immunity against L. major infection in the absence of CD11a

The preceding studies indicated that lack of CD11a markedly impairs the ability of Th2 cells to home to lungs, but that Th1 cell homing was largely unaffected. To address more rigorously the function of Th1 cells in the absence of CD11a, we challenged CD11a−/− and wild-type mice with the obligate intracellular pathogen L. major, eradication of which requires intact Th1 cell function (32). As expected, fully inbred BALB/c mice, which develop a predominant Th2 response against L. major (33), were unable to control infection as judged by the progressive rise in footpad size over 6 wk and recovery of large numbers of organisms from both footpads and spleens (Fig. 3, A–C). In contrast, C57BL/6 mice, which manifest a predominant Th1 response against the parasite (33), showed early arrest of disease and had significantly fewer parasites recovered from footpads and spleen (Fig. 3, A–C). However, by these same criteria, C57BL/6-background CD11a−/− mice showed superior disease control and markedly fewer footpad parasites relative to both inbred mouse strains (Fig. 3, A–C).

Similar experiments were performed in which L. major-infected BALB/c-background CD11a−/− mice were compared with wild-
FIGURE 6. Differential expression of integrins CD11a and CD29 on ex vivo-derived Th2 cells. Rag-2<sup>−/−</sup> mice were reconstituted with in vitro-conditioned Th2 cells and challenged intranasally with saline or A. fumigatus/OVA allergen to induce allergic lung inflammation. Single-cell suspensions were then prepared from lung and spleen. A. After initially gating on KJ1-26<sup>+</sup> CD4<sup>+</sup> cells to identify the transferred Th2 cells, expression of CD11a and CD29 was determined by flow cytometry from individual mice challenged intranasally with saline (saline challenge) or A. fumigatus/OVA allergen (allergen challenge) as indicated. B. The percentage of all lung Th2 cells expressing CD29 and CD11a was determined from four mice of the allergen-challenged group. *p < 0.05 compared with lung of saline-challenged mice. Data are representative of three independent experiments.

Differential integrin expression determines adherence of Th1 and Th2 cells to cognate addressins

The preceding analysis of two entirely distinct, but T cell-dependent, models of inflammation demonstrate that lack of CD11a has no effect on Th1 cell development and homing. In contrast, CD11a deficiency profoundly perturbs the recruitment of Th2 cells, while their development is also not impaired. To address the mechanism for the differential importance of CD11a for Th effector cell function shown by these studies, we reasoned that either lack of expression or failure of activation of other homing integrins or their endothelial addressins would explain the homing defect observed only in CD11a null Th2 cells. To assess these possibilities, we first determined the expression of both CD11a and an entirely unrelated homing integrin commonly expressed on T cells, CD29, on OVA-specific Th cells as they became progressively committed to either the Th1 or Th2 lineage, a process that evolves over several rounds of stimulation (34). OVA-specific cells were chosen because of their ability to maintain a Th1 or Th2 phenotype over several generations (35). OVA-specific Th cells as they became progressively committed to either the Th1 or Th2 lineage, a process that evolves over several rounds of stimulation (34). OVA-specific cells were chosen because of their ability to maintain a Th1 or Th2 phenotype over several weeks of stimulation (data not shown). Receptor analysis on OVA-specific T cells stimulated under both Th1- and Th2-biasing conditions over 4 wk of stimulation revealed that both CD29 and CD11a were detectable in Th1 cells but Th2 cells selectively lost expression of CD29 while preserving expression of CD11a (Fig. 4, A and B). Selective loss of CD29 on Th2 cells was specific because CD29 was present on all Th1 cells at all time points examined (Fig. 4, A and B; data not shown).

To ascertain whether the loss of CD29 on differentiated Th2 cells is functionally significant, we determined the adherence of Th1 and Th2 cells to CD54 and CD106, the major ligands for CD11a and CD29 integrins, respectively, using static and dynamic
CD54 is required for Th2 cell-mediated allergic lung disease. Indicated groups of wild-type (WT) and CD54−/− mice were administered 1 × 10⁶ splenic CD4 T cells obtained from allergen-immunized syngeneic wild-type mice and then challenged intranasally with allergen (■) or saline (□) over 2 wk. Airway hyperreactivity by PC200 (A), total BAL eosinophils (B), and secreted airway glycoproteins (C) were assessed within 18 h after the final allergen challenge (n = 4). *p < 0.05 compared with saline-challenged and KBA-treated mice.

Enhanced CD11a and reduced CD29 expression on lung Th2 cells following allergen challenge

Because CD54 and CD106 are both up-regulated in lung tissues in the asthma model (D. Corry, unpublished data), the preceding in vitro studies indicated that loss of CD29 expression, as opposed to differential expression of lung addressins, most likely explained the selective failure of CD11a−/− Th2 cells to home to lung. To determine whether Th2 cells lose expression of CD29 in vivo, DO11.10 TCR-transgenic Th2 cells conditioned for 7 days in vitro were adoptively transferred into Rag-2−/− deficient mice. DO11.10 cells were chosen because of the need to restimulate the transferred T cells in vivo with a physiologically relevant allergen to elicit allergic lung disease. After 2 wk of challenge with A. fumigatus/OVA allergen, Th2 cells from lungs and spleen were assessed for expression of CD29 and CD11a. Th2 cell-reconstituted Rag-2−/− mice showed allergic inflammation typical of wild-type mice challenged with the same allergen, including eosinophilia and glycoprotein hypersecretion in BAL fluid compared with saline-challenged mice (Fig. 5).

Analysis of CD11a and CD29 expression on adoptively transferred CD4+ Th2 cells isolated from lung revealed uniformly a high-level expression of CD11a (Fig. 6). As expected, few CD4+ T cells were recovered from lungs of saline control mice (Fig. 6, top panel). Th2 cells that were isolated from spleens showed two distinct populations of high- and low-CD11a expression (data not shown). Moreover, whereas all Th1 cells expressed CD29 (Fig. 4 and data not shown), less than half (a maximum of 45%) of lung Th2 cells expressed CD29 following allergen challenge (Fig. 6). Thus, lung Th2 cells analyzed ex vivo show progressive loss of CD29 and concomitant up-regulation of CD11a with exposure to airway allergen. These findings agree with in vitro analysis of integrin expression on Th1 and Th2 cells (Fig. 4) and correlate with specific localization of Ag-specific Th2 cells to lungs following allergen challenge.

LFA-1 inhibitors block allergic lung disease

The preceding studies establish a mechanism by which Th2 cells become dependent on CD11a for homing due to the selective
down-regulation or inactivation of alternate homing integrins such as CD29. This mechanism predicts that wild-type Th2 cells are markedly dependent on endothelial CD54 and T cell CD11a for their recruitment to sites of allergen challenge and therefore their ability to elicit allergic inflammation. To assess each of these possibilities, we performed additional in vivo experiments using CD54-deficient mice and wild-type mice given distinct LFA-1 inhibitors. To address the role of CD54 in the allergic lung disease model, we adoptively transferred wild-type CD4 T cells derived from spleens of previously immunized wild-type mice to either wild-type or CD54-deficient mice (17) and challenged the reconstituted mice with \textit{A. fumigatus}/OVA allergen (Fig. 7). With this protocol, airway hyperreactivity and BAL fluid eosinophils and glycoproteins were readily induced only in wild-type mice. In contrast, all of these features of allergic lung disease were significantly diminished in CD54-deficient mice (Fig. 7). These results support the concept that CD54 is the major intravascular receptor required for recruitment of Th2 cells to lung and induction of allergic lung disease.

To address the relative importance of LFA-1 on wild-type Th2 cells in vivo, allergen-sensitized mice were administered two inhibitors of LFA-1, a blocking mAb (KBA) and a small molecule (LFA703) derived from the cholesterol-lowering agent lovastatin (25, 26). Compared with lovastatin, LFA703 powerfully inhibits the LFA-1-CD54 interaction, but no longer inhibits 3-hydroxy-3-methyl-glutaryl-CoA reductase activity (26). Similar to CD11a- and CD54-deficient animals and irrespective of the mouse strain,
Allergen used, or LFA-1 inhibitor, LFA-1 blockade reduced or abrogated all features of allergic lung disease, including airway hyperresponsiveness, BAL fluid eosinophilia, and BAL glycoprotein secretion (Figs. 8 and 9, A–C).

LFA703 had no effect on the total number of IL-4- and IFN-γ-secreting cells generated in lung and spleen, nor did it diminish the fraction of IFN-γ-secreting cells that were recruited to lung (Fig. 9, D–F). In contrast, LFA703 markedly decreased the fraction of total IL-4-secreting cells recruited to the lung (Fig. 9G). Thus, LFA-1 inhibitors acutely inhibit allergic lung inflammation and selectively reduce lung IL-4 responses. Together, these findings demonstrate that the CD11a-CD54 interaction is required for robust allergic lung inflammation and, along with results from CD11a null animals, support the concept that Th2, but not Th1, cells selectively require CD11a for their recruitment to lung.

Discussion

In this study, we have analyzed the contribution of CD11a to Th1 cell development and function in vitro and Th cell-dependent disease using two well-characterized in vivo models and describe a major mechanism by which Th2 cells show selective dependence on CD18 integrins. Our studies confirm findings from CD18-deficient animals that CD18 integrins and, especially LFA-1, are not required for either T cell activation or effector differentiation under the conditions studied. Moreover, Th1 homing in vivo was entirely preserved in the absence of LFA-1 and correlated with expression on wild-type cells of both CD18 and CD29 integrins and the ability to adhere in vitro to multiple endothelial addressins. In contrast, Th2 cells showed preferential expression of CD11a and selective adherence to CD54, which correlated with reduced potential for allergic inflammation and lung IL-4 responses in the absence or blockade of either CD11a or CD54. Together, these findings provide a unique molecular mechanism that explains the Th2 cell-specific immune defects observed in CD18- and CD11a-deficient mice (Fig. 10).

These findings confirm and extend our previous studies of CD18-deficient and inhibited mice (15). Of the four known CD18 integrins, all of which are found on human and murine T cells (35–38), only CD11a-deficient mice manifest an impaired allergic lung phenotype that is substantially similar to that of CD18-deficient animals; mice deficient in CD11b, CD11c, and CD11d all show robust allergic lung responses to fungal allergens (Ref. 39 and J. Prince and D. Corry, unpublished data). We previously demonstrated that the reduced allergic lung disease in CD18-deficient animals was due to markedly impaired recruitment of Th2 cells to lungs (15). Because CD18 and CD11a reciprocally require each other for expression on T cells (D. Corry and S.-H. Lee, unpublished data), findings from both CD18 and CD11a null mice reflect the absence of the heterodimeric LFA-1 complex. Therefore, our studies together establish that LFA-1, interacting largely through CD54, is the principal CD18 integrin controlling Th2 homing and allergic inflammation, but do not exclude important functions other than homing regulated by other CD18 integrins.

Our studies are further consistent with previous investigations that demonstrated superior control of Listeria monocytogenes infection in the absence of CD11a (40, 41). These observations together indicate that Th1 responses are generally enhanced in the absence of CD18 or CD11a (40, 42). Augmented IL-12 production by LFA-1-deficient granulocytes was thought to explain the enhanced anti-listerial effect seen (41). Although we cannot exclude this mechanism from our studies of L. major-infected mice, its relative importance is diminished given the requirement of LFA-1 for recruitment of granulocytes (16).

Other studies have also suggested that LFA-1 is required for maintenance or survival of memory T cells (43). However, we have shown clearly that LFA-1-deficient TCR-transgenic T cells may be maintained for at least 6 wk in vitro and that LFA-1 null mice maintain control over L. major for at least 10 wk in vivo, demonstrating that memory responses are maintained without LFA-1. Instead, enhanced Th1 responses observed in the absence of LFA-1 are best explained by the lack of recruited Th2 cells that otherwise interfere with Th1 function. Thus, rather than functioning as a costimulatory molecule for either naive or memory T cells, our detailed dissection of the murine LFA-1 complex demonstrates that its major role is to control the homing of Th2 cells under the conditions studied.

Our studies also confirm previous investigations showing enhanced VLA-2 (CD49b/CD29) expression on Th1 cells and reduced expression of this integrin complex on Th2 cells (44), but we show here the functional consequences of differential expression of homing integrins on Th2 cells. At least some LFA-1-deficient Th2 cells were capable of reaching the lung following allergen challenge (Fig. 2f), but this LFA-1-independent homing response is explained by the subset of Th2 cells that retain expression of CD29 integrins after only 2 or 3 wk of stimulation (Figs. 4 and 6). Nonetheless, the marked efficacy of acute LFA-1 blockade indicates that even at very early time points, Th2 cells are predominantly dependent on LFA-1 regardless of their degree of CD29 expression. The virtually complete loss of CD29 with additional rounds of Ag challenge suggests that LFA-1 blockade would become progressively more effective at inhibiting allergic inflammation over time, a hypothesis that will be tested as part of future investigations.

Our findings indicate the existence of novel mechanisms that differentially regulate the expression of T cell integrins in a lineage-specific manner. Ligation of Th2 cell-specific chemokine receptors may specifically activate LFA-1, and not other integrins such as VLA-4, to a high-affinity conformation required for efficient homing (45, 46), but this process may also enhance the expression of CD11a with repeated allergen exposure. This mechanism, or another, might be particularly relevant to relatively immature Th2 cells that express VLA-4, but possibly in an inactive conformation, and may also determine the suppression of CD29 on Th2 cells. Further understanding of these mechanisms will clarify the molecular processes that critically regulate the homing of T cell subsets and shed additional light on the pathogenesis of a wide
range of infectious and noninfectious immune processes driven by T cells. Our findings have important implications for the therapy of human diseases driven by T cell effector subsets. Diverse human infectious illnesses such as severe leishmaniasis, poorly controlled Mycobacterium tuberculosis and Mycobacterium leprae infection, nocardiosis, and likely many others are thought to result from an imbalance in T cell subsets marked by excessive numbers of Th2 cells and their cytokines (47–54). Moreover, allergic diseases such as asthma that result from predominant Th2 cell activation are among the most important chronic diseases in the world today (55). Although manipulation of CD29 integrins potentially compromises immune surveillance and infectious disease control (56), our findings suggest that inhibition of CD11a-dependent homing has the potential to resolve many chronic infectious ailments and attenuate allergic disease driven by Th2 cells.

Acknowledgments
We thank J. Xu and Y. Qian for technical assistance and B. W. McIntyre and J. R. Rodgers for helpful discussions.

Disclosures
The authors have no financial conflict of interest.

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