# Regulation of inflammation by collagen-binding integrins α1β1 and α2β1 in models of hypersensitivity and arthritis

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**Adhesive interactions play an important role in inflammation by promoting leukocyte attachment and extravasation from the vasculature into the peripheral tissues. However, the importance of adhesion molecules within the extracellular matrix–rich environment of peripheral tissues, in which cells must migrate and be activated, has not been well explored. We investigated the role of the major collagen-binding integrins, α1β1 and α2β1, in several in vivo models of inflammation. mAb's against murine α1 and α2 were found to significantly inhibit effector phase inflammatory responses in animal models of delayed-type hypersensitivity (DTH), contact hypersensitivity (CHS), and arthritis. Mice that were α1-deficient also showed decreased inflammatory responses in the CHS and arthritis models when compared with wild-type mice. Decreased leukocyte infiltration and edema formation accompanied inhibition of antigen-specific models of inflammation, as nonspecific inflammation induced by croton oil was not inhibited. This study demonstrates the importance in vivo of α1β1 and α2β1, the collagen-binding integrins, in inflammatory diseases. The study also extends the role of integrins in inflammation beyond leukocyte attachment and extravasation at the vascular endothelial interface, revealing the extracellular matrix environment of peripheral tissues as a new point of intervention for adhesion-based therapies.**

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## Introduction

By promoting leukocyte attachment and extravasation from the vasculature and into the peripheral tissues, adhesive interactions play a key role in inflammation (1). A further critical step in the inflammatory cascade, which has not been extensively explored, occurs within the peripheral tissues. Here infiltrating and resident cells need to migrate towards the site of infection, recognize the foreign antigen, and undergo cellular activation to perform their effector functions. To assess the importance of interstitial adhesive interactions in inflammation, in isolation from the role adhesive interactions play in leukocyte recruitment, we focused on the major collagen-binding integrins, α1β1 and α2β1.

Cells must migrate through both the interstitial environment and basement membranes, and it is this environment, rich in extracellular matrix molecules, in which they are activated. The most abundant of these molecules are collagens, accounting for approximately 75% of skin's dry weight (2). The major cell surface receptors for collagens are the α1β1 and α2β1 integrins, with α1β1 showing a preference for type IV collagen, and α2β1 showing a preference for type I collagen (3, 4). The α1β1 and α2β1 integrins are also able to interact weakly with laminin (5).

The expression of α1β1 in the adult is largely confined to mesenchymal cells, notably smooth muscle cells, fibroblasts, hepatocytes, and microvascular endothelium (6, 7), whereas α2β1 is predominantly epithelial in distribution (8). Analysis of α1β1 and α2β1 expression on immune cells reveals that little detectable α1β1 or α2β1 is expressed on peripheral blood lymphocytes (5, 9, 10). Monocytes and platelets both express moderate amounts of α2β1, whereas α1β1 is expressed weakly on monocytes (5). Consistent with the initial description of α1β1 and α2β1 as very late antigens, long-term activated lymphoid cells can express these receptors. Specifically, IL-2–activated T-cell clones express both α1β1 and α2β1 after several weeks of in vitro culture (5), and infiltrating T cells express α1β1 in a variety of chronic inflammatory settings; these include the rheumatoid synovium of arthritis patients (PBL)(5, 9, 11), the lungs of sarcoidosis patients (5), and atherosclerotic plaques (10).

Targeted null mutations for α1 and α2 have been generated, with the α2 null mutation being embryonic lethal (H. Gardner, unpublished results). Although the α1-deficient mice showed no gross developmental effects, fibroblasts from these mice showed a specific

deficiency in attachment to collagen type IV, as well as increased collagen expression resulting from loss of feedback regulation (12, 13). Because of the restricted expression of α1β1 and α2β1 on immune cells, most of the information on the role of α1β1 and α2β1 relates to the function of these integrins on nonimmune cells. The α1β1 integrin, but not α2β1, is linked via the Shc adaptor protein to the mitogen-activated protein kinase pathway and is responsible for regulating cell survival and cell cycle progression (14). In vitro, both α1β1 and α2β1 play a role in cell migration (12, 15) and in the reorganization and contraction of collagen matrices, which are important in wound healing (16–18). Finally, in vivo studies have shown that α1β1 and α2β1 are important in angiogenesis (19), and that α1β1 is involved in intestinal graft-versus-host disease (20).

Given the functional importance of the α1β1 and α2β1 collagen-binding integrins in nonimmune cells, the presumed role of the extracellular matrix environment to the inflammatory process, and the fact that long-term activated immune cells can express these integrins, we tested the importance of α1β1 and α2β1 integrin function in animal models of inflammation. Using function-blocking mAb's against murine α1 and α2, both α1β1 and α2β1 were found to significantly inhibit inflammatory responses in animal models of delayed-type hypersensitivity (DTH), contact hypersensitivity (CHS), and arthritis. Similar decreased inflammatory responses were seen in α1-deficient mice in models of CHS and arthritis. These results emphasize the importance of extracellular matrix–binding integrins in inflammation.

## Methods

*Mice*. Six- to eight-week-old Balb/c female mice were purchased from Taconic Farms (Germantown, New York, USA), and α1 integrin–deficient mice on a Balb/c background were obtained as previously described (12).

*Monoclonal antibodies*. These function-blocking mAb's to murine integrin chains were prepared in an azide-free and low-endotoxin format: PS/2 (rat anti-α4) (21), Ha31/8 (hamster anti-α1) (22), Ha1/29 (hamster anti-α2) (22), and the hamster group II control mAb Ha4/8 (hamster anti-KLH) (22). Function-blocking mAb's to murine antigens were also purchased as no-azide, low-endotoxin preparations from PharMingen (San Diego, California, USA): M1/70 (rat anti-CD11b), Ha2/5 (hamster anti-β1 integrin chain), and 3E2 (hamster anti–ICAM-1). Murine TNF-R55 receptor human Ig fusion protein and control Ig fusion protein were provided by J. Browning (Biogen Inc., Cambridge, Massachusetts, USA) (23). For immunohistochemistry, Ha4/8 and Ha31/8 mAb's were fluorescently labeled using the Alexa 488 protein labeling kit as recommended by the manufacturer (Molecular Probes Inc., Eugene, Oregon, USA); phycoerythrin (PE)-conjugated mAb's, 145-2C11 (hamster anti-CD3e), M1/70 (rat anti-CD11b), and RB6-8C5 (rat anti–Ly-6G/Gr-1), were from PharMingen.

*Flow cytometry*. Cells were incubated with primary antibody, washed, resuspended in FITC-conjugated anti-hamster Ig secondary antibody (Caltag Laboratories Inc., Burlingame, California, USA), and analyzed on a FACStar$^{PLUS®}$ flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, California, USA).

*Adhesion assay*. Balb/c splenocytes were cultured with 20 ng/mL IL-2 for 7–12 days. Adhesion of cells to collagen type I and IV was performed as described previously (17).

*Delayed-type hypersensitivity*. Sheep red blood cell–induced (SRBC-induced) DTH was performed as described (24). Mice were immunized subcutaneously (s.c.) on the back with $2 \times 10^7$ SRBC in 100 μL PBS on day 0. The mice were challenged on day 5 by injecting $10^8$ SRBC in 25 μL PBS s.c. into the right hind footpad. Footpad thickness was measured with an engineer's caliper 20 hours after antigen challenge, and the degree of footpad swelling was calculated as: percent increase = [(right footpad thickness after antigen challenge/uninjected left footpad thickness after antigen challenge) – 1] × 100. To block the effector phase of the SRBC-induced DTH response, mAb (100 μg) was given intraperitoneally (i.p.) 1 hour before antigen challenge on day 5.

*Contact hypersensitivity*. Mice were sensitized by painting 100 μL 0.5% FITC (Sigma Chemical Co., St. Louis, Missouri, USA) in 1:1 acetone/dibutylphthalate onto the shaved back on day 0. Animals were challenged 10 days later with the application of 5 μL 0.5% FITC onto both sides of each ear. Ear swelling response was determined by ear thickness measured with an engineer's caliper at the time of antigen challenge (day 10) and 24 hours later, and the results were calculated as percent increase in ear thickness. To block the effector phase of the CHS response, mAb (250 μg) was given i.p. 4 hours before antigen challenge on day 10. Mice that were antigen sensitized and ear challenged with vehicle only, or mice that were ear challenged without prior sensitization served as negative controls (having never exceeded a 2% increase in ear thickness).

*Irritant dermatitis*. Mice were painted with 5 μL of 0.8% croton oil (ICN Radiochemicals Inc., Costa Mesa, California, USA) in acetone on both sides of each ear; mAb (250 μg) was given 4 hours before the application of the irritant. Ear swelling was measured 24 hours later, compared with ear thickness before croton oil application, and results reported as above. Mice painted with acetone only (vehicle control) served as a negative control.

*Anti-collagen mAb-induced arthritis*. Arthrogen-collagen–induced arthritis antibody kits were purchased from Chondrex LLC (Redmond, Washington, USA), and arthritis was induced using an established protocol (25, 26). Mice were injected i.p. with a cocktail of 4 anti-collagen type II mAb's (0.5 mg each) on day 0, followed by i.p. injection of 25 μg LPS on day 3. After 3–4 days, the mice developed swollen wrists, ankles, and digits. mAb (250 μg) or Ig fusion protein (200 μg) was administered i.p. starting on day 0 and continuing every third day for the duration of the experiment. Severity of arthritis in each limb was scored as follows: 0 = normal; 1 = mild

redness, slight swelling of ankle or wrist; 2 = moderate swelling of ankle or wrist; 3 = severe swelling including some digits, ankle, and foot; 4 = maximally inflamed.

*Histology.* Samples were excised, fixed in 10% formalin-buffered saline, decalcified, embedded in paraffin, sectioned, and then stained with either hematoxylin and eosin or toluidine blue using standard techniques. For immunohistochemical staining, acetone-fixed frozen sections (10-μm thick) were blocked in a 3% BSA/PBS solution for 30 minutes at room temperature. Slides were washed and sections incubated with 5 μg/mL of Alexa 488–labeled mAb in 3% BSA/PBS for 1 hour at room temperature. Slides were then washed in PBS and mounted in Citifluor (Ted Pella Inc., Redding, California, USA). Two-color immunohistochemistry was performed with the inclusion of PE-conjugated mAb during the primary staining step. The stained sections were examined by dual immunofluorescent microscopy (Leica, Wetzlar, Germany).

## Results

*Expression and functional blockade of α1β1 and α2β1 on activated leukocytes*. Given the key role leukocytes play in inflammation, we tested whether the anti-α1 and anti-α2 mAb's were capable of blocking leukocyte adhesion to collagens. To obtain leukocytes expressing α1β1 and α2β1, murine T cells were stimulated in vitro with IL-2 for 7–12 days. These cells expressed high levels of both α1 and α2 (Figure 1a), and bound well to both collagen type IV– and type I–coated surfaces (Figure 1b). Adhesion to type IV collagen was partially inhibited by anti-α1 mAb alone and not by anti-α2 mAb alone. In contrast, adhesion to type I collagen was completely inhibited by anti-α2 mAb, and anti-α1 mAb alone showed only partial inhibition. Both anti-β1 mAb and the combination of anti-α1 and anti-α2 mAb's completely inhibited adhesion to collagen types I and IV. Having demonstrated that α1β1 and α2β1 integrins are expressed on activated T cells and that anti-α1 and α2 mAb's are able to functionally block leukocyte adhesion to collagens, we used these mAb's to investigate the in vivo role of these integrins in animal models of inflammation.

*Inhibition of DTH responses by anti-integrin mAb's*. SRBC-induced DTH is a well-characterized in vivo model of inflammation (24). SRBC-sensitized mice received anti-integrin mAb 1 hour before footpad antigen challenge, and inflammation was assessed 20 hours later as measured by increased footpad thickness. PBS and control hamster Ig–treated mice showed a 60%–70% increase in footpad thickness (Figure 2a). Compared with control hamster Ig treatment, anti-α1 or anti-α2 mAb's resulted in a 76% and 61% inhibition in footpad thickness, respectively. The combination of anti-α1 and anti-α2 mAb's resulted in 76% inhibition, demonstrating little additive effect over anti-α1 or anti-α2 mAb alone. These findings were confirmed histologically as footpads from control mAb-treated mice showed marked edema, whereas footpads of mice treated with anti-α1, anti-α2, or a combination of anti-α1 and anti-α2 mAb's resulted in a marked reduction in footpad thickness (Figure 2b).

Further histologic analysis of the SRBC-induced DTH response confirmed the ability of anti-α1 and anti-α2 mAb treatment to modulate the elicited inflammatory response (Figure 3). An unchallenged footpad from an SRBC-sensitized mouse (Figure 3a) showed virtually no inflammatory cellular infiltrate when compared with an SRBC-challenged footpad from the same mouse (Figure 3b). Treatment of SRBC-sensitized mice with anti-α1 and anti-α2 mAb, either alone or combined, greatly reduced the number of these infiltrating cells found in SRBC-challenged footpads when compared with control mAb-treated mice (Figure 3, c–f). Closer examination of the infiltrating cells revealed most cells to be composed of neutrophils, with some monocytes and lymphocytes present, and confirmed that anti-α1 and anti-α2 mAb treatment greatly decreased the numbers of these cells (Figure 3, g and h).

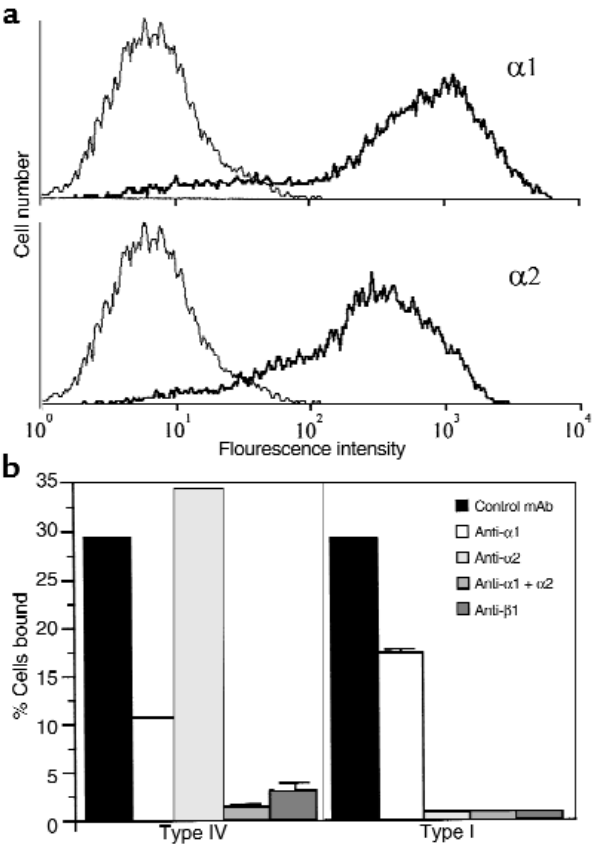


**Figure 1**
Collagen-binding integrins α1β1 and α2β1 on activated leukocytes. (**a**) Flow cytometric analysis of α1 and α2 integrin expression on IL-2–activated splenocytes (day 11). Cells were labeled with anti-α1 mAb, anti-α2 mAb, or nonbinding control mAb (gray lines), and followed by FITC anti-hamster immunoglobulin. (**b**) Effect of anti-α1 and anti-α2 mAb's on leukocyte adhesion to collagen. IL-2–activated splenocytes were treated with the indicated mAb's and then plated onto either type IV or type I collagen-coated wells. Adhesion assays were done in triplicate; 1 of 3 representative experiments is shown.

Immunohistochemistry was performed to more precisely determine the nature of the infiltrating cells and whether they express collagen-binding integrins (Figure 4). Our efforts were restricted to examining α1 expression because of the lack of a functional anti-α2 mAb for use in acetone-fixed frozen sections. Infiltrating cells from an inflamed footpad of an untreated mouse were examined for expression of α1 integrin and cell lineage markers (Figure 4). We found that α1 integrin was expressed on many infiltrating leukocytes (Figure 4a). Dual immunohistochemistry was used to identify the nature of the infiltrating cells and the distribution of α1 expression (Figure 4b). Using cell lineage markers, the infiltrate was found to be largely composed of granulocyte/monocytes (Mac-1$^+$), with many of these cells being neutrophils (Gr-1$^+$), along with a smaller number of T lymphocytes (CD3$^+$) (Figure 4b). Expression of α1 integrin was found among all 3 subsets of cells, with α1 expressed on a subset of Mac-1$^+$ granulocyte/monocytes, a subset of Gr-1$^+$ neutrophils, and on the majority of infiltrating CD3$^+$ T lymphocytes (Figure 4b). Detailed immunohistochemical analysis revealed that although anti-α1 and anti-α2 mAb treatment reduced the numbers of infiltrating cells, no change in the cellular composition of the infiltrate was seen (data not shown). Immunohistochemistry staining with a FITC anti-hamster mAb confirmed the ability of the anti-α1 and anti-α2 mAb's to localize to the inflamed footpad (data not shown).

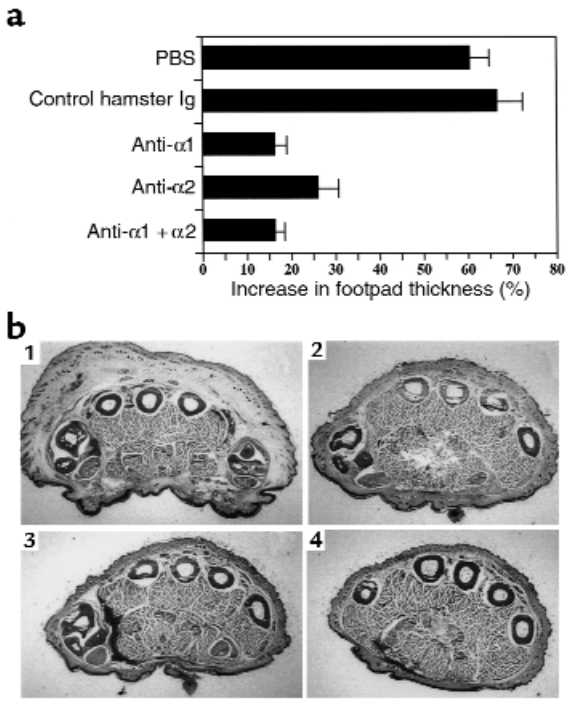


**Figure 2**
Effect of anti-α1 and anti-α2 mAb's on the effector phase of DTH. (**a**) Inhibition of DTH response by administration of anti-α1 and anti-α2 mAb at antigen challenge. Footpad thickness was measured 20 hours after antigen challenge, and results are shown as percent increase in footpad thickness ± SEM. Groups of 10 mice per condition were used; 1 of 8 representative experiments is shown. (**b**) Histologic analysis of inflamed footpads (cross-section) showing reduced edema upon treatment with anti-integrin mAb. Formalin-fixed tissue sections are from footpads of SRBC-sensitized mice 20 hours after challenge with SRBC. Mice were treated 1 hour before challenge with either control hamster Ig (part **1**), anti-α1 (part **2**), anti-α2 (part **3**), or anti-α1 and anti-α2 mAb's (part **4**).

*Inhibition of CHS effector responses by anti-integrin mAb's.* Because CHS is mechanistically distinct from DTH and involves different effector cells, we investigated what effect anti-α1 or anti-α2 mAb's had on the effector phase of the CHS response in both wild-type and α1-deficient mice (Figure 5). FITC-sensitized mice received anti-integrin mAb's 4 hours before antigen challenge to the ear, and inflammation was assessed 24 hours later, as measured by increased ear thickness. FITC-sensitized wild-type mice demonstrated a 60%–70% increase in ear thickness 24 hours after antigen challenge (Figure 5). Compared with control mAb, treatment of wild-type mice with anti-α1 or anti-α2 mAb's resulted in 37% and 57% inhibition in ear swelling, respectively (Figure 5). The combination of anti-α1 and anti-α2 mAb's resulted in slightly greater inhibition of ear swelling (65%). Consistent with the findings of Scheynius et al. (27), anti–ICAM-1 mAb treatment resulted in 51% inhibition of ear swelling. The triple combination of anti-α1, anti-α2, and anti–ICAM-1 mAb's showed a similar level of inhibition as seen with the dual combination of anti-α1 and anti-α2 mAb's (data not shown). In agreement with the mAb-based inhibition data, the effector phase of CHS was significantly reduced in α1-deficient mice (30% increase in ear thickness) as compared with wild-type mice (60%–70% increase in ear thickness) (Figure 5). Finally, mAb blockade of α2 in the α1-deficient mice resulted in a slightly increased inhibition of ear swelling, consistent with the results seen in wild-type mice treated with a combination of anti-α1 and anti-α2 mAb's. Histologic analysis of inflamed ears revealed that both edema formation and leukocytic infiltration were inhibited by anti-α1 and anti-α2 mAb treatment (data not shown).

Consistent with the finding that α1β1 and α2β1 can be expressed on IL-2–activated splenocytes, analysis of lymph nodes from antigen-sensitized mice revealed α1 and α2 to be expressed exclusively on CD44$^{hi}$ LFA-1$^{hi}$–activated CD4$^+$ and CD8$^+$ T cells (data not shown). Treatment of mice with anti-α1 and anti-α2 mAb's did not result in deletion of these cells, as the numbers of activated T cells in both spleen and lymph nodes were unaffected in response to antigen sensitization in the CHS model (data not shown). In addition, effector cells were not functionally deleted as prolonged treatment of antigen-sensitized mice with anti-α1 and anti-α2 mAb's (day 10–16) did not affect the inflammatory response of mice challenged with antigen at day 20 (data not shown).

*Irritant dermatitis is not inhibited by α1β1 or α2β1.* The effect of anti-α1 and anti-α2 mAb's on irritant dermatitis was studied to further exclude the possibility

that the inhibitory effect seen in both the DTH and CHS models of inflammation is caused by a general anti-inflammatory effect mediated by these mAb's (Figure 6). Balb/c mice were treated with anti-integrin mAb 4 hours before application of 0.8% croton oil onto the ears. Ears of mice treated with croton oil showed an increase in ear thickness 24 hours later (48%), when compared with mice receiving vehicle only. Toxic ear swelling caused by croton oil was not significantly affected in mice pretreated with anti-α1 or anti-α2 mAb's when compared with either PBS or control mAb-treated animals (Figure 6). Histologic examination of the croton oil–treated ears revealed no differences in numbers or types of infiltrating cells or edema formation in mice treated with anti-α1 or anti-α2 mAb's, as compared with control mAb-treated mice or PBS-treated mice (data not shown).

*Inhibition of arthritis by α1β1 and α2β1.* Because α1β1 is well expressed on infiltrating cells in the synovium of arthritis patients, we examined whether anti-α1 or anti-α2 mAb's would be inhibitory in an accelerated model of arthritis previously described (25, 26). This model involves injection of a cocktail of anti-collagen type II mAb's into mice, followed later by LPS administration, resulting in the development of arthritis over the next 3–7 days. Mice were given anti-integrin mAb every third day, starting at day 0, and then were scored for the development of arthritis every third day. Severe arthritis developed in all mice within 72 hours after LPS injection and persisted for more than 3 weeks. Neither an injection of anti-collagen mAb's alone, nor an injection of LPS alone induced arthritis. Mice receiving control mAb treatment displayed equally severe arthritis as than seen in PBS-treated mice (Figure 7a). In contrast, treatment with anti-α1 mAb alone resulted in a marked reduction (79%) in arthritis, which lasted the duration of the experiment. Treatment with anti-α2 mAb alone also had a beneficial effect, resulting in a 37% decrease in the arthritic score as compared with control mAb-treated mice. The combination of anti-α1 and anti-α2 mAb's resulted in a similar degree of inhibition as that seen with anti-α1 mAb alone. Reduction of arthritic score with anti-α1 mAb treatment was seen in all mice and compares favorably with several other mAb-based treatments for arthritis, such as soluble TNF receptor Ig fusion protein (28), anti–Mac-1 (29), anti-α4 (30), and anti–ICAM-1 (31) (Figure 7a). In agreement with mAb-based data showing an important role for α1β1 in arthritis, untreated α1-deficient mice showed significant reduction in arthritic score when compared with wild-type mice (Figure 7b). Joints from wild-type arthritic mice (day 8) receiving either control mAb or anti-α1 mAb treatment were compared visually and histologically with joints from a healthy untreated mouse (Figure 7c). Visually, joints from control mAb-treated mice demonstrated redness and swelling of the entire foot including digits, whereas anti-α1 mAb–treated mice showed little, if any, signs of inflammation in either joints or digits. Histologic examination showed severe changes in control mAb-treated arthritic joints, with extensive infiltration of the subsynovial tissue with inflammatory cells, adherence of cells to the joint surface, and marked cartilage destruction as evidenced by proteoglycan loss (Figure 7c). Consistent with previous reports (25, 26), most of the infiltrating cells in this model are neutrophils. Anti-α1 mAb treatment of mice dramatically reduced the amount of inflammatory infiltrate and the degree of cartilage destruction (Figure 7c).

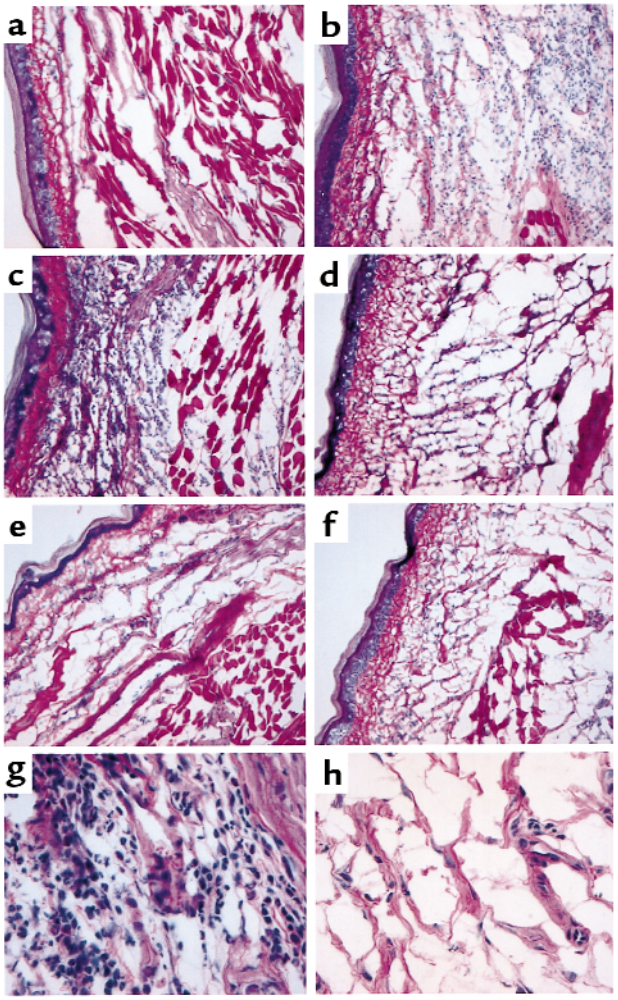


**Figure 3**
Administration of anti-α1 or anti-α2 mAb's inhibits leukocyte infiltration into footpads during a DTH response. The experiment was performed as described in Figure 2. Footpads were excised 20 hours after antigen challenge, and tissue sections were stained with hematoxylin and eosin. Tissue sections are from footpads of either unchallenged mice (**a**) or SRBC-sensitized mice challenged with SRBC (**b**–**h**). Mice were treated 1 hour before challenge with either PBS (**b**), control hamster Ig (**c**, **g**), anti-α1 (**d**), anti-α2 (**e**), or a combination of anti-α1 and anti-α2 mAb's (**f**, **h**). (**a**–**f**) ×100; (**g**–**h**) ×400.

## Discussion

Recent studies in animal models of asthma suggest an emerging paradigm shift regarding the role of leukocyte adhesion molecules in inflammation. These stud-

ies indicated that despite the importance of α4β1-VCAM-1 interactions in promoting leukocyte exit from the blood stream, the efficacy of anti-α4 mAb treatment in asthma is linked to α4-expressing cells in the interstitial tissues (32, 33). Using several in vivo models of inflammation, we investigated the importance of adhesion molecules in the extracellular matrix–rich environment of peripheral tissues. We chose to concentrate on the collagen-binding integrins, α1β1 and α2β1, as collagens are major components of the extracellular matrix and basement membranes. The fact that these integrins are not involved in leukocyte-endothelial interactions also allowed us to clearly validate the concept that adhesion molecules play an important role in peripheral tissues.

Using 3 well-defined inflammatory models (DTH, CHS, arthritis), we have demonstrated that anti-α1 or anti-α2 mAb treatment of antigen-sensitized mice just before antigen challenge significantly inhibited the inflammatory response — decreasing edema and leukocyte infiltrate, and, in the case of arthritis, also preventing cartilage destruction. The degree of inhibition seen in these models with anti-α1 mAb, anti-α2 mAb, or both combined (generally 60%–80% inhibition) compares favorably with that seen with other integrin-based therapies, whether compared in parallel or historically. For example, treatment of mice with anti–ICAM-1 mAb in vivo demonstrates a 50% inhibition of CHS response (Figure 5) (27) and a 30%–50% inhibition of arthritic score (Figure 7) (31). Treatment of mice with anti-α4 mAb in vivo has demonstrated approximately a 50% inhibition in DTH (C.L. Nickerson-Nutter, unpublished results) and CHS responses (34), and a 40%–50% inhibition of arthritic scores (Figure 7) (30). Indeed, the effectiveness of anti-α1 mAb treatment in inhibiting arthritis is comparable to that seen with TNF receptor Ig fusion protein. The decreased inflammation seen in the CHS and arthritis models with the α1-deficient mice was similar to the degree of inhibition seen with anti-α1 mAb treatment of wild-type mice, and confirmed, in an mAb-independent manner, the importance of α1β1 in inflammation.

The mechanism by which anti-α1 and anti-α2 mAb's modulate the effector phases of these inflammatory responses is unknown. The ability of anti-α1 and anti-α2 mAb's to inhibit T-cell–dependent antigen-specific inflammation (DTH and CHS), but not nonspecific inflammation caused by croton oil suggests that the mAb's can act at the level of the antigen-specific activated T cell. The effector cells in DTH and CHS responses have been identified as activated $CD4^+$ and $CD8^+$ T cells, respectively (35, 36). Regarding integrin expression on these effector cells, not only are α1β1 and α2β1 well expressed on in vitro–activated T cells, but in the CHS model in vivo–activated T cells from lymph nodes of antigen-sensitized mice also selectively expressed both these integrins; and in the DTH model, the majority of infiltrating T cells at the site of inflammation expressed α1β1. The inhibitory effect of anti-α1 and anti-α2 mAb's in the collagen mAb–induced model arthritis also suggests that these integrins can affect inflammation by acting on activated monocytes/neutrophils. The collagen mAb–induced arthritis model is thought to represent a neutrophil/monocyte-based model of inflammation (25, 26), and this is consistent with our finding that anti–Mac-1 (CD11b) mAb treatment is effective at inhibiting arthritis in this model. The arthri-

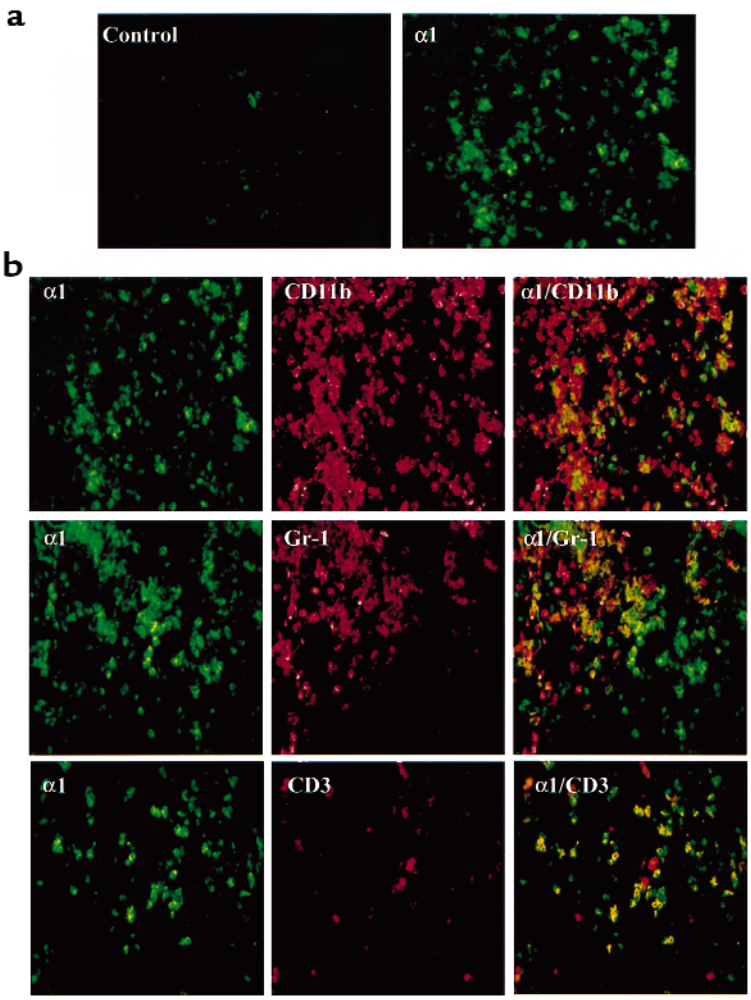


**Figure 4**
Integrin α1β1 is expressed on infiltrating leukocytes in footpads during a DTH response. Immunohistochemical staining of infiltrating leukocytes from an untreated inflamed footpad 20 hours after antigen challenge. (**a**) Serial sections stained directly with Alexa488-conjugated control mAb and anti-α1 mAb. (**b**) Dual immunofluorescent staining with Alexa488-conjugated anti-α1 mAb and PE-conjugated cell lineage–specific mAb's. PE-conjugated mAb's were specific for granulocytes/monocytes (anti-CD11b), neutrophils (anti-Ly6G/Gr-1), and T lymphocytes (anti-CD3). ×400.

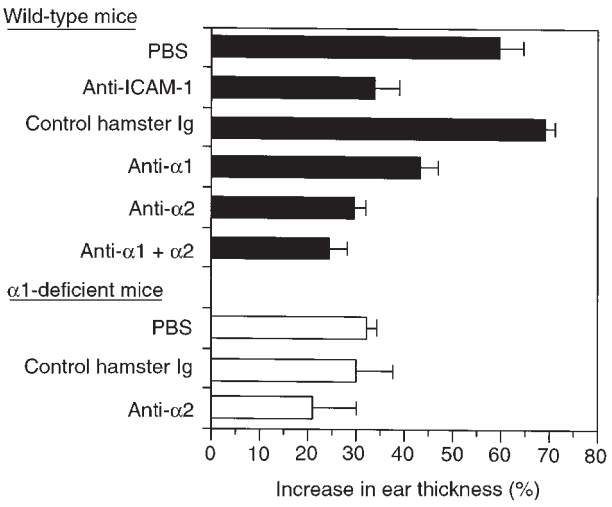


**Figure 5**
Effect of anti-α1 and anti-α2 mAb's on the effector phase of CHS in wild-type and α1-deficient mice. FITC-sensitized mice were treated with the indicated mAb's 4 hours before FITC challenge. Ear thickness was measured at baseline and 24 hours later, and results are shown as percent increase in ear thickness ± SEM. Data for wild-type and α1-deficient mice is the average of 10 and 2 experiments, respectively, with each experiment consisting of groups of 5 mice per condition.

tis model involves LPS-mediated activation of neutrophils and monocytes, and although resting monocytes express little α1β1 and moderate levels of α2β1, LPS-activated monocytes rapidly upregulate α1β1 expression (37). The rapid upregulation of α1β1 on activated monocytes, along with the novel finding of α1β1 on infiltrating neutrophils at sites of inflammation, may explain the striking effect of anti-α1 mAb treatment in this model.

Given the fact that α1β1 and α2β1 are well expressed on appropriate effector cells in all 3 inflammatory models, several mechanisms of action can be postulated. Disruption of integrin/matrix interactions can affect leukocyte recruitment into the tissue itself (endothelial/leukocyte adhesion), migration of cells within the inflamed tissue, priming and activation of cells via ligation to matrix, and apoptosis of cells (32). Disruption of endothelial/leukocyte adhesion as a possible mechanism is ruled out because both α1β1 and α2β1 are collagen and laminin receptors with no known ligands on endothelial cells. With regards to an apoptotic mechanism of action, the fact that extended treatment of mice with anti-α1 and anti-α2 mAb's did not result in either deletion of cells or loss of immunological memory suggests that other mechanisms may be responsible. The disruption of cell migration within tissues and the effects on cellular priming and activation within tissues are 2 possibilities that deserve closer examination.

Integrin receptors clearly transduce signals upon engagement of ligand. Integrin-mediated binding to collagen provides a costimulatory signal for T-cell activation after CD3 cross-linking, which is thought to mimic antigen engagement; these signals include proliferation (5, 9) and increased production of TNF-α (38) and IFN-γ (G. Chi-Rossi et al., unpublished results). It has been demonstrated that TNF-α– and IFN-γ–producing activated $CD4^+$ and $CD8^+$ T cells are the effector cells responsible for DTH and CHS responses (36, 39). In addition, in vivo evidence has shown that both TNF-α (39) and IFN-γ (40) are directly responsible for driving the effector phases of CHS and DTH, and that TNF-α plays a central role in collagen mAb–induced arthritis (Figure 7). Given the expression of α1β1 and α2β1 on a variety of activated cells — including T cells, monocytes, and neutrophils — their possible role in promoting cytokine secretion, and thereby effector cell function, is intriguing.

In vitro, α1β1 and α2β1 integrins have also been shown to function in cell migration (12, 15) and in reorganization and contraction of collagen matrices (16, 17). However, in certain cell types, such as leukocytes and dendritic cells, migration through 3-dimensional collagen matrices may be largely integrin independent, involving highly transient interactions with collagen that lack the typical focal adhesions and matrix reorganization seen in other cells (41). Similarly, the combination of anti-α1 and anti-α2 mAb's was unable to block the antigen-dependent migration and activation of Langerhans cells in in vitro explant cultures. In vivo, this was evidenced by both Langerhans cell trafficking studies and a failure of anti-α1 and anti-α2 mAb's to block the sensitization phase of the CHS response (A.G. Sprague and A.R. de Fougerolles, unpublished results). The potential involvement of α1β1 or α2β1 in the in vivo migration of other cell types and the role other integrins may play in cellular migration requires further study. Whether the inhibition of leukocyte infiltration into inflammatory sites that is seen with both anti-α1 and anti-α2 mAb treat-

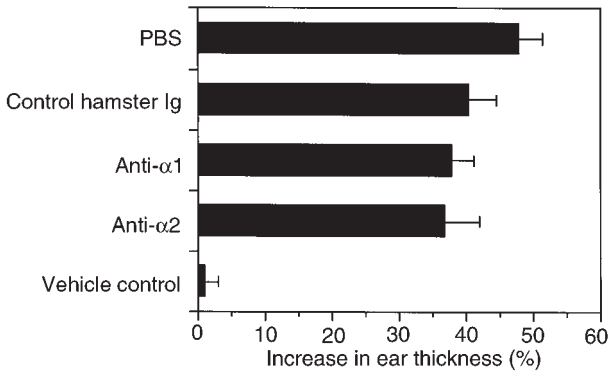


**Figure 6**
Effect of anti-α1 and anti-α2 mAb's on croton oil–induced nonspecific inflammation. Mice were treated with the indicated mAb's 4 hours before ear painting with croton oil. Ear thickness was measured at baseline and 24 hours later, and results are shown as percent increase in ear thickness ± SEM. Groups of 5 mice per condition were used; 1 of 3 representative experiments is shown.

ment represents a direct inhibitory effect on cell migration or is a consequence of disruption of a cellular activation event that then leads to decreased leukocyte recruitment is unclear.

The fact that short-term in vitro– and in vivo–activated T cells can express both α1β1 and α2β1, and the fact that infiltrating leukocytes at the site of inflammation express α1β1, clearly indicates that these integrins are expressed at the proper time and in the proper place to play an important role in inflammation. Their importance in the inflammatory process is underscored by the dramatic effects that both mAb-based inhibition and genetic deletion of these integrins has on several different in vivo models of inflammation. Although these integrins are the major cell surface receptors for collagen, ligand interactions with molecules other than collagen (such as laminin) may also be important in regulating the inflammatory process. By demonstrating the in vivo importance of α1β1 and α2β1 integrins in inflammatory diseases, this study extends the role of integrins in inflammation beyond leukocyte attachment and extravasation at the vascular endothelial

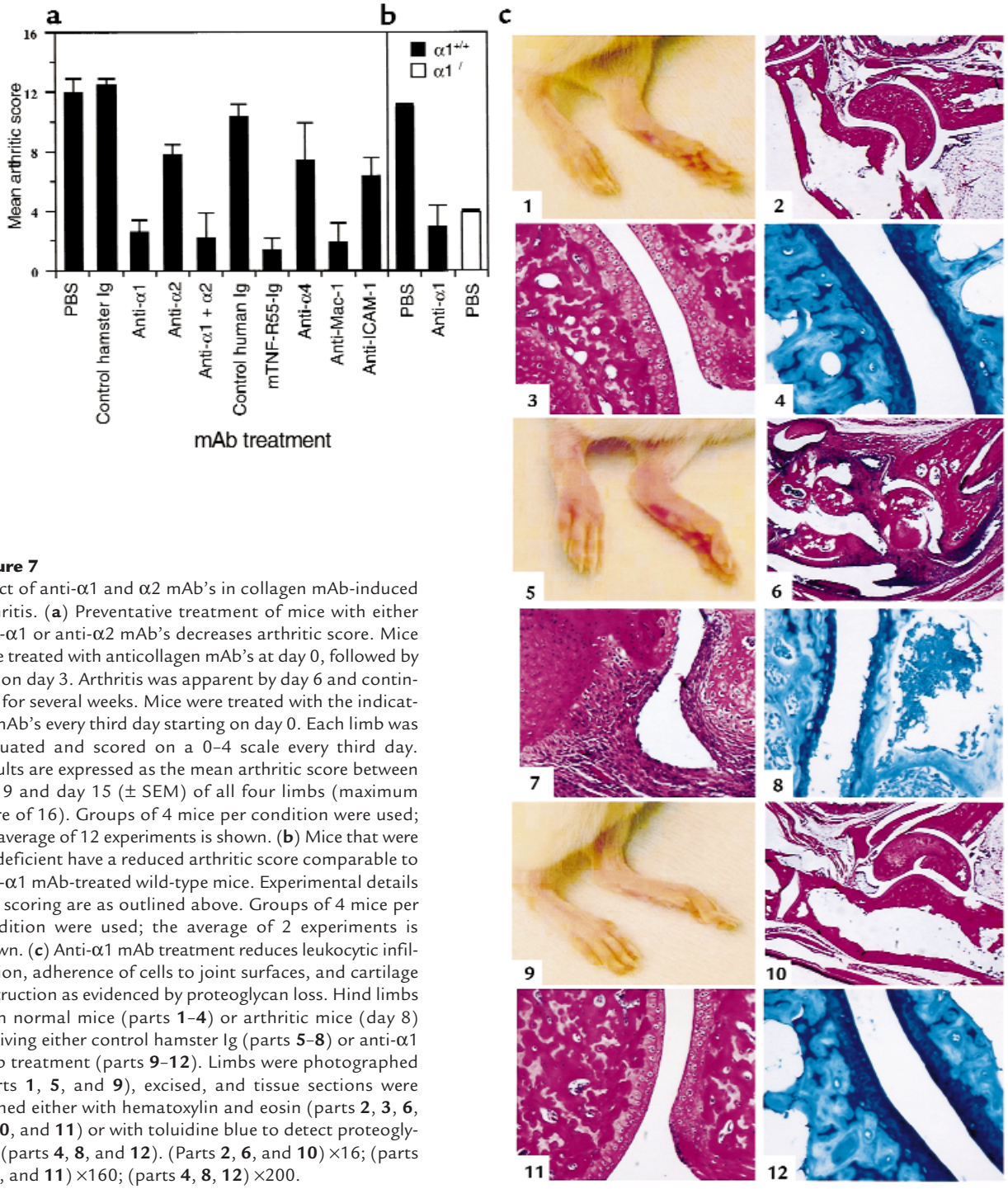


**Figure 7**
Effect of anti-α1 and α2 mAb's in collagen mAb-induced arthritis. (**a**) Preventative treatment of mice with either anti-α1 or anti-α2 mAb's decreases arthritic score. Mice were treated with anticollagen mAb's at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated with the indicated mAb's every third day starting on day 0. Each limb was evaluated and scored on a 0–4 scale every third day. Results are expressed as the mean arthritic score between day 9 and day 15 (± SEM) of all four limbs (maximum score of 16). Groups of 4 mice per condition were used; the average of 12 experiments is shown. (**b**) Mice that were α1 deficient have a reduced arthritic score comparable to anti-α1 mAb-treated wild-type mice. Experimental details and scoring are as outlined above. Groups of 4 mice per condition were used; the average of 2 experiments is shown. (**c**) Anti-α1 mAb treatment reduces leukocytic infiltration, adherence of cells to joint surfaces, and cartilage destruction as evidenced by proteoglycan loss. Hind limbs from normal mice (parts **1**–**4**) or arthritic mice (day 8) receiving either control hamster Ig (parts **5**–**8**) or anti-α1 mAb treatment (parts **9**–**12**). Limbs were photographed (parts **1**, **5**, and **9**), excised, and tissue sections were stained either with hematoxylin and eosin (parts **2**, **3**, **6**, **7**, **10**, and **11**) or with toluidine blue to detect proteoglycan (parts **4**, **8**, and **12**). (Parts **2**, **6**, and **10**) ×16; (parts **3**, **7**, and **11**) ×160; (parts **4**, **8**, **12**) ×200.

interface. Also, by highlighting the importance of the matrix-rich peripheral tissue environment to immune responses, it reveals peripheral tissues as a new point of intervention for adhesion-based therapies.


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