Intestinal Mast Cell Progenitors Require CD49dβ7 (α4β7 Integrin) for Tissue-specific Homing

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Abstract

Mast cells (MCs) are centrally important in allergic inflammation of the airways, as well as in the intestinal immune response to helminth infection. A single lineage of bone marrow (BM)-derived progenitors emigrates from the circulation and matures into phenotypically distinct MCs in different tissues. Because the mechanisms of MC progenitor (MCp) homing to peripheral tissues have not been evaluated, we used limiting dilution analysis to measure the concentration of MCp in various tissues of mice deficient for candidate homing molecules. MCp were almost completely absent in the small intestine but were present in the lung, spleen, BM, and large intestine of β7 integrin–deficient mice (on the C57BL/6 background), indicating that a β7 integrin is critical for homing of these cells to the small intestine. MCp concentrations were not altered in the tissues of mice deficient in the αE integrin (CD103), the β2 integrin (CD18), or the recombination activating gene (RAG)-2 gene either alone or in combination with the interleukin (IL)-receptor common γ chain. Therefore, it is the α4β7 integrin and not the αEβ7 integrin that is critical, and lymphocytes and natural killer cells play no role in directing MCp migration under basal conditions. When MCp in BALB/c mice were eliminated with sublethal doses of γ-radiation and then reconstituted with syngeneic BM, the administration of anti-α4β7 integrin, anti-α4 integrin, anti-β7 integrin, or anti–MAdCAM-1 monoclonal antibodies (mAbs) blocked the recovery of MCp in the small intestine. The blocking mAbs could be administered as late as 4 d after BM reconstitution with optimal inhibition, implying that the MCp must arise first in the BM, circulate in the vasculature, and then translocate into the intestine. Inasmuch as MCp are preserved in the lungs of β7 integrin–deficient and anti-α4β7 integrin–treated mice but not in the small intestine, α4β7 integrin is critical for tissue specific extravasation for localization of MCp in the small intestine, but not the lungs.

Key words: αE/CD103 integrin • β2/CD18 integrin • c-*kit* • stem cell factor • MAdCAM-1

Introduction

Mast cell progenitors (MCp)* arise in the bone marrow (BM) and transit through the circulation to the peripheral tissues where they mature (for reviews, see references 1 and 2). A single lineage of committed progenitors gives rise to mature MCs, which can be divided into two predominant phenotypes based on their expression of distinct secretory granule protease arrays (3, 4). Constitutive or connective tissue MCs (CTMCs) are found in many connective tissues, especially around blood vessels and in the peritoneal cavity, where they have been clearly implicated in the innate immune response to bacteria (5, 6). T cell cytokine-dependent reactive or mucosal MCs (MMCs) appear at mucosal interfaces such as in the intestine, where their numbers are amplified after a parasitic infection to mediate rejection of certain helminths such as *Trichinella spiralis* (7–10). The presence, in situ, of all mature MCs requires an intact stem cell factor (SCF)/c-*kit* (the cell surface tyrosine kinase receptor for SCF) pathway; animals lacking either

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**Abbreviations used in this paper:* BM, bone marrow; BMMC, bone marrow-derived MC; ICAM, intercellular adhesion molecule; MC, mast cell; MCp, MC progenitor; MMC, reactive or mucosal MC; MNC, mononuclear cell; SCF, stem cell factor.

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component possess few CTMCs and produce few reactive MMCs in response to a helminth infection (10–15).

Studies evaluating the localization of MCp in peripheral tissues have been limited by the fact that these cells cannot yet be defined and enumerated on the basis of morphology. Thus, the numbers of MCp in tissues have been determined with limiting dilution analysis with IL-3–enriched medium and monitoring of MC colony formation after 2 wk (16–19). However, Rodewald et al. recently identified circulating, committed mast cell progenitors in mouse fetal blood, and showed that their proliferation ex vivo occurred only when the medium contained both SCF and IL-3 and not either cytokine alone (20). These rare circulating fetal mast cell progenitors stain weakly positive for metachromatic granules with toluidine blue, do not have high affinity Fc receptors for IgE (FcεRI), are c-*kit* high and Thy-1 low by immunodetection, and do not respond to cytokines that promote the development of other hematopoietic lineages. The combination of SCF and IL-3 also provided greater estimates of MCp than the individual cytokines in BM and blood of rats and in the mesenteric lymph nodes of mice infected with a helminthic parasite (21, 22). Although earlier analyses with media containing IL-3 in limiting dilution assays may not have detected all the MCp in different tissues, they nonetheless revealed that the MCp concentration (defined as the number of MCp per 10^6 mononuclear cells [MNCs]) in the intestine equaled that in the BM (17–19). Because the large reservoir of MCp in intestinal tissue could be a local source of MMCs for clearance of worms during helminth infection, we hypothesized that there would be an intestinal-selective homing requirement for MCp in normal mice. And as no studies had evaluated the membrane signals controlling the movement of BM-derived MCp into the small intestine under normal conditions, we investigated the integrin and c-*kit*/SCF requirements for the appearance of these cells in the small intestine.

In this study, we optimized the limiting dilution assay by comparing the effect of SCF, IL-3, and several other cytokines in various combinations on enumeration of the concentration of MCp and established that SCF plus IL-3 provides the maximum MCp counts in two peripheral tissues, the intestine and the lung. We then used this assay to analyze the concentration of MCp in the intestine of gene-disrupted or naturally deficient mouse strains to identify requirements for particular integrins and growth factors in the homing of MCp to these tissues. Finally, we confirmed the integrin requirement with irradiated, BM-reconstituted mice and various blocking mAbs. We conclude that the membrane signals provided to MCp by c-*kit* and α4β7 integrin are needed for intestinal MCp, with the later directing tissue-selective homing to the intestine.

Materials and Methods

Animals. BALB/c (BALB/cAnNTac), and BALB/c–recombination activating gene (RAG)-2$^{-/-}$ (C.129(B6)-*Rag2^{tm1}* N12), and C57BL/6-RAG-2/γ*c* double knockout (C57BL/6J × C57BL/10SgSnAi)-[KO]*gamma C*-[KO]*Rag2*) mice were obtained from Taconic. WBB6F1/J-*Kit^{W}*/*Kit^{W-v}* (W/Wv), WCB6F1/J-*Kitl^{Sl}*/*Kitl^{Sl-d}* (Sl/Sld), β2 integrin chain-deficient (B6.129S7-*Itgb2^{tm1Bay}*), β7 integrin chain-deficient (C57BL/6-*Itgb7^{tm1Cgn}*) and intercellular adhesion molecule (ICAM)-1-deficient (B6.129S7-*Icam1^{tm1Bay}*) mice, and the wild-type control mice for each of these strains were obtained from The Jackson Laboratory. The BALB/c-αE integrin knockout mice are maintained in the Dana-Farber Cancer Institute (DFCI) animal facility and derived as described (23). All mice were 6–12 wk old when used in these experiments. All animal protocols have been reviewed and approved by the DFCI Animal Care and Use Committee in accordance with the Public Health Service Policy and provisions of the Animal Welfare Acts.

Media and Cytokines. Cells were cultured in complete medium with cytokines as previously reported for the culture of mouse BM-derived MCs (BMMCs; references 24 and 25). The complete medium is composed of RPMI 1640 supplemented with 10% fetal calf serum (Sigma-Aldrich), glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), pyruvate (1 mM), nonessential amino acids, Hepes (10 mM), and 2-ME (50 μM). Cytokines are added as indicated. Most mouse recombinant cytokines (SCF, IL-3, IL-4, IL-9, IL-10) were prepared using baculovirus vectors. Supernatants from infected cultures of Hi5 insect cells (Invitrogen) were tested for each cytokine by ELISA (R&D Systems) and/or by proliferative action on IL-3–derived BMMCs. The concentrations of SCF and IL-3 were determined by respective comparison to recombinant mouse SCF obtained from PeproTech and recombinant mouse IL-3 obtained from Endogen. For the limiting dilution analysis of MCp described below, SCF was used at a final concentration of 200 ng/ml and IL-3 at a final concentration of 10 ng/ml. Mouse recombinant IL-6 (PeproTech) was used at a final concentration of 10 ng/ml. The other cytokines, IL-4, IL-9, and IL-10, were used at the concentration giving maximum coproliferation of BMMCs with IL-3.

Sublethal Irradiation with BM Reconstitution and mAb Blocking Protocol. Animals were irradiated in groups of 12 in a Gammacell-40 irradiator (Atomic Energy of Canada Limited, Ottawa, Canada) for 4.5 min; this procedure resulted in an average exposure of 5 gray (500 rads). For reconstitution, 2 h after irradiation, the mice received intravenous injections of 10 million BM cells that had been isolated from the femurs and tibias of syngeneic or congenic animals, washed, and resuspended in complete medium.

The anti-α4 integrin mAb PS/2 (rat IgG2b-κ, American Type Culture Collection no. CRL-1911) and the anti-α4β7 integrin mAb DATK32 (rat-IgG2a, American Type Culture Collection no. HB-294) were obtained from American Type Culture Collection and grown as an ascites. DATK32 was also obtained in purified form from BD PharMingen. Purified anti–MAdCAM-1 (rat IgG2a, clone MECA-367), anti-β1 integrin (anti-CD29) mAb (clones Ha2/5 [Armenian Hamster IgM] and 9EG7 [rat IgG2a]), anti-β7 integrin mAb (rat IgG2a-κ, clone FIB27), and the Armenian hamster control mAb (anti-TNP, IgM-κ) were obtained from BD PharMingen. Purified anti-αE integrin mAb (rat IgG2a, clone M290) was purified in-house (23, 26). An isotype control rat IgG2a-κ mAb (rat anti–mouse IgD) was purchased from Biosource International. All mAbs containing sodium azide were dialyzed against HBSS to eliminate the azide. Except where noted, animals received 100 μl of the mAb diluted in HBSS, intraperitoneally, every other day. For the initial experiments, the first injection was administered 4 h after the BM cells had been received. Subsequently, it was found that the same results were obtained if the initial administrative was delayed until the second day.

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Limiting Dilution Analysis. Previous investigators analyzed MCp in the small intestine by using either the cells liberated by agitation from the villous epithelium or by discarding this population and digesting the residual tissue with enzymes to isolate cells from the crypt epithelium and lamina propria (16–19). To determine the entire population of MCp, we analyzed MCp in MNC populations isolated from the small intestine by enzymatic digestion without discarding the villous epithelial cells. In brief, the small intestine was removed, flushed with 30–40 ml of HBSS, opened with scissors, rinsed in HBSS, and chopped with a scalpel. The tissue was transferred to 50-ml tubes, resuspended in 40 ml of complete medium containing 120 μg/ml collagenase (crude type 1A; Sigma-Aldrich) and 120 μg/ml of collagenase/dispase (Sigma-Aldrich), and digested with shaking for 25 min at 37°C. Undigested cell clumps were allowed to settle and were subjected to a second round of enzymatic digestion. The liberated cells were spun down, resuspended in 44% Percoll (Sigma-Aldrich), overlayed on a 67% Percoll layer, and spun at 400 *g* for 20 min at 4°C. The MNCs were harvested from the interface and washed in complete medium. The viable cell counts were determined by trypan blue dye exclusion on a hemocytometer. MNCs were isolated from the lung and large intestine by enzymatic digestion in a similar manner. For the large intestine, the entire colon including the cecum was taken for analysis. MNCs were obtained from the BM by flushing the tibia and femurs with complete medium and from the spleen by gentle grinding between two frosted glass slides. Cells from each of these tissues were fractionated on Percoll gradients. MNCs were diluted serially in complete medium, and 100 μl of each dilution was added to 24 wells of standard 96-well microtiter plates (Corning). Each well then received 100 μl of 3,000 rad gamma-irradiated syngeneic spleen cells as feeder cells plus cytokine(s). Feeder cell concentrations between 10^5 and 10^6 cells per well gave equivalent results; ~10^5 feeder cells per well were used throughout. The cytokine combination of SCF and IL-3 was used throughout these studies unless otherwise indicated. In all experiments, tissues from two or three animals were pooled for the analysis.

Cultures were incubated in a humidified 37°C incubator with 5% CO_2 for 10–14 d. Colonies grown in SCF plus IL-3 could be detected after 10–12 d, while those developed in IL-3 or in SCF plus IL-6 plus IL-10 (Triad) were always detected after 12–14 d because of their slower growth rates. Other cytokine combinations tested, SCF plus IL-4 or IL-9 or IL-10 or all four cytokines together, did not result in the formation of increased numbers of MCp colonies from intestinal MNCs. Because several combinations of IL-3 plus the other cytokines also did not give greater estimations of the MCp concentration than that achieved with SCF plus IL-3, SCF plus IL-3 was the cytokine combination used in all experiments unless otherwise indicated. MCp colonies were detected with an inverted microscope and were easily distinguished as nonadherent, medium sized cells (16–18). The presence of MCp colonies in wells scored as positive for MC growth was validated histologically for all conditions with toluidine blue and/or chloroacetate esterase staining of cytocentrifuge preparations of the individual wells (4). Several wells scored as negative for MC growth but containing other colonies were also initially evaluated histologically to confirm the ability to distinguish MCp colonies from those of other cells. Several methods for determining the concentration of MCp in the isolated MNC preparations were compared. The L-Calc™ computer program (Stem Cell Technologies Inc.) is commercially available. Also, using the trend function in commercially available spreadsheet programs, the number of MNCs plated that results in 37% of the wells negative for a MCp colony was estimated from the plot of the log of the fraction of nonresponding cultures versus the cell number plated. Finally, as described in Crapper and Schrader (18), the concentration can be estimated by dividing the log of the fraction of nonresponding cultures by the cell number plated per well. All three methods gave very similar results (variation <10% in three different comparisons), and thus we routinely estimated the MCp concentration (MCp per 10^6 MNCs) in the various tissues with the trend function in Microsoft Excel™. The percent inhibition was calculated with the following formula: $100 - [100 \times$ (experimental MCp concentration/control MCp concentration)].

Results

Comparison of MCp Concentrations in the Tissues of C57BL/6 and BALB/c Mouse Strains Cultured with Different Cytokine Combinations. As an initial assessment of cytokines to be used for the optimal analysis of tissue MCp concentrations, we compared IL-3 alone, the cytokine triad of SCF plus IL-6 plus IL-10, and SCF plus IL-3. The cytokine triad is active on early Sca1$^+$ stem cells and generates less mature cells, termed early metamastocytes, from BM than IL-3 alone (25), while SCF plus IL-3 is active on fetal blood promastocytes that do not respond to either cytokine alone (20). We determined the MCp concentration in the C57BL/6 (BL/6) and BALB/c mouse strains, which are the background strains of the various genetic mutants used in these studies. Similar concentrations of MCp were found in the intestine of both strains when the MNCs were cultured in either SCF plus IL-6 plus IL-10 or IL-3 alone (Fig. 1). However, SCF plus IL-3 provided a significantly greater concentration of MCp from the intestine of BALB/c mice

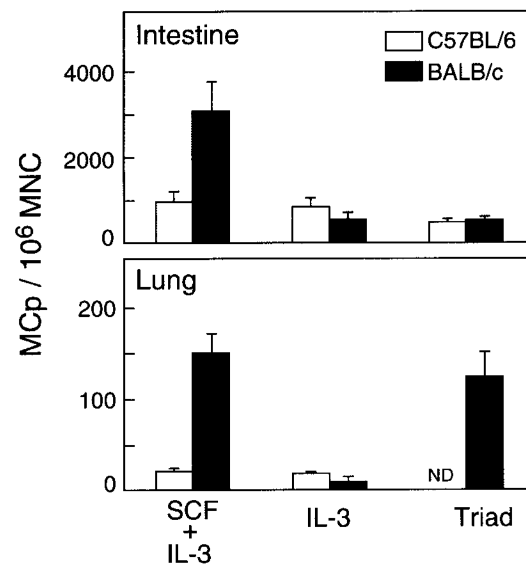

Figure 1. Concentrations of MCp in the small intestine and lung of C57BL/6 (white bars) and BALB/c (black bars) mice. MNCs were isolated from the intestine or the lung of normal C57BL/6J or BALB/c mice by enzymatic digestion. The MCp concentration is the number of MCp per 10^6 MNCs as determined by limiting dilution analysis with the cytokine combinations of SCF plus IL-3, IL-3 alone, or SCF plus IL-6 plus IL-10 (Triad). The values are the means ± SEM for five experiments. ND, not done.

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Table I. *Concentrations of MCp in Tissues of MC-deficient Mice*

Strain		MCp/10^6 MNCs (SEM)		
		Tissue		
		BM	Intestine	Spleen
SCF plus IL-3–responsive MCp				
WCB6F1	(+/+)	345 (90)	939 (241)	47
WCB6F1-*Kitl*^{Sl}/*Kitl*$^{Sl\text{-}d}$	(Sl/Sld)	145 (88)	0 (0)	23
WBB6F1	(+/+)	198	739	39
WBB6F1-*Kit*^{W}/*Kit*$^{W\text{-}v}$	(W/Wv)	224 (18)	0 (0)	13
IL-3–responsive MCp				
WCB6F1	(+/+)	311 (2)	878 (352)	81
WCB6F1-*Kitl*^{Sl}/*Kitl*$^{Sl\text{-}d}$	(Sl/Sld)	113 (24)	0 (0)	14
WBB6F1	(+/+)	188 (13)	583 (127)	30 (13)
WBB6F1-*Kit*^{W}/*Kit*$^{W\text{-}v}$	(W/Wv)	246 (33)	0 (0)	11 (1)

The values are the average MCp concentration for the indicated tissue. The intestine refers to the small intestine only. Numbers in parentheses are the standard error of the mean (same as 1/2 range) from two experiments; numbers standing alone are from a single experiment. For the mutant strains, the historical designation is provided in parentheses.

($P = 0.009$), but not from BL/6 mice ($P = 0.5$), than that obtained with IL-3 or SCF plus IL-6 plus IL-10. The concentration of intestinal MCp detected with SCF plus IL-3 is about threefold greater than in BM of BALB/c mice (3,057 ± 757 versus 924 ± 155 MCp/10^6 MNCs, mean ± SEM, $n = 5$ and $n = 6$, respectively), but the two concentrations are equivalent in BL/6 mice (876 ± 223 versus 992 ± 57 MCp/10^6 MNCs, $n = 5$ and $n = 2$, respectively). None or very few colonies were detected in cultures containing SCF alone. In contrast to the large concentration of intestinal MCp, the concentration of MCp in the lung is 20–50-fold less in both mouse strains (Fig. 1, note different scale), although, again, the BALB/c mice show many more SCF plus IL-3 responsive progenitors in this tissue than does the BL/6 strain. The concentration of IL-3 responsive progenitors is comparable for the lung of both strains and similar to the concentration of SCF plus IL-3 responsive progenitors in the BL/6 strain. In the BALB/c mice, comparable concentrations of MCp are detectable with SCF plus IL-6 plus IL-10 or SCF plus IL-3 in the lung. As SCF plus IL-3 was optimal for the BALB/c strain and sufficient for the BL/6 strain, this combination was used for all subsequent assays of tissue MCp concentrations.

Role of c-Kit and SCF on Intestinal MCp Concentrations. W/Wv and Sl/Sld mice have <1% of the number of mature tissue MCs, constitutive or reactive, found in wild-type control mice and yet provide comparable numbers of IL-3 developed, BM-derived MCPs of an identical protease phenotype (14, 15, 27, 28). However, their intestinal MCp concentrations had not been assessed. Because the W/Wv mice have a mutant c-*kit*, we used both SCF plus IL-3 and IL-3 alone to analyze the concentration of tissue MCp in both strains. The response to IL-3 alone in the wild-type controls was the same as that to SCF plus IL-3 in all the tissues studied (Table I), likely reflecting their hemizygous BL/6 background. The BM concentrations of MCp for the W/Wv mice were similar to those of their wild-type controls (28), whereas the Sl/Sld mice had about one-half the BM MCp concentration of their controls (Table I). No intestinal MCp were detected for either mutant strain, a finding compatible with a defect in translocation or survival of peripheral tissue-localized MCp in both strains. The negative data were based on plating more than 4 × 10^6 MNCs and 2 × 10^6 MNCs for the W/Wv and Sl/Sld mouse strains, respectively, without the appearance of a single MCp colony. These results are similar to those reported for the intestine of Ws/Ws (c-*kit* deficient) rats (21) and more dramatic than those reported for the intestine of Wf/Wf mice (18). In contrast, the spleen, with an open vascular system as well as an extramedullar hematopoietic potential, contained MCp, but at a level below that of their wild-type controls.

Role of αE, β2, and β7 Integrins on Intestinal MCp Concentrations. Mice with various gene disruptions have been used to establish the role of various integrins, particularly β7 and β2 integrins, in the trafficking of T cells into the gut-associated lymphoid tissues (23, 29–31). We took advantage of such mice to investigate the role of αE, β7, and β2 integrins on MCp trafficking to the intestine and lung. The β7 integrin chain deficiency resulted in a virtual ablation of the MCp reservoir in the small intestine without a

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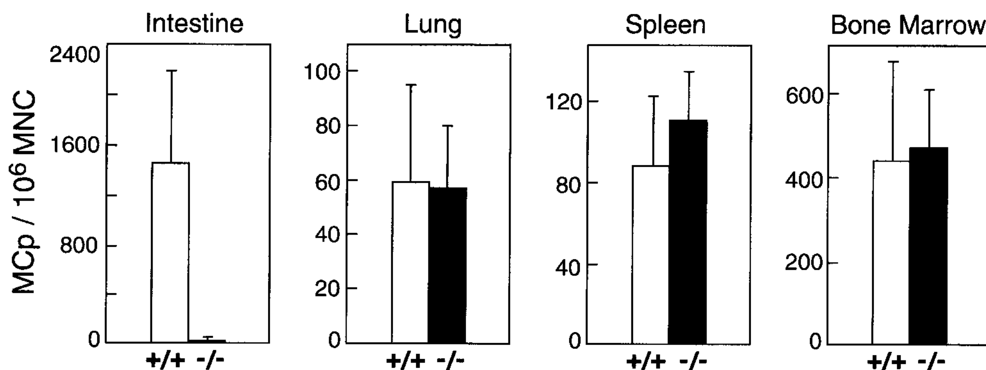

Figure 2. Concentrations of MCp in the small intestine, lung, spleen, and BM of wild-type (C57BL/6, +/+, white bars) control and β7 integrin-deficient mice (−/−, black bars) evaluated in parallel. The data are from three separate experiments and the values are the mean ± SEM. SCF plus IL-3 was used to determine MCp concentration, expressed as the number of MCp/10^6 MNCs.

Table II. *Concentrations of MCp in Tissues of αE and β2 Integrin-deficient and ICAM-1–deficient Mice*

Strain		MCp/10^6 MNCs		
		BM	Intestine	Lung
C57BL/6J	(+/+)	557	980	22
B6.129S7-*Itgb2*	(β2 integrin$^{-/-}$)	305	740	N.D.
B6.129S4-*Icam1*	(ICAM-1$^{-/-}$)	414	904	N.D.
BALB/c	(+/+)	924	3,051	149
BALB/c-CD103$^{-/-}$	(αE integrin$^{-/-}$)	998	3,207	126

The values are the MCp concentration in the listed tissues from the indicated strains of mice. The numbers from the E integrin-deficient mice are the average of four experiments for the BM and small intestine and three experiments for the lung. The values for the +/+ animals are those reported in Fig. 1 as MCp concentrations obtained with SCF plus IL-3. The values for the β2 integrin-deficient, and ICAM-1–deficient mice are from a single experiment. N.D., not done.

reduction in the lung, spleen, or BM MCp concentrations (Fig. 2). MCp concentrations in the large intestine were similar in both strains as well (282 and 392 MCp/10^6 MNCs for BL/6 and β7 integrin-deficient mice, respectively, in a single analysis). The average concentration of MCp in the small intestines of β7 integrin-deficient mice was only 26 ± 22 MCp/10^6 MNCs (mean ± SEM, n = 3), whereas BL/6 mice analyzed in parallel had an average of 1,440 ± 770 MCp/10^6 MNCs. The β7 integrin chain is found associated with the α4 (CD49d) and the αE (CD103) integrin chains (23, 26, 29, 32). Unlike the deficiency in the β7 integrin chain, a deficiency in the αE integrin chain had no effect on intestinal MCp concentrations (Table II). Normal concentrations of MCp also were observed in the lung and BM of these animals. Normal concentrations of MCp were observed in the intestine and BM in both β2 integrin-deficient and ICAM-1–deficient mice as well (Table II). Thus, under basal conditions, intestinal MCp trafficking requires the α4β7 integrin but apparently not the αEβ7 or β2 integrins.

The selective deficiency of intestinal MCp in mice with a disruption of the β7 integrin gene could reflect a direct impairment of extravasation of these cells or be indirect due to an altered availability of another cell type. We addressed this issue with mice lacking all mature lymphocytes and NK cells, available as RAG-2 plus IL-receptor common γ chain double-deficient mice (RAG-2/γc$^{-/-}$; references 33 and 34). RAG-2/γc-deficient mice have normal or increased concentrations of MCp in the small intestine, the lung, and the BM compared with the BL/6 control mice (Fig. 3). Mice deficient in only RAG-2 (on the BALB/c background), which lack mature lymphocytes, also had increased concentrations of MCp when compared with their BALB/c controls (data not shown). Therefore, the presence of MCp in peripheral tissues is not dependent on the availability of lymphocytes or NK cells.

Blocking of Intestinal MCp Reconstitution by Anti-integrin mAbs. Kitamura and others have demonstrated that BM reconstitutes the MC populations in W/Wv MC-deficient mice and that BM MCp are very sensitive to gamma-irradiation (11, 15, 35, 36). Thus, we evaluated the loss and recovery of MCp in the BM, intestine, and lung of BALB/c mice exposed to sublethal irradiation, with and without reconstitution by administration of syngeneic BM cells. In the absence of BM reconstitution, the recovery of the MCp concentration in the small intestine is slow (Fig. 4). With BM reconstitution, the recovery of intestinal MCp reaches 20% by day 7, 40% by day 9, and normal levels by day 11 (Fig. 4). The recovery of the MCp concentration in the BM of the reconstituted mice occurs even more rapidly, with normal levels obtained 4 d after irradiation, the earliest time point evaluated (data not shown, n = 3). The levels of MCp were ~50% of normal in the lung 7 d after sublethal irradiation and BM reconstitution (data not shown, n = 2). The partial recovery of MCp concentrations in the intestine and lung 7–9 d after sublethal irradiation and BM reconstitution provided a reference against which the effect of administering blocking mAbs to various integrins could be quantitated.

The high affinity mAb PS/2 (rat IgG2b) recognizes and blocks the binding function of both α4β1 and α4β7 integrins, whereas the mAb DATK32 (rat IgG2a) recognizes a combinatorial determinate and blocks binding only by the

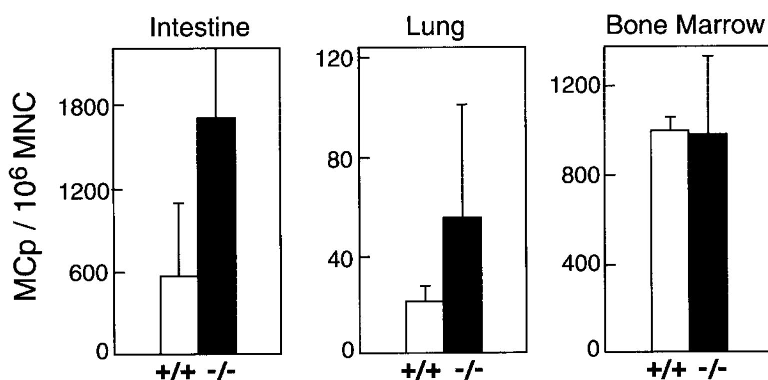

Figure 3. Concentrations of MCp in the small intestine, lung, and BM of wild-type (C57BL/6, +/+, white bars) controls and RAG-2/IL-receptor common γ-chain (γc) double-deficient mice (−/−, black bars). The data are the mean ± 1/2 range of MCp/10^6 MNCs from two experiments using SCF plus IL-3 in which concentrations in wild-type and deficient mice were determined in parallel.

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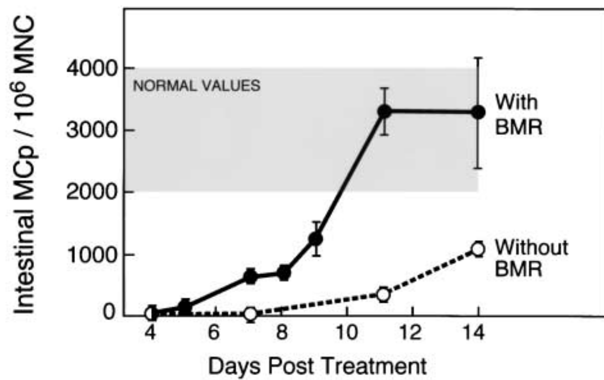

Figure 4. Recovery of MCp in the small intestine of BALB/c mice after sublethal irradiation with (filled circles) or without (open circles) BM reconstitution (BMR). Values for MCp concentrations without BM reconstitution are the mean ± 1/2 range of two experiments. Values for animals receiving BM reconstitution are the mean ± SEM of the following number of experiments: day 7, $n = 12$; day 8, $n = 9$; day 9, $n = 6$; day 11, $n = 5$; day 14, $n = 4$. MCp concentrations were determined with SCF plus IL-3. The shaded area represents the mean ± SEM concentration of MCp in normal BALB/c small intestine (from Fig. 1, SCF plus IL-3).

α4β7 integrin (32, 37). The dose-dependent effect of administering anti-α4 (PS/2) and anti-α4β7 (DATK32) integrin mAbs on the recovery of MCp in the intestine was determined with the sublethal irradiation and BM reconstitution procedure and compared with the effect of a control mAb, rat anti–mouse IgD (rat IgG2a). All doses greater than 1 μg (given intraperitoneally every other day) of either the anti-α4 or the anti-α4β7 integrin mAb caused ~70% inhibition of MCp reconstitution (Fig. 5). As a dose of 30 μg of either mAb consistently gave ~70% inhibition, this dose was chosen for subsequent experiments. No effect on BM MCp concentrations was observed in mice given either PS/2 or DATK32 (data not shown).

Because the recovery of MCp in recipient BM preceded recovery in the intestine, the effectiveness of delaying the introduction of the blocking mAb on the recovery of intestinal MCp was determined. The blocking mAb to the combinatorial epitope of α4β7 integrin (DATK32) was fully effective when the first dose was delayed to day 2 or even day 4 and was partially effective with a single dose on day 6 as compared with the initial administration on day 0 when MCp concentrations were assessed on day 7 (Fig. 6). Comparable inhibition also was obtained in one experiment in which intestinal MCp were quantitated on day 11 after sublethal irradiation and BM reconstitution and the administration of anti-α4β7 integrin mAb was initiated on day 2 or 6. The administration of DATK32 beginning on day 2 gave 92% inhibition, and on day 6 gave 76% inhibition relative to the effect of an irrelevant isotype-matched mAb given beginning on day 2. As the administration of blocking mAb could be delayed for several days, the reconstituting syngeneic cells required conditioning in the recipient BM for lineage development before distribution of MCp to the intestine.

To evaluate the integrin specificity of MCp recovery in the intestine after sublethal irradiation and BM reconstitution, we compared the inhibition obtained with anti-β7 integrin (FIB27, rat IgG2a), anti-αE integrin (M290, rat IgG2a), or anti-β1 integrin (9EG7, rat IgG2a) mAb to that obtained with the isotype matched anti-α4β7 integrin (DATK32) mAb. The MCp concentration was evaluated 7 or 8 d after sublethal irradiation and BM reconstitution (Fig. 7). The anti-α4β7 integrin mAb DATK32 blocked MCp reconstitution in the intestine by 74 ± 5% (mean ±

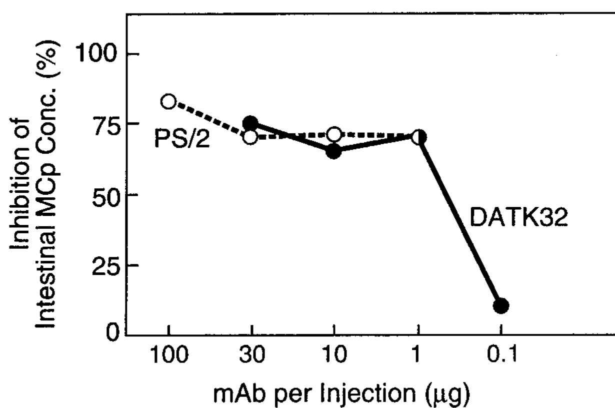

Figure 5. Effect of the blocking anti-integrin mAb, PS/2 (anti-α4, rat IgG2b, open circles) and DATK32 (anti-α4β7, rat IgG2a, filled circles), on the recovery of MCp in the small intestine of BALB/c mice. MCp concentrations were determined 9 d after sublethal irradiation and BM reconstitution and are expressed as the percent inhibition of MCp concentrations in the intestine relative to mice treated with a control mAb (rat anti–mouse IgD, IgG2a) or HBSS. mAb was diluted in HBSS and administered intraperitoneally every other day starting 4 h after BM reconstitution. The results are the average inhibition for two or three experiments with the following n values: For PS/2, 100 μg, $n = 2$; 30 μg, $n = 3$, 10 μg and 1 μg, $n = 1$; for DATK32, 30 μg, $n = 3$, for 10 μg, 1 μg, and 0.1 μg, $n = 1$. The average concentration of intestinal MCp for mice given the control mAb or HBSS was 903 ± 311 MCp/10^6 MNCs (mean ± SEM, $n = 5$).

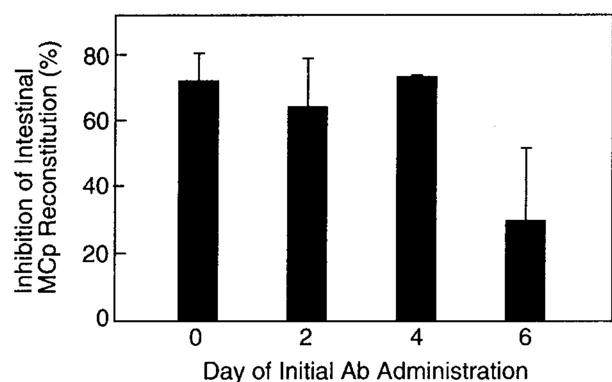

Figure 6. Effect of delaying the time of initial administration of the blocking mAb DATK32 (anti-α4β7 integrin) on the recovery of MCp in the small intestine of BALB/c mice. The MCp concentrations were determined 7 d after sublethal irradiation and BM reconstitution and are expressed as the percent inhibition of MCp concentrations in the intestine relative to the mice treated with the isotype-matched rat anti–mouse IgD mAb. The control mice had an average MCp concentration of 632 ± 206 MCp/10^6 MNCs (mean ± SEM, $n = 6$). Experimental values are the mean ± SEM of four (day 0 and day 2) or two (day 4 and day 6) experiments.

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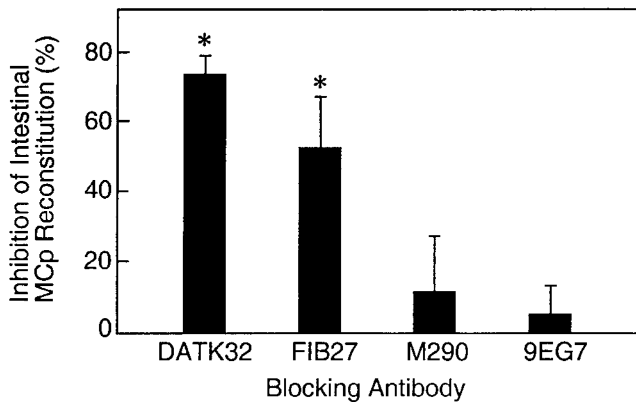

Figure 7. Comparison of the inhibition by mAb to α4β7, β7, αE, or β1 integrins of the recovery of MCp in the small intestine of BALB/c mice. Animals received 30 μg of the indicated mAb, DATK32, FIB27, M290, or 9EG7, directed against α4β7, β7, αE, or β1 integrins, respectively, starting 0 or 2 d after sublethal irradiation and BM reconstitution. Values represent the mean ± SEM percent inhibition relative to control animals treated in parallel. MCp concentrations were analyzed 7 or 8 d after sublethal irradiation and BM reconstitution. Means are from seven (DATK32), three (FIB27, M290), or four (9EG7) experiments. The mean MCp concentration for the control mice was 622 ± 65 MCp/10^6 MNCs (mean ± SEM). Asterisks indicate statistically significant inhibition relative to mice injected with anti-β1 integrin mAb ($P < 0.03$).

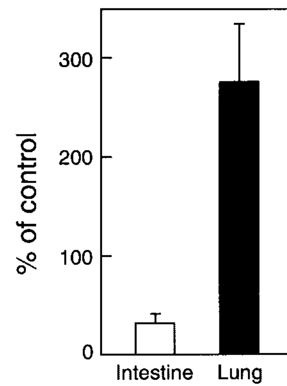

Figure 8. Inhibition by mAb to α4β7 integrin of the recovery of MCp in the small intestine of BALB/c mice is accompanied by increased MCp in the lung. Animals received 30 μg of the mAb, DATK32, directed against the α4β7 integrin, or a control injection of HBSS or isotype matched rat mAb, starting 2 d after sublethal irradiation and BM reconstitution. Values represent the mean ± SEM ($n = 4$) of the MCp concentration in the indicated tissues expressed as a percentage of the value obtained from control animals treated in parallel. MCp concentrations were analyzed 7 d after sublethal irradiation and BM reconstitution. The MCp concentrations for the control mice were 1,068 ± 390 and 252 ± 51 MCp/10^6 MNCs (mean ± SEM) for the intestine and lung, respectively.

SEM, $n = 7$), and the anti-β7 integrin mAb blocked reconstitution by 53 ± 14% ($n = 3$). In contrast, neither of the isotype-matched mAbs reacting with αE (CD103) or β1 (CD29) integrins had any significant effect on MCp recovery in the intestine (11 ± 16% inhibition, $n = 3$, and 5 ± 8% inhibition, $n = 4$, respectively). Similar results were also obtained with the hamster anti–mouse β1 integrin mAb, Ha2/5 (hamster IgM, mean % inhibition ± SEM = −7 ± 19%, $n = 4$). The inhibition obtained with DATK32 was statistically significant compared with that obtained with M290 and 9EG7 ($P \leq 0.001$), whereas the inhibition obtained with FIB27 was significantly different from that obtained with 9EG7 ($P = 0.026$), as analyzed by the Student's *t* test. As MAdCAM-1 is a high endothelial cell ligand for α4β7 integrin in the small intestine, we also examined the blocking of intestinal MCp recovery by anti–MAdCAM-1 mAb (MECA-367). After sublethal irradiation and BM reconstitution, administration of anti–MAdCAM-1 mAb beginning on day 2, with analysis on day 7, revealed a 45 ± 5% suppression (mean ± SEM, $n = 3$) compared with mice receiving an isotype-matched control mAb.

The evidence that anti-α4β7 or anti-α4 integrin, in contradistinction to the findings for αE or β1 integrins, blocked reconstitution of MCp in the small intestine led to a parallel assessment of the role of α4β7 integrin for homing of MCp to the lung. Whereas mice receiving anti-α4β7 integrin restored only 30.9 ± 9.6% (mean ± SEM) of their MCp in the small intestine, the lung concentration of MCp was increased to 275.1 ± 57.5% relative to isotype-matched control mAb injected mice in four separate experiments assayed on day 7 after sublethal irradiation and BM reconstitution (Fig. 8).

Discussion

To facilitate our study of the mechanisms of MCp localization to tissues, we sought to identify conditions of culture that would maximize the proliferation of MCp dispersed from tissues. It was recently reported that the combination of SCF and IL-3 was superior to either cytokine alone for the detection, by ex vivo culture, of fetal MC lineage progenitors isolated from 14.5-d-old embryos or MCp isolated from tissues of helminth-infected rodents (20–22). We found that SCF plus IL-3 induced the growth of ~sixfold more MCp than IL-3 alone in the intestines of BALB/c mice, but similar concentrations in the intestines of C57BL/6 mice (Fig. 1). SCF plus IL-3 also induced the growth of more MCp colonies from the intestines than SCF plus IL-6 plus IL-10, a cytokine triad that induces the proliferation of progenitors from the BM that are less mature than the cells induced by IL-3 alone (25). In comparison to the intestines, there were dramatically fewer MCp in lung tissue of both strains after culture with SCF plus IL-3. Further, SCF plus IL-6 plus IL-10 detected MCp concentrations in the lungs of BALB/c mice that were almost equal to those obtained by culture with SCF plus IL-3. The different cytokine-driven proliferation characteristics for MCp in these two tissues and between these two strains of mice likely indicates that there is different tissue-directed maturation and/or a different tissue-selective homing requirement for localization and differentiation of the common circulating MCp.

As an initial approach to characterize the integrin requirements for MCp homing and retention in the small intestine and other peripheral tissues, MCp concentrations were evaluated in mice that lacked specific adhesion molecules due to targeted disruptions. These studies focused on adhesion molecules that have been implicated in T cell homing or localization to the small intestine, such as the α4β7, αEβ7, and αLβ2 integrins, which bind to MAdCAM-1, E-cadherin, and ICAM-1, 2, and 3, respectively (23, 26, 29–32, 37–41). In β7 integrin gene-disrupted mice, there was a marked reduction in the small intestinal MCp concentration, to less than 5% of that in wild-type C57BL/6 control mice, while the MCp concentration was

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preserved in their BM, spleen, lung (Fig. 2), and large intestine. In contrast, disruption of the αE integrin gene, the β2 integrin gene, or the ICAM-l gene had no effect on the concentration of MCp in the small intestine (Table II). As αE integrin deficiency did not result in altered MCp numbers, it seemed highly likely that α4β7, rather than αEβ7, was the integrin required for the localization of MCp to the small intestine. However, the maturation of reactive MMCs in the intestine requires T cells (7–10, 42–44) that are also dramatically diminished in number in the β7 integrin-deficient mice (29, 44). Therefore, it was important to determine if MCp localization also required T cells, in which case the impact of β7 integrin chain deficiency on MCp concentrations might be an indirect one due to the concurrent loss of intestinal T cells in these animals. In contrast to the impact of β7 integrin chain deficiency, the MCp concentrations were actually increased in the small intestine and lung of RAG-2/γc and RAG-2 gene disrupted mice compared with their wild-type controls, C57BL/6 or BALB/c mice, respectively (Fig. 3). Thus, MCp localization in the intestine did not depend on the presence of T (or B) lymphocytes or NK cells, and the impact of β7 integrin chain deficiency on MCp localization to the small intestine is a direct effect on this cell. Moreover, because β7 integrin chain deficiency did not affect the concentration of MCp in the BM, large intestine, or lung, these findings indicate that α4β7 integrin plays a selective role in MCp cell homing to the small intestine.

The evidence from gene-disrupted mice that the α4β7 integrin was needed for the presence of intestinal MCp did not establish that its role was directed to the transendothelial movement of lineage progenitors out of the vasculature and into the intestine. Therefore, we turned to the use of blocking mAbs in animals rendered deficient in MCp by sublethal irradiation and undergoing reconstitution in the BM and at peripheral sites by the administration of syngeneic BM. The small intestinal (Fig. 4) and lung MCp were highly radiosensitive, as shown previously for BM MCp (36). Spontaneous recovery after sublethal irradiation required more than 14 d for BM (data not shown), and the peripheral tissues reached only one-third that of the BALB/c control mice at d 14 (Fig. 4). After the administration of syngeneic BM, the full recovery of MCp concentrations in recipient BM occurred by d 4, at which time neither intestine nor lung had detectable MCp. 7–9 d after sublethal irradiation and BM reconstitution, recovery of intestinal and lung MCp concentrations were consistent and in the 20–50% range (Fig. 4). The administration of anti-integrin mAb either to the α4 integrin chain or to a combinatorial epitope on α4β7 integrin inhibited this recovery by ~70% (Figs. 5–8), even when the initial administration was delayed until the fourth day after sublethal irradiation and BM reconstitution (Fig. 6), a time when recipient BM was fully recovered. This finding indicates that progenitors suitable for translocation to the intestine via the vasculature were first developed or conditioned in the recipient BM.

The inhibition of intestinal homing of MCp by anti-α4β7 integrin mAb was substantial at both day 7 and even at day 11 with every other day administration of blocking mAb from day 2 to the day before the assay. In contrast, mAb to αE or β1 integrin subunits had no effect (Fig. 7), indicating that homing to the intestine requires α4β7 integrin and not α4β1 or αEβ7 integrins. That mAbs to α4β7 integrin and to MAdCAM-1 each inhibited reconstitution of MCp in the small intestine, whereas mAb to αE was without effect (Fig. 7), identifies a critical pairing for homing to this tissue. The inhibition of recovery of intestinal MCp by mAb to MAdCAM-1 after sublethal irradiation and BM reconstitution also implies that the inhibitory effect obtained with mAb to the α4β7 integrin can be attributed to blocking transmigration out of the vasculature rather than to cytolysis or sequestration. This interpretation would also be compatible with the findings of a threefold increment in pulmonary MCp in association with blocking of intestinal MCp recovery by mAb to α4β7 integrin (Fig. 8). The deficiency in small intestinal but not lung or large intestinal MCp in β7-null mice and the failure of mAb to α4β7 integrin to inhibit lung MCp recovery indicates that the movement of MCp into these two peripheral tissues is regulated differently than for the small intestine. Furthermore, these findings indicate that circulating MCp have a common phenotype and that in the absence of elicited inflammation, selection is based on tissue-regulated constitutive differences. Finally, the normal concentrations of MCp in the small intestine of β2 integrin and ICAM-1 null mice (Table II) distinguish the homing of MCp from that of intraepithelial T cells (31).

The critical role of the α4β7 integrin-dependent MCp reservoir is evident from the impaired intestinal mastocytosis and clearance of the *T. spiralis* worm burden recently reported for the β7–integrin chain-deficient animals (44). That a delayed, attenuated MC amplification subsequently occurred in the intestine after infection with *T. spiralis* in spite of the paucity of resident MCp demonstrated by us likely reflects recruitment via vascular changes associated with the inflammatory response. The T cell recruitment to the intestine was also attenuated (44), revealing an α4β7 integrin requirement previously recognized through other studies (26, 29, 30, 32, 37–41). Because our analysis does not reveal any T cell requirement for homing of MCp to peripheral tissues (Fig. 3), we interpret the *T. spiralis* challenge study in β7 integrin-deficient mice to support the critical role of the physiologic homing of α4β7 integrin-positive MCp to the small intestine for their subsequent T cell–driven maturation. This homing of MCp poised to respond to an appropriate inflammatory stimulus in this tissue is unlike the T cell localization to the lamina propria that primarily involves recruitment of activated cells (30–32, 37–41).

Like the β7 integrin-deficient mice, mice with a heritable disruption of c-*kit* or its ligand, SCF, have reduced concentrations of intestinal MCp, despite substantial concentrations of MCp in their BM (Table I). Thus, a functional c-*kit* also is required for the localization of intestinal MCp. Based upon in vitro, in situ, and ex vivo studies, the activities of the *c-kit*/SCF pathway include developmental maturation, chemotaxis, adhesion, and cytoprotection of

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mature MC in peripheral tissues and/or BMMCs (45–51). In particular, BMMCs derived from W/Wv mice lack a fully functional c-*kit* but still have a chemotactic response to SCF (48). Thus, soluble SCF could send a translocation signal to W/Wv MCp. However, the injection of W/Wv mice with SCF does not result in the appearance of local mature MC in these mice, suggesting that SCF is required for a function other than the induction of migration of MCp (51). Although cutaneous application of phorbol 12-myristate 13-acetate (52) or intestinal helminthic infection (10–12) can elicit local mature MC in the c-*kit*/SCF-deficient mice, the numbers are minimal relative to normal mice. Taken together, these findings suggest that c-*kit*/SCF-deficient mice lack intestinal MCp because SCF provides a signal required for the survival of MCp in this tissue and therefore the maturation of any translocated MCp is not possible. Thus, the physiologic intestinal reservoir of MCp requires both an intact cytoprotective c-*kit*/SCF pathway and a tissue-selective homing of MCp that express $\alpha 4\beta 7$ integrin.

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