CD11c⁺ Cells Are Required for Antigen-Induced Increase of Mast Cells in the Lung

Joakim S. Dahlin, Ricardo Feinstein, Yue Cui, Birgitta Heyman and Jenny Hallgren

*J Immunol* 2012; 189:3869-3877; Prepublished online 12 September 2012;
doi: 10.4049/jimmunol.1201200
http://www.jimmunol.org/content/189/8/3869

Supplementary Material  http://www.jimmunol.org/content/suppl/2012/09/12/jimmunol.1201200.DC1

References  This article cites 31 articles, 16 of which you can access for free at:
http://www.jimmunol.org/content/189/8/3869.full#ref-list-1

Subscription  Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions  Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts  Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
CD11c⁺ Cells Are Required for Antigen-Induced Increase of Mast Cells in the Lung

Joakim S. Dahlin,* Ricardo Feinstein,† Yue Cui,* Birgitta Heyman,* and Jenny Hallgren*

Patients with allergic asthma have more lung mast cells, which likely worsens the symptoms. In experimental asthma, CD11c⁺ cells have to be present during the challenge phase for several features of allergic inflammation to occur. Whether CD11c⁺ cells play a role for Ag-induced increases of lung mast cells is unknown. In this study, we used diphtheria toxin treatment of sensitized CD11c-diphtheria toxin receptor transgenic mice to deplete CD11c⁺ cells. We demonstrate that recruitment of mast cell progenitors to the lung is substantially reduced when CD11c⁺ cells are depleted during the challenge phase. This correlated with an impaired induction of endothelial VCAM-1 and led to a significantly reduced number of mature mast cells 1 wk after challenge. Collectively, these data suggest that Ag challenge stimulates CD11c⁺ cells to produce cytokines and/or chemokines required for VCAM-1 upregulation on the lung endothelium, which in turn is crucial for the Ag-induced mast cell progenitor recruitment and the increase in mast cell numbers. The Journal of Immunology, 2012, 189: 3869–3877.

P atients with allergic asthma have a Th2 type of immune response leading to, for example, mucus production, airway hyperresponsiveness, infiltration of inflammatory cells, and increased levels of allergen-specific IgE (1). Tissue-resident mast cells are activated and release mediators that participate in the allergic response (2), and the numbers of mature mast cells are increased in the human asthmatic lung (3–5). In experimental models, mice lacking mast cells have attenuated airway responses, also pointing to an important role for mast cells in the development of allergic asthma (6–8). Mast cells arise from mast cell progenitors (MCp) originating from the bone marrow. During normal conditions these home to peripheral organs via the blood and mature in situ (9). Naïve mice, or mice sensitized to an Ag but not challenged, have few MCp and mature mast cells in the airways (10–12). However, when sensitized mice are challenged, the influx of MCp to the lung increases 10–30-fold (10, 12). This is followed by an increase in mature mast cells in the tracheal intraepithelium 1 wk later (11). Therefore, the allergen-induced increase in mature lung mast cells seen in both asthmatic patients and in mouse models is presumably preceded by an increased migration of MCp into the lung, referred to in this study as MCp recruitment.

In the lung, dendritic cells and alveolar macrophages express CD11c (13). Using an experimental model of allergic asthma in which mice were sensitized with OVA-pulsed dendritic cells followed by OVA aerosol challenge, van Rijt et al. (14) showed that depletion of CD11c⁺ cells during the challenge phase reduces production of Th2 cytokines, eosinophilia, goblet cell hyperplasia, and airway hyperresponsiveness. This prompted us to investigate whether CD11c⁺ cells play a role in the recruitment of MCp to the lung and in the subsequent increase of mast cells. Small amounts of diphtheria toxin (DT) deplete CD11c⁺ cells in CD11c-DTR mice but not in wild-type strains (15). Using mice transiently depleted of these cells during the challenge phase, we demonstrate in this study that CD11c⁺ cells are required for Ag-induced recruitment of MCp to the lung and for the appearance of mature mast cells. The diminished MCp recruitment correlated with lower induction of VCAM-1 on the lung endothelium. A plausible explanation is that Ag challenge induces production of cytokines and/or chemokines by dendritic cells and alveolar macrophages crucial for VCAM-1 upregulation on the lung endothelium, which in turn is required for Ag-induced MCp recruitment (10).

Materials and Methods

Animals

Age- and sex-matched female or male mice with an age of at least 6 wk were used in the experiments. The mice were bred and maintained at the National Veterinary Institute in Uppsala, Sweden. BALB/c mice were originally acquired from Bommice (Ry, Denmark), and hemizygous CD11c-DTR mice on a BALB/c background were acquired from The Jackson Laboratory (Bar Harbor, ME). CD11c-DTR mice were genotyped as previously described (16). The Ethics Committee in Uppsala approved all experiments performed.

Experimental protocol

The mice were immunized with 10 μg OVA grade V (Sigma-Aldrich, St. Louis, MO) adsorbed to 1 mg Alum (Pierce, Rockford, IL, USA) in 200 μl PBS i.g. on days 0 and day 7. On days 17, 18, and 19, the mice were challenged with 1% OVA (w/v) in PBS for 30 min using a nebulizer (PARI, Starnberg, Germany). To deplete CD11c⁺ cells during the challenge phase, 250–400 ng DT (depending on the batch; Sigma-Aldrich) was injected i.p. in 200 μl PBS on days 16 and 18. The dose of DT used was chosen as the lowest possible that still efficiently depleted lung CD11c⁺ cells. Day 20, the lungs were analyzed for MCp by limiting dilution and for other cell populations by flow cytometry. In the experiments where bone marrow chimeric mice were used (see Fig. 4), the lungs were removed on day 27 for analysis of mature mast cells.
CD11c+ CELLS REGULATE LUNG MAST CELL NUMBERS

Generation of bone marrow chimeras

CD11c-DTR mice were euthanized and the femurs and tibiae were removed. The bone marrow was flushed out with complete RPMI (RPMI 1640, 10% heat-inactivated FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 10 µg/ml gentamicin, 0.1 mM nonessential amino acids, 2 mM l-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, and 20 µM 2-ME; all from Sigma-Aldrich) using a 27-gauge needle. The cells were centrifuged and resuspended in PBS (pH 7.4). After determining the concentration of viable cells with trypan blue exclusion on a hemacytometer, the cells were centrifuged and resuspended in PBS. Ten million bone marrow cells in 200 µl were injected i.v. into sublethally irradiated (7.5 Gy) wild-type BALB/c mice. The mice were allowed to rest for at least 6 wk before the experiments. The bone marrow chimeric mice were only used in the experiments illustrated in Fig. 4.

Preparation of lung and bone marrow mononuclear cells

The mice were euthanized by an overdose of isoflurane (Schering-Plough, Farum, Denmark) followed by dislocation of the neck. The lungs were flushed with 10 ml PBS administered through the right ventricle of the heart. A single-cell suspension was prepared by chopping the lungs with scalpel blades and enzymatic degradation of tissue by using 1500 U collagenase type IV (Life Technologies, Paisley, Scotland, U.K.) in 10 ml complete RPMI (described above) at 37°C. After 20 min, 30 ml complete RPMI was added. The released cells were removed by pipetting, and the undigested tissue was subjected to a total of three digestions. Released cells were centrifuged for 10 min at 200 × g and resuspended in 44% Percoll (Sigma-Aldrich). The solution was underlayed with 67% Percoll and the gradient was centrifuged for 20 min at 500 × g. The Percoll gradient separation was repeated from the second to the third digestions. Mononuclear cells (MNC) from the interfaces were extracted by pipetting, washed in complete RPMI, and viable cells were counted on a hemacytometer using trypan blue exclusion. For preparation of bone marrow MNC, the femur and tibia were obtained from the right hind leg. The bone marrow was flushed out with complete RPMI and spun down for 10 min at 200 × g. The pellet was subjected to one round of Percoll purification as described above.

Quantification of MCP

A 2-fold serial dilution of MNC in two 96-well tissue culture plates was performed, starting at 20,000 cells per well in 24 wells. The MNC were supplemented with 20–40 ng/ml IL-3 from X63 supernatant (17A, 20–40 ng/ml recombinant murine stem cell factor (PeproTech, Rocky Hill, NJ), and feeder cells from 30 Gy gamma-irradiated total splenic cells. IL-3 concentration in X63 supernatant was measured by ELISA. After culture for 10–12 d, the numbers of wells scored positive for colony growth were counted, and the concentration of MCP was quantified as described earlier (10, 12, 18).

Flow cytometry

Unless indicated, lung cells were preincubated with CD16/CD32 (2.4G2; BD Biosciences, Franklin Lakes, NJ) in FACS buffer (PBS supplemented with 2% heat-inactivated FCS) to prevent unspecific binding. The following Abs were used for identification of dendritic cells and alveolar macrophages: I-Ad-PE (AMS-32.1; BD Biosciences) and CD11c-allophycocyanin (HL3; BD Biosciences) or CD11c-PE-Cy7 (N418; eBioscience). Culture supernatants (from the second and third digestions) were stained with anti-c-Kit-PE-Cy7 (2B8; eBioscience), anti-mouse (m) F4/80-PE-Cy5 (10F11; BD Biosciences), anti-mouse (m) Gr-1-APC (RB6-8C5; eBioscience), anti-mouse (m) CD44-PE (3E5; BioLegend), anti-mouse (m) CD19-PE-Cy5 (eBio1D3; eBioscience), and anti-mouse (m) CD3-PE (17A2; BD Biosciences). The remaining 2.5 ml was subjected to one round of Percoll purification as described above. The remaining 2.5 ml was subjected to one round of Percoll purification as described above. The released cells were removed by pipetting, and the undigested tissue was subjected to a total of three digestions. Released cells were centrifuged for 10 min at 200 × g and resuspended in 44% Percoll (Sigma-Aldrich). The solution was underlayed with 67% Percoll and the gradient was centrifuged for 20 min at 500 × g. The Percoll gradient separation was repeated from the second to the third digestions. Mononuclear cells (MNC) from the interfaces were extracted by pipetting, washed in complete RPMI, and viable cells were counted on a hemacytometer using trypan blue exclusion. For preparation of bone marrow MNC, the femur and tibia were obtained from the right hind leg. The bone marrow was flushed out with complete RPMI and spun down for 10 min at 200 × g. The pellet was subjected to one round of Percoll purification as described above.

Immunohistochemistry

The paraffin-embedded lung sections were deparaffinized, hydrated in ethanol and water, and thereafter incubated with 30% hydrogen peroxide in 50 ml TBS (0.05 M Tris-HCl with 9% NaCl [pH 7.6]) for 20 min to inactivate endogenous peroxidase. After washing three times in TBS, the slides were heated in Dako Target Retrieval Solution (Dako, Glostrup, Denmark) in a microwave oven (7 min at 750 W plus 14 min at 350 W). VCAM-1 (H-276) rabbit polyclonal IgG (sc-8304; Santa Cruz Biotechnology, Santa Cruz, CA) was diluted 1:30 in 1% BSA and detected using EnVision+ system HRP/rabbit (K4003; Dako) according to the instructions by the manufacturer. The difference in VCAM-1 staining of the lung endothelium was blindly semiquantified by giving the sections a score between 0 and 4 (with 4 corresponding to intense staining of all vessels; 3, strong staining of most vessels; 2, a few vessels strongly stained; 1, a few vessels weakly stained; 0, no staining observed). For the score of 0 (no staining), see the control staining in Fig. 5D.

Preparation of lung cells for quantification of mast cells with flow cytometry

The left lung lobe was chopped with scalpel blades and enzymatically degraded with 750 U collagenase IV in 2.5 ml complete RPMI for 20 min at 37°C. After digestion, 10 ml complete RPMI was added, and the released cells were centrifuged for 10 min at 200 × g. The remaining 2.5 ml was subjected to another degradation, and all released cells were pooled, centrifuged for 10 min at 200 × g, and resuspended in 1 ml RBC lysis buffer (0.16 M NH4Cl, 10 mM KHCO3, 1 mM EDTA in distilled H2O) for 1 min on ice. The cells were washed in complete RPMI and resuspended in FACS buffer. After counting the viable cells on a hemacytometer, cells were kept overnight at 4°C. The next day, the cells were centrifuged for 10 min at 200 × g, resuspended in FACS buffer, and 2 million cells were filtered through a nylon mesh into FACS tubes. Cells were washed in FACS buffer and stained for mast cells (described above in the paragraph headed Flow cytometry).

Statistics

The results were analyzed with Prism 5.0d (GraphPad Software). All comparisons between groups were made by two-tailed Student’s t tests, except for Fig. 5C, which was analyzed by a two-tailed Mann–Whitney U test. Correlations were made with a Pearson test. Means ± SE of mean are considered significant.

Results

Lung dendritic cells and alveolar macrophages in CD11c-DTR mice are depleted by DT

To study the involvement of CD11c+ cells in the Ag-induced recruitment of MCP to the lung, we used CD11c-DTR mice that can be transiently depleted of CD11c+ cells by administration of DT. The CD11c-DTR mice were sensitized with OVA-Alum on days 0 and 7 and subsequently divided into three groups: one was left unchallenged, which was challenged with OVA aerosol on days 17–19, and the third group was treated with DT during the challenge phase (Fig. 1A). To test whether DT treatment led to depletion of CD11c+ cells in our model, lung MNC from the three groups were analyzed 24 h after the final challenge. Sensitized but not challenged CD11c-DTR mice had low levels of CD11c+MHC
class II (MHC-IImhi) dendritic cells. These cells increased both in frequency and in total number after challenge (Fig. 1B–D), whereas the frequency and total number of CD11c*MHC-IImlo alveolar macrophages were similar in the two groups (Fig. 1B, 1E, 1F). As expected, the sensitized and challenged CD11c-DTR mice treated with DT during the challenge phase had a severe reduction of both CD11c*MHC-IImhi dendritic cells and CD11c*MHC-IImlo alveolar macrophages (Fig. 1B–F). In summary, Ag challenge leads to a substantial increase in CD11c*MHC-IImhi dendritic cells whereas CD11c*MHC-IImlo alveolar macrophages are not significantly increased. Both cell types are depleted after treatment with DT.

**CD11c+ cells are required for Ag-induced MCp recruitment to the lung**

Analogous to previous observations in wild-type mice (10, 12), sensitized and challenged CD11c-DTR mice that were not depleted of CD11c+ cells had a higher frequency and higher total numbers of MCp per lung (368 ± 58 MCp/10⁶ MNC; 2964 ± 381 MCp/lung) than did mice that were sensitized but not challenged (28 ± 5 MCp/10⁶ MNC; 72 ± 22 MCp/lung) (Fig. 2A, 2B). Remarkably, when CD11c+ cells were depleted during the challenge phase, the MCp recruitment to the lung was severely impaired (Fig. 2A, 2B). The frequency of lung MCp was reduced with 78% and the total number of MCp per lung with 74% as compared to sensitized and challenged mice not treated with DT. This substantial reduction was specific because the DT treatment did not reduce the total number of MNC in the lung (Fig. 2C).

**Mast cells and their progenitors do not express CD11c and they are not killed by DT**

To ensure that the DT-induced effects on MCp recruitment were not due to an unspecific toxic effect, we performed a series of control

---

**FIGURE 1.** Lung dendritic cells and alveolar macrophages are depleted by DT. (A) CD11c-DTR mice were either only sensitized (Sens) to OVA, sensitized and challenged (Ch) with OVA aerosol, or sensitized, challenged, and treated with DT according to the protocol. (B) Representative plots of flow cytometric analysis of lung MNC stained with the indicated Abs. The CD11c*MHC-IImhi dendritic cells (DC) within the black gate were quantified as percentage of MNC (C) and total number per lung (D). CD11c*MHC-IImlo alveolar macrophages (AM) within the gray gate were quantified as a percentage of MNC (E) and total number per lung (F). The graphs show 5–12 individual mice per group pooled from four independent experiments. **p < 0.01, ***p < 0.001, ****p < 0.0001. The data represent the means ± SEM.
CD11c+ cells are required for Ag-induced MCp recruitment to the lung. CD11c-DTR mice were either only sensitized (Sens), sensitized and challenged (Ch), or sensitized, treated with DT, and challenged. One day after the third challenge, the frequency (A) and total number (B) of lung MCp were assessed. (C) Number of MNC per lung. The graphs show 5–12 individual mice per group pooled from four independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. The data represent the means ± SEM.

In summary, DT treatment of wild-type mice did not lead to depletion of CD11c+ dendritic cells or CD11c+ alveolar macrophages or to reduced MCp recruitment. Neither MCp nor mature mast cells express detectable levels of CD11c, and they are not killed by DT in vivo.

CD11c+ cells are required for MCp recruitment to the lung in sublethally irradiated wild-type mice reconstituted with CD11c-DTR bone marrow

Previous studies have demonstrated that the Ag-induced recruitment of lung MCp is followed by intraepithelial mast cells that appear in the trachea 1 wk after challenge (11). To investigate whether the absence of CD11c+ cells during the challenge phase leads to reduced numbers of mature mast cells in the airways, we therefore had to wait 11 d after the first DT treatment. However, CD11c-DTR mice die 6–7 d after DT injection owing to DTR expression on nonhematopoietic cells (20). Therefore, we had to generate bone marrow chimeric mice. Sublethally irradiated wild-type mice were reconstituted with CD11c-DTR bone marrow and then treated according to the protocol (Fig. 4H). The frequency and total number of MCp and MNC in the lung increased with challenge in sensitized CD11c-DTR chimeric mice (Fig. 4A–C) in a similar way as observed in nonchimeric CD11c-DTR mice (Fig. 2). For unknown reasons the MCp frequency was 3-fold higher and the total number of lung MCp was 7-fold higher in sensitized and challenged CD11c-DTR chimeric mice than in nonchimeric CD11c-DTR mice (compare Fig. 2A and 2B with Fig. 4A and 4B). Importantly, depletion of CD11c+ cells with DT during the challenge phase resulted in a markedly reduced recruitment of MCp to the lung in chimeric CD11c-DTR mice (Fig. 4A, 4B), similar to what was seen in the normal CD11c-DTR mice (Fig. 2A, 2B). Thus, overall the MCp recruitment in chimeric mice closely resembled that in nonchimeric mice, making us confident that this model is relevant for the study of mature mast cells.

CD11c+ cells are required for Ag-induced increase in mature lung mast cells

To quantify the effect of CD11c+ cell depletion on the appearance of mature mast cells, the four right lung lobes from each mouse were analyzed for mature mast cells with histology. Naïve or only sensitized CD11c-DTR chimeric mice had very few toluidine blue-positive mast cells in their lungs (Fig. 4D, 4G). However, after sensitization and challenge these numbers increased by 8-fold (Fig. 4E, 4G). Interestingly, the newly recruited mature mast cells were distributed in patches in the alveolar parenchyma (Fig. 4E). The increase in mast cells was dependent on the presence of CD11c+ cells, as it was completely abrogated after treatment with DT (Fig. 4F, 4G). In parallel with the study of the lung sections, mast cells in the left lung lobes were quantified with flow cytometry. Mast cells were identified as non-autofluorescent CD3–CD19–CD34–c-Kit+c-FceRI+ cells (Fig. 4I). Sensitized and challenged chimeric CD11c-DTR mice had 1518 ± 397 mast cells per 106 lung cells (Fig. 4J) and 4810 ± 1039 mast cells per left lung lobe (Fig. 4K). Depletion of CD11c+ cells during the challenge phase led to a 65% reduction in the frequency of mast cells, as well as a 57% reduction of mast cells per left lung lobe (Fig. 4J, 4K). There was a strong correlation between the number of toluidine blue-positive mast cells in histological sections and the mast cell numbers obtained by flow cytometry (Fig. 4L). In summary, both MCp recruitment to the lung (measured 1 d after challenge) and the number of mature mast cells in the lung experiments. Our approach was to compare the effects of DT in wild-type mice (which do not express the DTR and therefore should not be affected by specific effects of DT) with those in CD11c-DTR mice. First, the effects of DT on CD11c+ lung cell populations were tested. The percentages of CD11c+MHC-IIhi dendritic cells in the sensitized, challenged, and DT-treated wild-type mice (Supplemental Fig. 1B) were comparable to those in sensitized and challenged wild-type mice without DT treatment (Supplemental Fig. 1A). Similarly, the same pattern was true for the percentage of CD11c+MHC-IIhi alveolar macrophages (Supplemental Fig. 1C, 1D). This showed that DT treatment was not generally toxic to CD11c+ cells. As expected, the levels of CD11c+ cells in DT-treated wild-type mice were severely reduced (Supplemental Fig. 1B, 1D). Second, putative toxic effects of DT on Ag-induced MCp recruitment were studied. Following DT treatment, wild-type mice had more lung MCp than did CD11c-DTR mice, although the MNC numbers were the same, suggesting that DT treatment does not affect MCp recruitment in wild-type mice (Fig. 3E). As expected, because CD11c+ cells populate the bone marrow, the number of MNC was reduced (Fig. 3F) and the frequency of MCp was higher (Fig. 3G). Third, mature mast cells obtained from cultured lung MCp were negative for CD11c surface expression (Fig. 3G). Third, mature mast cells obtained from cultured lung MCp were negative for CD11c expression (Fig. 3H). Fourth, to exclude that DT nonspecifically depleted mature mast cells, the tongue that contains a high number of mast cells also in naïve mice was chosen as a control organ. Clearly DT treatment of sensitized and challenged CD11c-DTR mice did not deplete mature mast cells (Fig. 3I–K).

In summary, DT treatment of wild-type mice did not lead to depletion of CD11c+ dendritic cells or CD11c+ alveolar macrophages or to reduced MCp recruitment. Neither MCp nor mature mast cells express detectable levels of CD11c, and they are not killed by DT in vivo.
measured 8 d after challenge) were severely impaired in the absence of CD11c+ cells during the challenge phase.

CD11c+ cells are required for Ag-induced VCAM-1 expression on the lung endothelium

Ag-induced recruitment of lung MCp is dependent on the upregulation of VCAM-1 on the endothelium (10). A possibility is that CD11c+ cells are crucial for MCp recruitment because they induce VCAM-1, and therefore the VCAM-1 expression on the lung endothelium was studied. Sections from Ag-sensitized and -challenged CD11c-DTR mice displayed strong VCAM-1 staining (Fig. 5A, 5C). In contrast, the VCAM-1 staining in CD11c-DTR mice that in addition were DT treated was considerably reduced (Fig. 5B, 5C), suggesting that CD11c+ cells were required for upregulation of VCAM-1.

Lung CD11c+ cells from Ag-sensitized and -challenged mice produce TNF-α

Because TNF-α is a known inducer of VCAM-1 (21), the CD11c+ lung cells were analyzed for TNF-α by flow cytometry. Mice were sensitized and challenged according to the protocol described in Fig. 1A. Twenty-four hours after the last challenge, most of the CD11c+MHC-IIhi dendritic cells and a substantial amount of the CD11c+MHC-IIlo alveolar macrophages stained positive for TNF-α (Fig. 6, bottom).
**Discussion**

In agreement with earlier studies (14, 22), our data show that Ag challenge increases the number of CD11c+ lung dendritic cells whereas the CD11c+ alveolar macrophages remain at a constant level. To quantify lung MCp, the MNC fraction is collected mainly to remove mature mast cells and the numerous eosinophils. Interestingly, depletion of CD11c+ cells did not affect the total number of MNC, although it dramatically reduced the numbers of MCp. In the previous studies of the impact of CD11c+ cells on the allergic response in the lung, no attempts to measure MCp or mature mast cells were made. In this study, we demonstrate that depletion of CD11c+ cells severely inhibited the Ag-induced recruitment of lung MCp. We wanted to test whether the inhibition of MCp recruitment to the allergic lung translated into a reduction also in the numbers of mature mast cells. Similarly to wild-type or CD11c-DTR mice, irradiated wild-type mice reconstituted with CD11c-DTR bone marrow had increased numbers of lung MCp after sensitization and challenge that was diminished with DT
A highly reproducible and unexpected finding was that they had 3-fold higher frequency and 7-fold more lung MCp than do nonchimeric CD11c-DTR mice after sensitization and challenge. We speculate that the increased level of MCp in CD11c-DTR chimeric mice is partly due to a greater capacity of CD11c-DTR bone marrow to form the mast cell lineage. Additionally, the adoptive transfer may affect the site of “storage” of MCp, which may cause an easier release of MCp into the blood upon challenge. However, this effect worked in our favor because for the first time, a tremendous increase in mature lung mast cells could be observed with this mild and acute protocol. The mature mast cells in sensitized and challenged chimeric CD11c-DTR mice were localized to the alveolar parenchyma. Interestingly, patients with atopic uncontrolled asthma also have more mast cells in the alveolar parenchyma as compared with healthy controls (5). Remarkably, sensitized mice that lacked CD11c+ cells during the challenge phase had significantly fewer mast cells 8 d after challenge. In fact, the numbers were as low as those seen in naive or only sensitized mice.

Because the effects of depleting mice of CD11c+ cells with DT had such a dramatic impact on the number of MCp, and later on the number of mature mast cells in the allergic lung, we performed control experiments to exclude possible direct effects of DT on mast cells and their progenitors. Our experiments altogether show that the reduced Ag-induced recruitment of MCp is not due to an unspecific toxic effect of the DT or to direct deletion of MCp or mast cells via CD11c-DTR. Rather, CD11c+ cells are required for Ag-driven recruitment of lung MCp. In support of our data, other studies have demonstrated that mast cells do not express CD11c (23, 24).

An interesting question is why CD11c+ cells are required for Ag-induced MCp recruitment to the lung. One candidate molecule that could cause such a dramatic decrement in Ag-induced MCp recruitment to the lung is VCAM-1 (10, 11). Mice lacking endothelial VCAM-1, or mice treated with Abs blocking VCAM-1 during the challenge phase, had severely impaired MCp recruitment to the lung when our protocol to induce lung inflammation was used (10). In this study, we observed a striking decrease in VCAM-1 staining in DT-treated CD11c-DTR mice as compared with controls not treated with DT.
VCAM-1 after treatment with DT constitutes a likely explanation for the impaired Ag-induced MCp recruitment in mice lacking CD11c+ cells. However, we cannot exclude that other mechanisms operating in parallel may be contributing to the reduction of Ag-induced MCp recruitment.

The next question was why depletion of CD11c+ cells during the challenge phase led to impaired upregulation of endothelial VCAM-1. One possibility is that VCAM-1 upregulation is dependent on a full-blown Th2 response, which will be abrogated in CD11c- cell-depleted CD11c-DTR mice subjected to the experimental OVA model (14). Similarly, the Th2 response was hampered upon transient CD11c+ cell depletion in house dust mite-induced experimental asthma and the priming phase of Schistosoma mansoni infection (25, 26). In fact, both IL-4 and IL-13 can induce VCAM-1 expression (27, 28). However, the Th2 cytokines IL-4, IL-5, and IL-13 are dispensable for the recruitment of MCp, at least when the cytokines are knocked out or neutralized one by one (29). Thus, the inhibition of Ag-induced MCp recruitment is not likely due to a reduced induction of Th2 cytokines in the mice transiently devoid of CD11c+ cells, although we cannot completely rule out that possibility. A cytokine that is known to be required for optimal MCp recruitment to the allergic lung is IL-9. However, neither mice treated with blocking Ab to IL-9 nor IL-9-deficient mice have reduced VCAM-1 expression on the vascular endothelium using the same model of Ag-induced allergic inflammation (29). Another cytokine that is crucial for the onset of allergic responses in mouse models of allergic airway inflammation is TNF-α (8, 30). TNF-α is a well-known and potent inducer of VCAM-1 (21). Recently, CD11c+ dendritic cells and alveolar macrophages were shown to be the major sources of TNF-α in a mouse model of Aspergillus fumigatus-induced airway inflammation (13). In agreement with this finding, lung CD11c+ dendritic cells and alveolar macrophages produce TNF-α in our model. Thus, a possible explanation for the reduced VCAM-1 expression may be that the reduction of CD11c+ cells leads to a reduction of TNF-α and, hence, less VCAM-1 upregulation. However, there are other powerful VCAM-1 inducers, one of which is the proinflammatory cytokine IL-1 (21). Furthermore, CD11c+ dendritic cells and alveolar macrophages also produce the chemokines CXCL1 (KC) and CXCL2 (MP-2), which bind to CXCR2 (31). Stromal expression of CXCR2 is required for upregulation of VCAM-1 on the endothelium and in turn for Ag-induced recruitment of lung MCp (11). Therefore, these chemokines also are likely to be involved in the CD11c+ cell-dependent upregulation of VCAM-1. These observations suggest that regulation of VCAM-1 expression is extremely complex, and probably several mechanisms operate redundantly.

To find out whether CD11c+ dendritic cells, alveolar macrophages, or both cell types are required for Ag-induced MCp recruitment, a large number of adoptive transfer experiments were performed (data not shown). Ag-sensitized and –challenged DT-treated CD11c-DTR mice were reconstituted with various CD11c+ cell populations. We tried unpulsed or OVA-pulsed dendritic cells and/or alveolar macrophages given by either intratracheal, i.v., and/or i.p. injection. We have also given DT by intratracheal injection and reconstituted through the same route. (Note that when DT is given by intratracheal injection instead of i.p., the Ag-induced MCp recruitment response is inhibited to the same degree.) However, these procedures all failed to repopulate the lungs with the CD11c+ cells as judged by flow cytometry. Not surprisingly, they also failed to rescue the MCp recruitment. It seems to be notoriously difficult to reconstitute the cells to the right place by adoptive transfer. To our knowledge, only one study has succeeded with adoptive transfer of dendritic cells to DT-depleted CD11c-DTR mice (14). However, in that study (14), unlike in our attempts, the CD11c-DTR mice were sensitized with OVA-pulsed dendritic cells of wild-type origin. Thus, the dendritic cells used for sensitization were not depleted by DT, and possibly the presence of wild-type dendritic cells makes it easier to restore the allergic response.

In summary, it seems likely that depletion of CD11c+ dendritic cells and alveolar macrophages leads to insufficient amounts of several cytokines and chemokines being produced in the allergic lung. This causes impaired VCAM-1 upregulation, which is a prerequisite for Ag-induced MCp recruitment and subsequent accumulation of lung mast cells. Our study also suggests that CD11c+ cells should be considered as targets in the treatment of patients with allergic asthma.

Acknowledgments

We thank Ingela Stake and colleagues at the Swedish National Institute for excellent care of the mice.

Disclosures

The authors have no financial conflicts of interest.

References


