Pulmonary CD103 expression regulates airway inflammation in asthma.

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Running title: CD103 and airway inflammation
Abstract

Although CD103+ cells recently emerged as key regulatory cells in the gut, the role of CD103 ubiquitous expression in the lung and development of allergic airway disease has never been studied. To answer this important question, we evaluated the response of Cd103-/− mice in two separate well-described mouse models of asthma (ovalbumin and House Dust Mite (HDM) extract). Pulmonary inflammation was assessed by analysis of broncho-alveolar lavage content, histology, and cytokine response. CD103 expression was analyzed on lung dendritic cells and T cell subsets by flow cytometry. Cd103+/− mice exposed to antigens developed exacerbated lung inflammation, characterized by increased eosinophilic infiltration, severe tissue inflammation and altered cytokine response. In wild type mice exposed to HDM, CD103+ dendritic cells are increased in the lung and an important subset of CD4+ T cells, CD8+ T cells and T regulatory cells express CD103. Importantly, Cd103+/− mice presented a deficiency in the resolution phase of inflammation, which supports and important role for this molecule in the control of inflammation severity. These results suggest an important role for CD103 in the control of airway inflammation in asthma.

Keywords: asthma, CD103, dendritic cells, T regulatory cells

Abbreviations: Dendritic cells (DCs); T regulatory cells (Tregs); House Dust Mite (HDM); Ovalbumin (OVA); Wild type (WT)
Introduction

Asthma is characterized by the presence of eosinophils in the lung and airways, a Th2-biased inflammatory response, increased airway responsiveness and remodeling of the airways (15). To date, research in the field has mainly focused on the cascade of events leading to the development of airway inflammation, which has greatly increased our understanding of asthma pathogenesis. Dendritic cells (DCs), responsible for antigen presentation to naïve CD4+ T cells (33), play a major role in the development of asthmatic airway inflammation. These cells patrol the lung, capture antigens and migrate to draining lymph nodes and local lymphoid tissue where antigen presentation occurs (11). This is partially coordinated through DC-expressed integrins, such as the alpha-E integrin CD103.

CD103 is expressed on a small population of DCs and on intra-epithelial T lymphocytes, including CD4+ and CD8+ T cells and T regulatory cells (Tregs) (8, 10, 17, 22, 25, 34). The ligand for CD103 is E-Cadherin, which is expressed on the basolateral face of all major epithelia, including lung (1), allowing for ligation with CD103+ cells in the parenchyma. CD103+ DCs are found in most organs, including the lung (9). Interestingly, in contrast to CD103- DCs, which are mostly qualified for innate immunity (8, 16), CD103+ DCs have a higher capacity to differentiate naïve T cells into Tregs in vitro in the presence of co-factors such as TGF-beta or retinoic acid (12).
The role of CD103 in the development of allergic airway inflammation in asthma remains unclear. Specifically, recent data suggested that CD103+ DCs induce allergic airway inflammation (27), which is in stark contrast to studies reporting an inhibitory role for this population in the lung and gut (7, 20, 21). Moreover, and importantly, the study by Nakano H. *et al* fails to account for the ubiquitous expression of CD103 on DCs and T cells, including Tregs. Here, for the first time, we shed a light on the importance of ubiquitous CD103 expression in the regulation of inflammation in asthma. Using *Cd103*-/- mice in two separate classical mouse models of asthma, we report that ubiquitous deletion of CD103 expression leads to a significant exacerbation of the inflammatory response, in contrast to the deletion of CD103 expression on DCs only (27). Additionally, we show that HDM exposure leads to an influx of CD103+ DCs into the lung and that loss of CD103 expression results in a delay in the resolution of inflammation. In summary, in opposition to a recent study suggesting that loss of CD103+ DCs may be an interesting target in the treatment of allergic diseases (27), our data reveal a crucial role for the expression of this molecule in the regulation of inflammatory responses to inhaled antigens, as well as for the regulation of airway responsiveness in a mouse model of asthma.
Methods

Animals

Cd103\(^{-/-}\) (B6.129S2(C)-Itgae\(^{im1Cmp}\)/J) and wild type littermates (WT) were obtained from Jackson Laboratories and kept in a specific pathogen-free animal unit (Biomedical Research Center, UBC, Vancouver, Canada and CRIUCPQ; Laval University, Québec, QC, Canada) for the duration of the experiments. Experiments were approved by local ethics committees and followed Canadian animal care guidelines.

Induction of asthma and assessment of airway inflammation

For the Ovalbumin (OVA) (Sigma-Aldrich, Oakville, ON, Canada) model of asthma, age and sex-matched WT and Cd103\(^{-/-}\) mice were sensitized with 100µL 0.2% OVA coupled to Al(OH)\(_3\) on days 1 and 8 as previously described (4, 5, 13, 28), and challenged intranasally with 50µl of 2% OVA diluted in saline on days 22, 23, 24, 26 and 28. For the House Dust Mite (HDM) (GREER, Lenoir, NC) model of asthma, mice were exposed to 50µl of 1.5mg/mL or 0.3mg/mL HDM antigen diluted in saline for 10 consecutive days, as previously described (30). Mice were sacrificed 24h after the last antigen exposure, tracheotomized with an 18G catheter, and a broncho-alveolar lavage (BAL) was performed by three separate injections/aspirations of 1mL saline. Total BAL cells were counted and differential counts obtained using Giemsa stain (HemaStain Set, Fisher Scientific, Kalamazoo, MI). A section of the left lobe was fixed in 10% formalin for histology studies. Hematoxylin/eosin-stained slides were blindly evaluated and a score of
0-5 was attributed for perivascular, peribronchial and parenchymal inflammation as per our previous studies (5).

Assessment of airway hyperresponsiveness

24hrs after the final challenge, WT and Cd103−/− mice were anaesthetized with ketamine/ xylazine, tracheotomised and intubated with an 18G catheter. Airway resistance (R) was measured with a Flexivent apparatus (SCIREQ, Montreal, Qc, Canada). Respiratory frequency was set at 160 breaths/min with a tidal volume of 0.2mL, and a positive end-expiratory pressure of 2-4mL H2O was applied. Increasing concentrations of methacholine (MCh) (0 to 2mg/kg) were administered via the jugular vein. R was recorded every 15 seconds by a snapshot measure. The % increase in R was calculated for each MCh dose.

Analysis of lung tissue

Leukocytes were obtained by digestion of lung tissue with 200U/mL Collagenase IV (Sigma, Oakville, ON, Canada) for 1.5hr at 37°C. Digested tissue was pressed through a 70µm cell strainer and leukocytes were enriched using a 30% Percoll gradient (GE Healthcare, Uppsala, Sweden). Red blood cells were lysed using ammonium chloride. Leukocytes were analyzed by flow cytometry for the presence of CD103+ DCs and T cells. Antibodies used were: anti-CD11b-Pe Cy7, anti-CD103-PE, anti-CD80-Biotin, anti-CD11c-APC (BD Biosciences, Pharmingen, San Diego, CA, USA), anti-CD90.2-
Biotin, Streptavidin-PerCP, anti-CD4-PB, anti-CD8-APC Cy7, Streptavidin-FITC, anti-MHC-II-Pac Blue, anti-CD11c-PerCP (BioLegend, San Diego, CA, USA), anti-NK1.1-Biotin, anti-CD19-Biotin (Ablab, Vancouver, B.C., Canada), anti-Foxp3-Biotin, anti-E-cadherin-Biotin, anti-CD25-APC (eBioscience, San Diego, CA, USA), and Streptavidin-AF700 (Invitrogen, Carlsbad, CA, USA).

Antigen-specific cytokine production

After leukocyte digestion, 5x10^5 total isolated lung cells from WT and Cd103^-^- mice exposed in vivo to OVA were stimulated with 1, 10 or 100 ng/ml OVA for 72hrs. Supernatants were collected and cytokine production was evaluated using a cytometric bead array mouse Th1/Th2 kit, or Inflammation kit (BD Biosciences, San Diego, California, USA), according to the manufacturer’s instructions.

Bone Marrow Derived Dendritic Cell Differentiation and Migration Assay

BMDDCs were obtained by differentiation of bone marrow monocytes in complete media (RPMI 1640, 10 % FBS, 10% GM-CSF (Ablab), 1% penicillin/streptomycin, 1% sodium pyruvate, and 40µM β-mercaptoethanol) for 8 days. 2.5x10^5 cells in 500µL of complete media were placed in 3µm inserts in 24-well plates and serial dilutions of CCL19 and CCL20 (Ablab) or SDF-1 (CXCL12) (Cedarlane, Burlington, ON, Canada) in 500µL of complete media were added into the lower chamber as chemo attractants.
After 18 hours of incubation (37 °C + 5% CO₂), cells remaining in the inserts and cells that migrated into the lower chamber were recovered and counted.

Assessment of In vivo Cell Trafficking Using OVA-AF647

At the basal level, mice were challenged with with 50uL of 1mg/mL OVA-AF647 (Life Technologies, Burlington, ON, Canada) diluted in saline. In the context of inflammation, mice were exposed to 50uL of 0.75mg/mL HDM antigen diluted in saline by intranasal instillation for 5 consecutive days. On day 5, mice were also challenged with 50uL of 1mg/mL OVA-AF647. All mice were euthanized 18 hours after the OVA-AF647 exposure. Lung leukocytes were isolated as described previously. Lymph node leukocytes were obtained by pressing digested tissue (200U/mL Collagenase IV for 1.5hr at 37°C) through a 70µm cell strainer. Purified leukocytes were analyzed by flow cytometry as described before.

Statistics

Data are presented as mean ± SE. Statistical analysis for multiple comparisons was performed using an ANOVA table followed by a Fisher post hoc test. Non-multiple comparisons were analyzed using unpaired T-tests. Statistical significance was determined at p ≤ 0.05.
RESULTS

Ubiquitous deletion of CD103 expression leads to exacerbation of OVA-induced allergic airway inflammation

The importance of CD103 ubiquitous expression in asthma has never been examined. Therefore, we set out to verify whether lack of CD103 expression would affect the development of airway inflammation in an OVA-induced mouse model of asthma. 

Cd103−/− mice developed exacerbated airway inflammation in response to OVA compared to wild type (WT) controls, as noted by an increase in total broncho-alveolar lavage (BAL) cells (Figure 1A) and eosinophil number (Figure 1B). When the airway responsiveness was studied, no difference was observed between WT and Cd103−/− mice at baseline (Figure 1C). However, after exposure to OVA, we noted an increase in methacholine-induced (MCh) airway hyperresponsiveness in Cd103−/− mice compared to WT (Figure 1C). Histological examination of hematoxylin/eosin-stained lung tissue demonstrated an increase in peribronchial, perivascular and parenchymal infiltrate in OVA-exposed Cd103−/− mice compared to WT mice (Figure 2). This demonstrates that lack of ubiquitous expression of CD103 leads to a significant exacerbation of the airway inflammatory response.

Lack of CD103 expression leads to altered cytokine production in response to OVA

Cytokine production is a key in determining the severity of airway inflammation. To verify if the exacerbated airway inflammation in Cd103−/− mice exposed to OVA was caused by alterations in the adaptive immune response, we performed ex-vivo cytokine
recall assays on total lung inflammatory cells isolated from whole lungs as previously
described by our team (5). *Cd103<sup>−/−</sup>* cells produced elevated quantities of IL-5 (Figure
3A), a Th2-associated cytokine promoting eosinophil infiltration in allergic diseases (24,
38, 39). Surprisingly, when the production of IFN-gamma was measured, we also
observed increases in this classical Th1 cytokine in *Cd103<sup>−/−</sup>* mice (Figure 3B) compared
to WT. This suggests that loss of CD103 leads to a non-specific deregulated and
overactive immune response to inhaled antigens.

*Ubiquitous deletion of CD103 expression exacerbates House Dust Mite antigen-induced
allergic airway inflammation*

As CD103 is expressed by intraepithelial DCs and T cells (8, 10, 17, 25, 34), which play
a major role in antigen presentation and local priming in asthma (37), we felt it was
important to verify whether the exacerbation phenotype observed in the OVA model of
asthma would also be observed in an HDM model where priming of the pulmonary
allergic response occurs locally. *Cd103<sup>−/−</sup>* mice exposed to HDM showed an increase in
airway inflammation compared to WT mice, as shown by increased BAL cells (Figure
4A) and eosinophil number (Figure 4B). Furthermore, *Cd103<sup>−/−</sup>* mice developed increased
tissue inflammation in response to HDM, characterized by an increase in peribronchial
and perivascular infiltrate (Figure 4C). To confirm that this was due to increased
sensitivity of *Cd103<sup>−/−</sup>* mice to HDM, we reduced the dose administered to 0.3mg/mL.
*Cd103<sup>−/−</sup>* mice indeed displayed an increased sensitivity to HDM, showing high levels of
leukocyte infiltration at a dose of 0.3mg/mL that only results in low levels of
inflammation in WT mice (Figure 4D and E). In our hands, HDM-exposed mice (WT and $Cd103^{-/-}$) did not demonstrate AHR compared to saline controls (unpublished observation).

*HDM exposure leads to infiltration of CD103$^+$ DCs and T cells*

Analyses of airway and tissue inflammatory response clearly revealed an exacerbated response to antigens in the absence of CD103 expression. To better understand the mechanisms behind this phenotype, we evaluated CD103 expression on DCs (Figure 5A) and T cell (Figure 5B) subsets in the lung, at baseline or in response to HDM antigen.

HDM exposure resulted in an influx of DCs into the lung, with a specific increase in CD103$^+$ DCs (Figure 5C). We did not observe any decrease in $Cd103^{-/-}$ mice lung total DC in response to HDM compared to WT mice, which was surprising as CD103 is known to play a role in cell retention in tissue (6, 19, 31). As expected in a mouse model of asthma, we noted strong CD103 expression on CD4$^+$ T cells in response to HDM, suggesting their importance in the pathology. We also noted a striking level of CD8$^+$/CD103$^+$ T cells, which was comparable to the level of CD4$^+$/CD103$^+$ cells (Figure 5D). In the gut, CD103 is also known to be expressed on Tregs (2, 22). We thus wanted to test whether pulmonary Tregs express CD103 in response to HDM: we found a clear population of CD103$^+$ Tregs upon HDM exposure (Figure 5D).
CD103 expression is involved in the resolution of inflammation in response to HDM

Our observations reveal an important role for ubiquitous CD103 expression in the regulation of airway inflammation in asthma. Moreover, we find CD103 expression on pulmonary Tregs. Tregs are known to play an important role in the resolution of inflammation, a process by which tissues return to homeostasis following an inflammatory state. Hence, we set out to test whether CD103 expression plays a role in the resolution of inflammation.

We found that on day 13 (i.e. 3 days after the last exposure to HDM), when WT mice show clear signs of declining inflammation, there is still persistent airway leukocyte infiltration in Cd103−/− mice. Surprisingly, on day 15, the number of leukocyte is similar between both mice strains (Figure 6A). However, as Cd103−/− mice do consistently present with higher levels of inflammation on day 11, the higher inflammation observed in these mice on day 13 could be a reflection of this exacerbated response on day 11. To verify if CD103 expression plays a role in the rate of resolution, we calculated an inflammatory index (expressed as the ratio of inflammation on day 13 or 15 divided by the total inflammation on day 11). Cd103−/− mice present a higher inflammatory index compared to WT mice at day 13, indicating a delayed resolution of inflammation (Figure 6A), which was characterized by sustained high levels of eosinophils in BAL of Cd103−/− mice (Figure 6B). Thus, CD103 may facilitate the initiation of the resolution phase in this model.
Interestingly, when CD103 expression was analyzed during the resolution phase, the number of CD103\(^+\) DCs (Figure 6C) and T cells (Figure 6D) was largely increased in the early phase of resolution (day 13) compared to day 15, where the resolution is almost complete. This further supports the importance of CD103 expression in the initiation of resolution.

**CD103 expression in DC trafficking and DC markers expression**

To pinpoint the role of CD103 expression on DCs in the exacerbation phenotype, we examined the influence of CD103 expression on DC function such as migration, *in vivo* trafficking, adhesion and antigen presentation. First, we reasoned that increased DC migration in the absence of CD103 expression could lead to the exacerbated phenotype in *Cd103\(^{-/-}\)* mice. To test this, we used bone marrow derived dendritic cells (BMDDCs) and *in vitro* migration assays in response to CCL19, SDF-1 and CCL20. Loss of CD103 expression had no effect on *in vitro* migration of BMDDCs to chemokines (Figure 7A), suggesting that loss of CD103 expression on DCs does not lead to any intrinsic defect on the *in vitro* migratory response to these chemokines.

However, since CD103 is an integrin which binds E-Cadherin *in vivo*, we postulated that lack of CD103 may lead to increased trafficking (via a lower retention in the lung) of DCs to the draining lymph nodes, which could lead to the exacerbated responses to HDM and OVA. To verify this, mice were challenged with AF647-coupled OVA at the basal level or in the context of airway inflammation and the % and total number of OVA-
AF647+ DCs were verified in the mediastinal lymph nodes as a marker of DC trafficking (as previously reported (3)). At basal level, there are no differences of migration of OVA-AF647+ DCs to the mediastinal lymph nodes between WT and Cd103−/− mice (data not shown). However, in the context of inflammation, we noted an increase in the % and total number of OVA-AF647+ DCs in the mediastinal lymph nodes (Figure 7B), which indicate that lack of CD103 allows DCs to traffic more readily to the draining lymph nodes to induce the antigen-specific response.

To further test the role of CD103 in DC function, we also evaluated lung DC markers that play a role in DC adhesion and antigen presentation. Interestingly, pulmonary DCs also express E-Cadherin (18, 32), which could lead to autologous binding of E-Cadherin expressed on airway epithelia or to other CD103+ cells. When E-Cadherin levels were compared between Cd103−/− and WT mice, we observed a significant increase in the frequency of E-cadherin+ DCs at baseline, followed by a trend to higher levels of E-Cadherin+ DCs upon exposure to HDM (Figure 7C). This could suggest a compensatory mechanism of CD103-deficient DCs to up-regulate E-Cadherin expression to allow binding at the epithelium. Lastly, we examined the expression level of the co-stimulatory markers CD80 in naïve and HDM-exposed mice. While HDM exposure results in an increase of CD80 expression on DCs in WT mice, DCs in Cd103−/− mice already display high levels of CD80 expression at baseline and do not increase following HDM exposure (Figure 7D).
Although there has been increasing interest on the role of CD103$^+$ cells in the
development of airway inflammation, to our knowledge, no study has ever evaluated the
effects of ubiquitous CD103 deletion and its role in allergy and airway inflammation.
Here, we report new data on CD103 function in the lung, suggesting that its expression is
crucial for the regulation of airway inflammation. At first glance, this may appear
contradictory to a previous report showing that CD103$^+$ DCs induce airway inflammation
in a HDM model of asthma (27). However, although the mice used in this study are
reported as “CD103$^+$ DCs-deficient”, they are also deficient in a number of other aspects
(35) and they do not reflect a specific deletion in CD103. Furthermore, the study does not
1) specifically report a function for CD103 expression on these cells but rather suggests a
role for this cell population in asthma and 2) take into consideration the expression of
CD103 on all cell subsets, including Tregs. We feel that our direct assessment of
CD103’s importance in asthma, using $\text{Cd103}^{-/-}$ mice, is a better fit for the evaluation of its
specific role in lung inflammation. Our results are of major importance since our findings
suggest that blocking CD103 expression or modulating CD103$^+$ cells function may lead
to heightened asthma severity, which is in stark contrast to the conclusion raised by this
previous study.

In the acute phase of inflammation, our data suggest a clear regulatory role for CD103 in
dampening allergic airway inflammation in response to both peripherally and locally-
administered antigens. Furthermore, our results propose an important role for CD103 in
the regulation of airway inflammation in general. Indeed, the observed increase in Th2 (IL-5) and Th1 (IFN-gamma) cytokine production points towards a general inflammatory exacerbation rather than the sole exacerbation of the Th2 allergic response.

Our results also suggest the importance of CD103\(^+\) cells in the regulation of inflammation, which is in accordance with most literature. Indeed, in the lung, CD103\(^+\) DCs are associated with a regulatory role (8, 12, 21) and with the development of tolerance (20). Furthermore, and in opposition with the study by Nakano, H. et al. (27), a recent study (29) showed that CD11b\(^+\) (but not CD103\(^+\)) DCs prime T cells to induce the Th2 inflammatory response to HDM antigen. To our knowledge, ours is the first study to examine T cell expression of CD103 in response to HDM exposure. Although CD103 expression on CD8 T cell in the lung has been reported in previous studies (14, 23, 26), the high quantity of CD8\(^+\)/CD103\(^+\) T cells found in the lung in response to HDM is intriguing as they are not known to play a major role in asthma. Interestingly, CD8\(^+\)/CD103\(^+\) T cells have recently been defined as regulatory T cells \textit{in vitro} (36), which may explain in part the exacerbated phenotype observed in \textit{Cd103}\(^{-/-}\) mice.

In addition to the exacerbated inflammatory response in the acute phase, we were struck by the important difference in the early resolution index. In humans, impaired resolution could lead to exacerbated and chronic inflammation with consequent inflammatory episodes caused by repeated exposure to antigens. Our results reveal an important role of ubiquitous CD103 expression in preventing this phenomenon.
Most of the literature on CD103 in asthma has focused on the role of CD103+ DCs. Here, we provide the first evidence that actual CD103 protein expression plays an important role in the regulatory function of this lung DC population. Although we do not note intrinsic differences in DC migration, we could not discard the role of CD103 in the retention of DCs in the lung, which ultimately influences cell trafficking. Even though our results clearly indicate that Cd103−/− mice do not present altered total DC numbers in the lung in response to HDM antigen, we noted a striking exacerbation in DC trafficking to the lymph nodes in these mice. This could be explained in part by the increased E-Cadherin expression on DCs in these mice, which may compensate for the lack of CD103 and insure proper DC seeding, but may not allow retention in situ once cells are activated by antigen capture. Co-stimulatory molecules, such as CD80, are important in DC-T cell interactions to promote a strong adaptive response. We do observe an increased expression of CD80 on Cd103−/− pulmonary DCs at baseline which may suggest that DCs which are retained in the lung also participate in the exacerbated phenotype. In aggregate, lack of CD103 expression may lead to increased trafficking potential to the lymph nodes and the consequent exacerbated response to antigens in asthma.

In conclusion, we demonstrated, using two well-described mouse models of asthma, that CD103 expression is crucial in regulating the severity of airway inflammation in asthma. The exacerbated phenotype in Cd103−/− mice was characterized by increased airway and tissue inflammation, increased cytokine production, and an influx of CD103+ cells in the lung. We also suggest that CD103 could be involved in the initiation of the resolution of inflammation and in regulating DC trafficking to the draining lymph nodes. These studies
reveal a crucial role for CD103 in dampening asthma severity, and that CD103 could ultimately be used as a therapeutic strategy in asthma.

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**Disclosures**

The authors declared no conflict of interest.
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FIGURE LEGENDS

Figure 1. Airway inflammation and lung function in WT and Cd103^{-/-} mice: OVA mouse model of asthma

WT and Cd103^{-/-} mice were exposed to OVA and the broncho-alveolar lavage (BAL) content as well as pulmonary function were evaluated. A) Total BAL cells in naive and OVA-exposed mice. B) % and total numbers/ml BAL of macrophages, lymphocytes, neutrophils and eosinophils (markers of asthma severity). C) Pulmonary function measurements in vehicle-exposed (saline) or OVA-exposed animals. The airway resistance (R) was measured after each dose of MCh. Results are expressed as % increase in R compared to the baseline R level. n = 4-6 mice per group. This experiment was repeated three times with similar results. * = p < 0.05

Figure 2. Histology: OVA mouse model of asthma.

WT and Cd103^{-/-} mice were exposed to OVA and a section of the left lobe was cut, fixed, embedded in formalin and stained with hematoxylin/eosin. A) Sections from naive mice reveal no differences at baseline in the infiltration of the lung tissue. Exposure of Cd103^{-/-} mice to OVA leads to impressive exacerbated infiltration of lung tissue with various leukocytes compared to WT animals. B) Histological score was obtained and compared between WT and Cd103^{-/-} mice. * : p = < 0.0001
Figure 3. Recall responses, lung inflammatory cells: OVA mouse model of asthma.

5x10^5 isolated lung cells from WT and Cd103^-/- mice exposed in vivo to OVA were stimulated with increasing doses of antigen for 72hrs. Supernatants were collected and cytokine production was evaluated using a cytometric bead array. Results are expressed as, on a cell basis, % release of A) IL-5 and B) IFN-gamma, from baseline (WT unstimulated cells). n = 3 separate experiments, with cells from 4-6 mice pooled in each experiment. * = p < 0.05

Figure 4. Airway inflammation in WT and Cd103^-/- mice: HDM mouse model of asthma.

Following HDM exposure (1.5mg/mL) for 10 days, airway inflammation was evaluated and compared between WT and Cd103^-/- mice. A) Total BAL cells in WT and Cd103^-/- HDM-exposed mice. B) Total number/ml BAL and % of macrophages, lymphocytes, neutrophils and eosinophils (markers of asthma severity). C) Histological sections and score reflecting the severity of tissue inflammation in WT and Cd103^-/- exposed to 1.5mg/mL HDM. D) Total BAL cells and E) differential counts obtained in WT and Cd103^-/- mice exposed to increasing doses of the antigen to further enhance the exacerbated phenotype in Cd103^-/- mice. n = 4-6 mice per group. This experiment was repeated four times with similar results. * = p < 0.05
DC, T cell subsets and CD103 expression analysis by flow cytometry is presented in A and B. A) FSC and SSC example obtained after lung harvest. DCs were identified as autofluorescence⁻ / NK1.1⁻, CD90.2⁻, CD19⁻ / CD11c⁺ / MHC-IIhi cells. CD103 expression on DCs was verified in WT samples using Cd103⁻/⁻ mice as control for CD103 staining. B) T cells were identified as SSClo / FSClo (not shown), then as CD4⁺ or CD8⁺. Tregs were identified as CD4⁺/Foxp3⁺/CD25⁺. CD103 expression on T cell subsets was verified in WT samples using Cd103⁻/⁻ mice as control for CD103 staining. C) Number of DCs per lung was calculated based on the % total DCs, CD11b⁺ DCs or CD103⁺ DCs, and compared between WT and Cd103⁻/⁻ mice, with or without exposure to HDM. D) Number of CD103⁺ CD4 and CD8 T cells and CD103⁺ Tregs found in lung of HDM-exposed WT mice. Cd103⁻/⁻ samples were used as negative stain controls. n = 4-6 mice per group; results are representative of at least three separate experiments. * = p < 0.05, in WT saline vs WT HDM for all graphs.

Figure 6. CD103 in the resolution of inflammation: HDM mouse model of asthma.

Mice were exposed to 1.5 mg/mL HDM for 10 days as described in the methods. On day 13 and 15, the resolution of airway inflammation was evaluated and compared between WT and Cd103⁻/⁻ mice. A) Total cells / ml BAL of WT and Cd103⁻/⁻ mice on day 13 and 15 and the resolution index expressed as a ratio of total cells / day 11 total cell counts. * = p < 0.05 compared to WT. B) Differential cell counts of macrophages, lymphocytes,
neutrophils and eosinophils (markers of asthma severity) / ml BAL of WT and Cd103<sup>−/−</sup> mice on day 13 and 15. * = p < 0.05 compared to WT. C) Total CD103<sup>+</sup> DCs and D) total CD103<sup>+</sup> CD4 T cells isolated from lung homogenates in mice at day 13 and 15 after the last exposure to HDM. * = p < 0.05 compared to day 15.

**Figure 7. Comparison of migration and expression of markers between WT and Cd103<sup>−/−</sup> DCs.**

A) BMDDCs were obtained and *in vitro* migration was stimulated with CCL19, SDF-1 and CCL20. Results are expressed as % of migration. Migration capacity to CCL19 (I) SDF-1 (II) and CCL20 (III) was compared between WT and Cd103<sup>−/−</sup> DCs. B) *In vivo* DC trafficking was compared between both strains using OVA-AF647 and flow cytometry. Results are presented as % (I) and total number (II) of OVA-AF647<sup>+</sup> DCs in the lymph nodes. The expression of C) E-Cadherin and D) co-stimulatory molecule CD80 was evaluated on total lung DCs isolated from WT and Cd103<sup>−/−</sup> mice exposed or not to HDM. Results are expressed as % cells expressing E-Cadherin (C) and mean fluorescence intensity (MFI), to represent the quantity of protein expressed on each cell, for CD80 expression (D). Results are representative of at least three separate experiments; for C) and D), n = 4-6 mice per group.
**A**

WT naive  
Cd103-/- naive  
WT OVA  
Cd103-/- OVA

**B**

I

- macrophages
- lymphocytes
- neutrophils
- eosinophils

II

- macrophages
- lymphocytes
- neutrophils
- eosinophils

**C**

Wild type OVA  
Cd103-/- OVA  
Wild type saline  
Cd103-/- saline

* = p < 0.05 compared to Wild Type mice
A

Wild Type

Cd103^{-/-}

Naive

OVA

B

Score

0  2  4  6  8  10  12  14  16

Wild Type  Cd103^{-/-}

*
**A**

- **% IL-5 release**
  - OVA (μg/ml)
  - Wild Type
  - Cd103-/-

**B**

- **% IFN-gamma release**
  - OVA (μg/ml)
  - Wild Type
  - Cd103-/-

* denotes statistical significance.
A) 

Inflammatory Index

- **DAY 11**
- **DAY 13**
- **DAY 15**

Wild type

Cd103⁻/⁻

Wild type

Cd103⁻/⁻

B) 

- **Macrophages**
- **Lymphocytes**
- **Neutrophils**
- **Eosinophils**

Wild type

Cd103⁻/⁻

C) 

CD103+ DCs x10³

- **DAY 13**
- **DAY 15**

Saline

HDM

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CD103+ CD4 T cells x10³

- **DAY 13**
- **DAY 15**

Saline

HDM