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Strain-specific requirement for eosinophils in the recruitment of T cells to the lung during the development of allergic asthma

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Eosinophils have been implicated as playing a major role in allergic airway responses. However, the importance of these cells to the development of this disease has remained ambiguous despite many studies, partly because of lack of appropriate model systems. In this study, using transgenic murine models, we more clearly delineate a role for eosinophils in asthma. We report that, in contrast to results obtained on a BALB/c background, eosinophil-deficient C57BL/6 ΔdblGATA mice (eosinophil-null mice via the ΔDblGATA1 mutation) have reduced airway hyperresponsiveness, and cytokine production of interleukin (IL)-4, -5, and -13 in ovalbumin-induced allergic airway inflammation. This was caused by reduced T cell recruitment into the lung, as these mouse lungs had reduced expression of CCL7/MCP-3, CC11/eotaxin-1, and CCL24/eotaxin-2. Transferring eosinophils into these eosinophil-deficient mice and, more importantly, delivery of CCL11/eotaxin-1 into the lung during the development of this disease rescued lung T cell infiltration and airway inflammation when delivered together with allergen. These studies indicate that on the C57BL/6 background, eosinophils are integral to the development of airway allergic responses by modulating chemokine and/or cytokine production in the lung, leading to T cell recruitment.

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Eosinophilia of the lung and airways has been observed in concurrence with other symptoms in models of allergic asthma, as well as in humans, and has been regarded as a cardinal feature of asthmatic responses (1, 2). However, the importance of eosinophils to the generation of allergic asthma has remained ambiguous despite the quantity of research that has been performed on the subject. The cytokine IL-5 has been shown to be important for eosinophil development (1, 3), and levels are elevated during asthmatic responses; however, studies in IL-5–deficient mice, which have reduced numbers of eosinophils, have yielded inconsistent results. On a C57BL/6 background lacking IL-5, airway hyperresponsiveness (AHR) is abolished, whereas, on the BALB/c background, AHR is either affected or not; this depends on the model used, perhaps because of residual numbers of eosinophils that may remain in the lungs (4–8). These studies suggest that in addition to IL-5, other factors may be required for regulation of eosinophils, and that perhaps eotaxins, including eotaxin-1, a chemokine that attracts eosinophils to sites of inflammation, may need to be blocked in combination with IL-5 to counteract the function of eosinophils in the lungs (3, 9).

T cells, particularly IL-4–, IL-5–, and IL-13–producing Th2 cells, have been shown to be important in allergic asthma, as introducing antigen-specific Th2 cells followed by antigen challenge is sufficient to cause AHR (10, 11). The independent administration of Th2 cytokines IL-4, -5, or -13 can also induce AHR (11–14). Evidence from mouse models suggests that IL-13 is necessary for mucous hypersecretion and AHR, and has also been shown to aid in eosinophil induction by eotaxin-1– and IL-5–dependent mechanisms (14–16). The relationship among these three factors is still under investigation, but studies in double-transgenic

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mice lacking IL-5 and eotaxin-1 have shown a defect in T cell IL-13 production (17).

Most recently, two research groups published conflicting data on the importance of eosinophils to the development of this disease. Using a transgenic cell ablation approach on a C57BL/6 background, Lee et al. found that eosinophils are integral to the development of airway inflammation and AHR (18). In contrast, Humbles et al. used the eosinophil-null mice via the ΔDblGATA1 mutation (ΔdblGATA) mouse, which lacks eosinophils (4), on a BALB/c background and determined that the absence of eosinophils did not protect mice from AHR development in an acute model of allergic inflammation, but are required for extensive airway remodeling (19). It is possible that different backgrounds have dissimilar responses to those observed for other genes, such as IL-4 and -5, and the development of allergic asthma (20). In this study, we have performed a more detailed analysis of the ΔdblGATA mice on C57BL/6 and BALB/c backgrounds. Our results show that the hallmarks of allergic asthma, including T cell infiltration of the lungs, Th2 cytokine production, and chemokine production, are reduced in C57BL/6 ΔdblGATA mice. Also unique to our study, we reconstituted ΔdblGATA mice with eosinophils to determine whether the characteristics observed were, indeed, caused by these mice lacking eosinophils. We determined that eosinophils are required for T cell infiltration as well as cytokine production in the lungs during allergic airway responses in C57BL/6 mice. Finally, we show that intranasal (i.n.) delivery of CCL11/eotaxin-1 rescued T cell recruitment and the development of AHR in C57BL/6 ΔdblGATA mice.

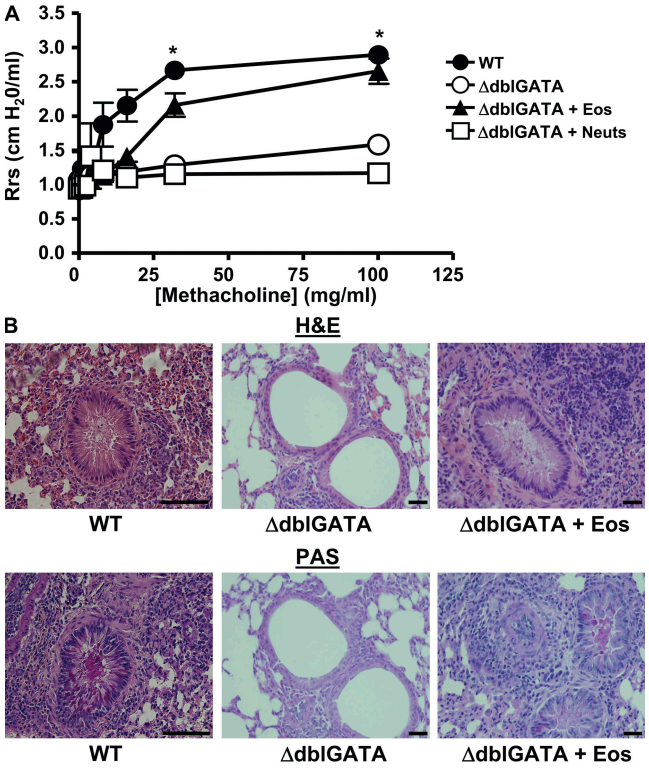

Figure 1. Eosinophils are required for the development of AHR, lung inflammation, and mucous production in response to allergic asthma induction in C57BL/6 ΔdblGATA mice. (A) C57BL/6 WT and ΔdblGATA mice were immunized and challenged i.n. with OVA. Some ΔdblGATA mice were given 1.5×10^6 eosinophils or neutrophils, followed by analysis of AHR by mechanical ventilation. *, $P < 0.05$ for WT versus ΔdblGATA alone ($n = 4$ mice/group, repeated 3 times). (B) Fixed and sectioned mouse lungs from the above groups were analyzed by HE or PAS stain. Bars: (WT) 50 µm; (all other panels) 20 µm.

RESULTS AND DISCUSSION

Eosinophils are required for the development of AHR and lung inflammation in C57BL/6, but not BALB/c, allergic asthma

We used OVA in a standard sensitization protocol in WT and ΔdblGATA mutant mice to induce allergic airway inflammation. 24 h after the last i.n. challenge, mice were subjected to mechanical ventilation for analysis of AHR. We found that WT mice on both C57BL/6 and BALB/c backgrounds, as well as BALB/c ΔdblGATA mice, showed an increase in AHR by mechanical ventilation, whereas C57BL/6 ΔdblGATA mice did not show a significant increase in this parameter by mechanical ventilation or whole body plethysmography (Fig. 1 A, Fig. 2 A, and Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20071836/DC1). Analysis of lung sections from these mice stained with hematoxylin and eosin (HE) showed that both WT mice and BALB/c ΔdblGATA mice exposed to OVA i.n. had elevated inflammation (Fig. 1 B and Fig. 2 B). In contrast, lungs from the C57BL/6 ΔdblGATA mice had significantly reduced inflammation (Fig. 1 B and Fig. S2 A). To determine if inflammation was accompanied by goblet cell mucous production in the airways, we analyzed similar sections stained with PAS. Again, as expected, there was mucous in the airways of WT and BALB/c ΔdblGATA lungs; however, there was little if any mucous detected in the airways of lungs from the C57BL/6 ΔdblGATA mice (Fig. 1 B, Fig. 2 B, and Fig. S2 B). These data indicate that on the C57BL/6, but not BALB/c, background, eosinophils are critical for the development of allergic airway inflammation, suggesting that different mouse strains have differing requirements for development of AHR.

Reduced $CD4^+$ T cell recruitment and Th2 cytokines in the lungs of C57BL/6 mice lacking eosinophils after airway challenge

$CD4^+$ T cells are recruited to the lung during chronic asthmatic responses, producing Th2 cytokines such as IL-4, -5, and -13 that perpetuate and exacerbate the pathology of this disease (11, 13, 14, 21). To determine if the underlying cause of the observed reduced AHR and pathology in the lungs of C57BL/6 ΔdblGATA mice was reduced recruitment of inflammatory cells to the lungs, we analyzed bronchoalveolar lavage fluid (BALF) from these mice for the presence of $CD4^+$ T cells. We found that C57BL/6 ΔdblGATA mice have significantly reduced numbers of $CD4^+$ T cells in the BALF (Fig. 3 A). This correlated with reduced expression of

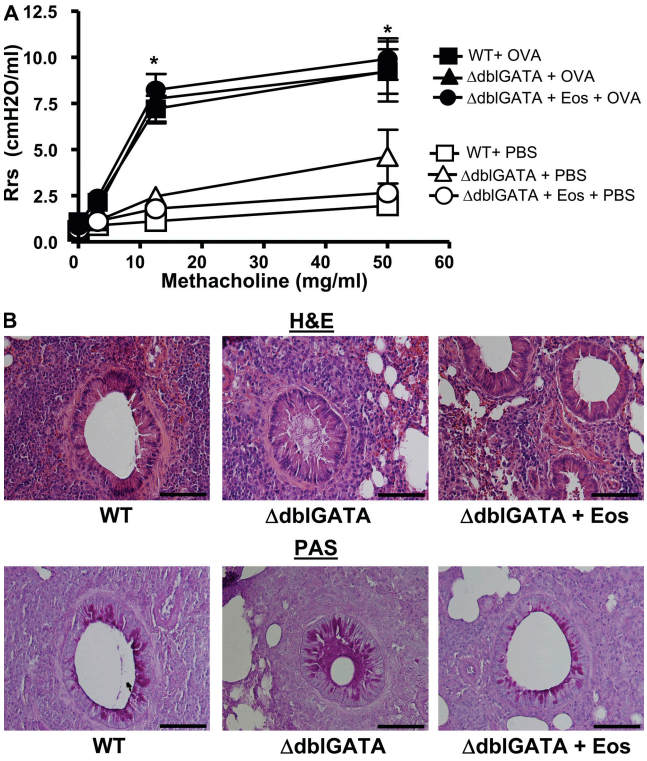

Figure 2. Eosinophils are not required for the development of AHR, lung inflammation, and mucous production during allergic asthma induction in BALB/c ΔdblGATA mice. (A) BALB/c WT and ΔdblGATA mice were treated as in Fig. 1 A, followed by analysis of AHR by mechanical ventilation (n = 7–8 mice/group for OVA-challenged mice; n = 4 Sham). *, $P < 0.02$ for WT and ΔdblGATA ± Eos versus PBS groups. (B) Mouse lungs from WT, ΔdblGATA, or ΔdblGATA + eosinophils treated as in Fig. 1 A, and analyzed by HE or PAS stain. Bars, 50 μm.

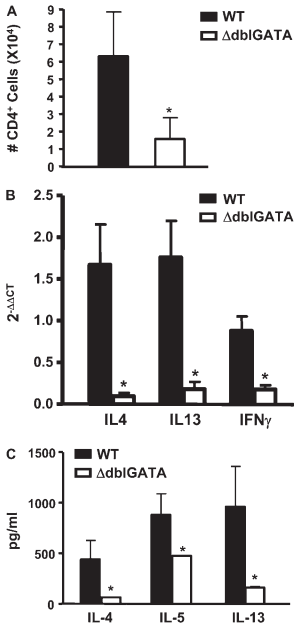

Figure 3. Eosinophils are required for the recruitment of CD4+ T cells to the lung in C57BL/6 ΔdblGATA in response to allergic airway inflammation. (A) Lungs from immunized and OVA-challenged C57BL/6 WT and ΔdblGATA mice (n = 3 mice/group, repeated 3 times) were analyzed for CD4+ T cells. (B) Lungs from WT and ΔdblGATA mice treated as in A were analyzed for mRNA for the indicated cytokines (n = 8 mice/group). (C) BALF from WT and ΔdblGATA mice treated as in A was analyzed for the indicated cytokines by ELISA (n = 3/group, repeated 3 times). *, $P < 0.05$ WT versus ΔdblGATA. Error bars are ± the SEM.

RNA for cytokines IL-4, IL-13, and IFN-γ in the lungs of C57BL/6 ΔdblGATA mice (Fig. 3 B). Protein levels of Th2 cytokines IL-4, -5, and -13 were also reduced in the BALF of C57BL/6 ΔdblGATA mice (Fig. 3 C). Thus, C57BL/6 ΔdblGATA mice are defective in recruitment of CD4+ T cells and the production of Th2 cytokines required for the induction of the disease.

Normal systemic immune responses in C57BL/6 ΔdblGATA mice upon OVA immunization and challenge

Reduction in Th2 cytokines in the lung could be the result of inefficient T cell activation or differentiation to Th2 cells. Because eosinophils have been suggested to serve as antigen-presenting cells under certain conditions (11, 17, 22, 23), we determined if splenic T cells from C57BL/6 ΔdblGATA mice were able to respond to OVA restimulation. We found that splenic T cells from both C57BL/6 WT and ΔdblGATA mice were able to respond to OVA stimulation similarly (Fig. S3 A, available at http://www.jem.org/cgi/content/full/jem.20071836/DC1). We also found that C57BL/6 ΔdblGATA mice were able to generate a Th2 response by class switching responding antibodies to the IgE isotype, as they generated similar levels of total IgE, as well as OVA-specific IgE, to WT mice (Fig. S3, B and C). Thus, T cell populations from these mice are capable of mounting an immune response, but are not able to migrate into the lungs to respond to OVA challenges.

Reduced expression of CCL7/MCP-3, CCL11/eotaxin-1, and CCL24/eotaxin-2 in lungs of C57BL/6 ΔdblGATA mice after airway challenge

One reason that T cells may not be able to migrate to the lungs during OVA challenge is reduced expression of chemokines critical for their migration into tissues (11). In particular, CCL7/MCP-3 and CCL11/eotaxin-1 have been shown to be important for recruitment of T cells into the lung during the development of allergic asthma (24–26). Eosinophils can induce proliferation and cytokine secretion from T cells (11, 17, 22, 23), as well as secrete T cell growth and chemotactic factors themselves, such as CCL11/eotaxin-1 and CCL24/eotaxin-2 (4). Analysis of lung RNA shows that C57BL/6 ΔdblGATA mice had a greatly reduced expression of these three chemokines (Fig. 4 A), which suggests that T cells are not recruited into the lungs of C57BL/6 ΔdblGATA mice because of a deficiency in chemokines able to aid migration of these cells to the lung.

Rescue of T cell recruitment and lung inflammation by transfer of eosinophils to C57BL/6 ΔdblGATA mice

To determine whether eosinophils are, indeed, required for recruitment of T cells and generation of allergic asthma symptoms on the C57BL/6 background, we performed i.v. transfers of 1.5×10^6 eosinophils, which were purified from the peritoneum of IL-5 transgenic mice, into OVA-immunized ΔdblGATA mice, followed by i.n. challenges with OVA. These mice were then analyzed for the development of AHR 24 h after the last challenge. We found that transfer of eosinophils into the C57BL/6 ΔdblGATA mice 6 h before the first i.n. challenge was able to rescue the development of AHR (Fig. 1 A), as well as lung inflammation and mucous production (Fig. 1 B). Transfer of eosinophils into C57BL/6 ΔdblGATA mice followed by challenge with PBS did not lead to AHR, the development of lung inflammation, or mucous production (unpublished data), indicating that eosinophil transfer in tandem with OVA challenge was required to rescue these responses. There were small numbers of T and B cells, as well as neutrophils, in our purified eosinophil population (unpublished data). We addressed this by transferring 1.5×10^6 neutrophils into C57BL/6 ΔdblGATA mice and challenging with OVA, which did not result in increases in AHR (Fig. 1 A), demonstrating that increased numbers of inflammatory cells are not sufficient to rescue allergic airway responses. In addition, we transferred IL-5 transgenic T cells into C57BL/6 ΔdblGATA mice. This did not result in increased airway inflammation or mucous production, indicating that rescue of lung airway inflammation is not caused by contaminating populations of T cells (Fig. S4, A and B, available at http://www.jem.org/cgi/content/full/jem.20071836/DC1).

Transfer of eosinophils, but not neutrophils or IL-5 transgenic T cells, followed by OVA challenge was also able to rescue the recruitment of T cells into the BALF and lungs of C57BL/6 ΔdblGATA mice (Fig. 4, B and C). In contrast,

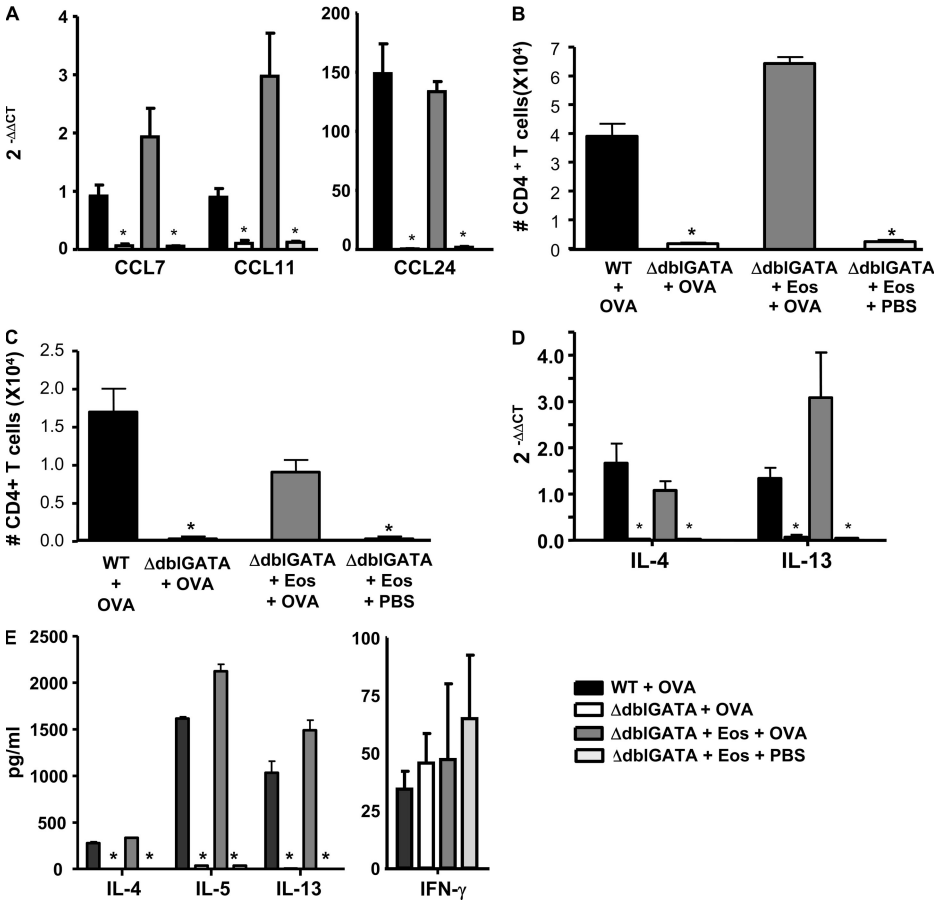

Figure 4. Eosinophils are required for expression of CCL7, CCL11, and CCL24, and CD4⁺ T cell recruitment in the lungs during allergic airway inflammation in C57BL/6 ΔdblGATA mice. (A) WT or ΔdblGATA mice were immunized. Some ΔdblGATA mice were given eosinophils, and then challenged with OVA or PBS; lungs were analyzed for mRNA for CCL7, CCL11, or CCL24 (n = 4–5 mice/group, repeated 2 times). *, $P < 0.05$ WT versus ΔdblGATA + OVA. (B) Lungs from WT or ΔdblGATA mice treated as in A were analyzed for CD4⁺ T cells. *, $P < 0.05$ WT versus ΔdblGATA + OVA (n = 3 mice/group, repeated 3X). (C) BALF from mice treated similarly as in A (n = 3 mice/group, repeated 3 times). (D) Lung cytokine mRNA analysis from mice treated similarly to those in A. *, $P < 0.05$ WT versus ΔdblGATA + OVA (n = 4–5 mice/group repeated 3X). (E) BALF from mice treated as in A analyzed for the indicated cytokines by ELISA. *, $P < 0.05$ WT versus ΔdblGATA + OVA (n = 4, repeated 3 times). Error bars are ± the SEM.

C57BL/6 ΔdblGATA mice that received eosinophils but were challenged with PBS had significantly fewer $CD4^+$ T cells than those challenged with OVA (Fig. 4, B and C). Transfer of eosinophils into C57BL/6 ΔdblGATA mice followed by OVA challenge resulted in the appearance of eosinophils in the lungs of C57BL/6 ΔdblGATA mice (unpublished data), and also rescued the expression of RNA for cytokines IL-4, -5, and -13. These mice actually displayed higher levels of IL-13 than WT mice challenged with OVA (Fig. 4 D).

Analysis of cytokine protein levels in the BALF of C57BL/6 ΔdblGATA mice showed that when these mice received eosinophils and were challenged with OVA, they produced levels of IL-4, -5, and -13 similar to those of WT mice (Fig. 4 E). C57BL/6 ΔdblGATA mice transferred with eosinophils and challenged with PBS had low levels of these cytokines, comparable to C57BL/6 ΔdblGATA mice challenged with OVA (Fig. 4 E). Eosinophil transfer-mediated rescue of $CD4^+$ T cell recruitment into the lung was also accompanied by rescue of CCL7/MCP-3, CCL11/eotaxin-1, and CCL24/eotaxin 2 expression (Fig. 4 A). WT C57BL/6 lung CCL17 levels were significantly higher than ΔdblGATA, but expression of this chemokine was not rescued by eosinophil transfer into these mice, indicating that eosinophils may not directly regulate CCL17 (Fig. S5 A, available at http://www.jem.org/cgi/content/full/jem.20071836/DC1), and CCL22 levels were equivalent in all groups (Fig. S5 A). Indeed, $CD4^+$ T cells that were recruited to the lungs of WT mice expressed as CCR3 (Fig. S5 B). These results suggest that eosinophils may modulate the expression of CCL11/24-eotaxin1/2, which are needed for recruitment of T cells into the lung during allergic airway inflammation. Our data imply that eosinophils may be required for low levels of secretion of CCL11/24-eotaxin1/2-Th2 cytokines in the lung, which could induce T cell migration and secretion of effector cytokines by these cells that can further amplify the recruitment of eosinophils and T cells into the lung in a feed-forward mechanism.

i.n. delivery of CCL11/eotaxin-1 rescues $CD4^+$ T cell recruitment to the lung and the development of AHR in C57BL/6 ΔdblGATA mice

Our aforementioned experiments revealed significantly reduced expression of the chemokine CCL11/eotaxin-1 in the lungs of OVA-challenged C57BL/6 ΔdblGATA mice. The reduction of this chemokine suggests a possible mechanism for the lower responses in these mice. To test if CCL11/eotaxin-1 is able to rescue T cell recruitment and the development of AHR, we delivered this chemokine to the lungs of C57BL/6 ΔdblGATA mice previously immunized with OVA over the 4 d of i.n. challenge, along with OVA. We found that CCL11/eotaxin-1 delivered with OVA was sufficient to induce AHR in C57BL/6 ΔdblGATA mice (Fig. 5 A). CCL11/eotaxin-1 delivery with CCL11/eotaxin-1 blocking antibody did not induce AHR in C57BL/6 ΔdblGATA mice challenged with OVA, indicating that the rescue was specific to this chemokine (Fig. S5 D). Analysis of the lungs of the C57BL/6 ΔdblGATA mice given CCL11/eotaxin-1 and OVA i.n. showed that this treatment also rescued T cell migration into the lungs, which did not occur in mice given i.n. CCL11/eotaxin-1 in combination with CCL-11/eotaxin-1 blocking antibody (Fig. 5 B). As expected, OVA plus i.n. CCL11/eotaxin-1 did not recruit eosinophils in the C57BL/6 ΔdblGATA mice because these mice lack these cells (Fig. S5 C). The chemokine receptor CCR3 most likely mediates this migration because this is the only receptor that has been reported to interact with these chemokines. Our data suggest that, in the absence of eosinophils, exposing mice to an allergic airway challenge results in the lack of production of appropriate chemokines; particularly eotaxins, which allow recruitment of T cells into the lung and contribute to the pathology of the disease.

In this investigation, we have provided evidence that eosinophils are required for the development of allergic airway responses and recruitment of T cells into the lungs after allergen challenge in C57BL/6 ΔdblGATA mice. These results add weight to other studies that have shown abrogation of the symptoms of allergic asthma in the absence or suppression of eosinophil function (4, 8). Lee et al. have provided evidence

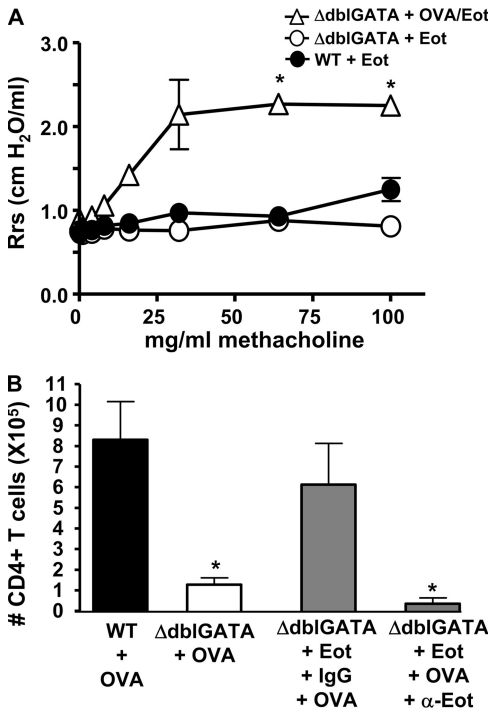

Figure 5. CCL11/eotaxin-1 can rescue AHR and T cell recruitment to the lung in C57BL/6 ΔdblGATA mice. (A) Immunized ΔdblGATA mice were given CCL11/eotaxin-1 during challenge with OVA. Alternatively, immunized WT C57BL/6 or ΔdblGATA mice were just given CCL11/eotaxin. This was followed by AHR analysis by mechanical ventilation ($n = 4$, repeated 2 times). *, $P < 0.05$ for ΔdblGATA + Eot/OVA versus WT or ΔdblGATA + Eot alone. (B) Lungs from immunized and i.n. OVA-challenged WT and ΔdblGATA mice or ΔdblGATA mice delivered eotaxin-1 with OVA, were analyzed for $CD4^+$ T cells. Some mice challenged with eotaxin/OVA also received anti-CCL11 blocking antibody i.n. ($n = 4$ mice/group, repeated 2 times). *, $P < 0.05$ WT versus ΔdblGATA + OVA. Error bars are ± the SEM.

in a transgenic eosinophil ablation approach in C57BL/6 mice that eosinophils are required to develop AHR and airway inflammation, as well as mucus secretion (18). This is in contrast to data provided by Humbles et al. in ΔdblGATA mutant BALB/c mice, suggesting that eosinophils are required only for airway remodeling (19). Our data in the ΔdblGATA mutant mice on the BALB/c and C57BL/6 backgrounds now indicate that the discrepancies observed between these two groups were most likely caused by strain differences in C57BL/6 and BALB/c mice. Indeed, our studies find that systemic Th2 responses are intact in the ΔdblGATA mutant mice on the C57BL/6 background, similar to that observed by Humbles et al. (19). The main difference we observe is the requirement for eosinophils in the acute model of allergic airway inflammation, and the recruitment of T cells into the lung. Indeed, we observed a reduction in recruitment of the number of T cells into the lungs of both C57BL/6 and BALB/c ΔdblGATA mice (Fig. 4 C and Fig. S6 B, available at http://www.jem.org/cgi/content/full/jem.20071836/DC1). However, although recruitment of T cells into the BALB/c ΔdblGATA mouse lungs was reduced compared with WT, the number of T cells in these lungs was significantly greater than controls, and the percentage of $CD4^+$ T cells was similar to WT (Fig. S6, A and B). Data from Voehringer et al. support this, showing in the Th2 *Nippostrongylus* model that T cell recruitment to BALB/c ΔdblGATA lungs is intact (27). Also, reduced T cell numbers did not prevent BALB/c ΔdblGATA from producing the Th2 cytokines IL-4 and -13 (Fig. S6, C and D). Although this is in agreement with Voehringer et al., Fulkerson et al. reported a reduction in IL-4 and -13 production in the lung in an *Aspergillus* model of asthma (28). Whether these differences are caused by the BALB/c strain having lower chemokine/cytokine requirements for recruitment of inflammatory cells in the airways and subsequent production of Th2 cytokines remains to be seen.

In contrast to the aforementioned research groups, our data also address the influence that eosinophils have over $CD4^+$ T cells in allergic asthma, suggesting that eosinophils are not just terminal effector cells, but are actively involved in the adaptive immune response by assisting in the recruitment of T cells to the lungs; this supports data that propose eosinophils can modulate the function of T cells in the allergic lung. Eosinophils resident in the lung during allergic responses are able to present antigen and traffic to local lymph nodes, where they colocalize with T cells; they can also induce proliferation and cytokine secretion from T cells (4, 11, 22, 23). In allergic mice deficient in eotaxin-1 and IL-5, there is reduced T cell production of cytokine IL-13, although these cells have normal cytokine production in general (17). Accompanying this, transfer of T cells defective in IL-13 production into eotaxin-1/IL-5 double knockout mice does not induce AHR, whereas transferring in vitro–differentiated, IL-13–producing T cells can overcome defects in eotaxin-1/IL-5 double knockout mice and induce asthma, suggesting that eosinophils may be linked to induction of IL-13 production in T cells during allergic airway responses (17). This latter study did not investigate whether $CD4^+$ T cells were actually recruited to the lungs in the eotaxin-1/IL-5 double knockout mice, and only determined that the levels of Th2-type cytokines in the lung were reduced. Together with these data, our findings indicate that instead of IL-13 production by T cells causing up-regulation of eotaxin that selectively recruits eosinophils to the lungs, as has been previously suggested (21), eosinophils are required to provide a stimulus, perhaps CCL11/24-eotaxin-1/2, for T cell migration and secretion of cytokines in the lungs.

While this work was under review, Jacobsen et al. reported similar findings in C57BL/6 PHIL mice, suggesting that T cell recruitment to the lung during generation of allergic airway responses is via an eosinophil-dependent mechanism (29). However, there were some differences between that study and our findings. Jacobsen et al. report that eosinophil transfer into primed and OVA i.n. challenged PHIL mice is not able to rescue recruitment of T cells to the lungs or Th2 cytokine production. This work is in direct contrast to data presented here in that eosinophil transfer to sensitized and i.n. OVA-challenged ΔdblGATA mice promotes T cell recruitment, as well as subsequent Th2 cytokine production in the lung. Jacobsen et al. attribute this defect in the PHIL mice to an inability to generate Th2 cells in the absence of eosinophils; however, they do not address whether other Th2 responses, such as induction of B cells to class switch for IgE production, are preserved as they are in the ΔdblGATA mice, nor do they attempt to transfer eosinophils during the initial OVA sensitization process to determine whether this could affect the generation of Th2 cells. This potential inability for PHIL mice to effectively generate a Th2 T cell response is an interesting finding that warrants further study of T cell responses in these mice to determine whether this observation is, indeed, eosinophil dependent or whether it indicates an underlying defect in antigen-specific T cell responses. Lastly, the mechanism by which T cells are recruited to the lung appears to differ in these two models of allergic airway responses. Although we find that eosinophils do not contribute to lung RNA levels of MDC or TARC in C57BL/6 ΔdblGATA mice, Jacobsen et al. show that PHIL mice have a defect in the production of these chemokines in the lung that can be rescued with transfer of in vitro–differentiated OT-II T cells and eosinophils to these mice (29). Conversely, Jacobsen et al. (29) found similar BAL levels of eotaxin-1 and 2 in PHIL and WT mice, whereas our data suggest that there is reduced production of these chemokines in the lungs of ΔdblGATA mice. In addition, we show that administration of eotaxin-1 with OVA to the lungs of ΔdblGATA mice rescues AHR and T cell recruitment to the lung. The reason for the discrepancy between the two mechanisms is not clear, but may perhaps be caused by the differing nature of the defects in these two mouse models. Regardless, data from both groups support the central hypothesis that eosinophils are required for the recruitment of T cells to the lung, and thus are not only terminal effector cells but also important modulators of allergic asthma.

MATERIALS AND METHODS

Mice. WT C57BL/6, BALB/c, ΔdblGATA on both backgrounds (19), and IL-5 transgenic mice on both backgrounds (a gift from J. Lee and N. Lee, The Mayo Clinic, Scottsdale, Arizona) (30) were used for these experiments. All experiments were approved by the Office of Research Protection's Institutional Animal Care and Use Committee at Pennsylvania State University.

OVA-induced allergic asthma model. Groups of mice (WT or ΔdblGATA) were immunized i.p. on day 0 and 5 with 50 μg/ml OVA (Sigma-Aldrich) complexed with aluminum hydroxide (10 μg OVA/1 mg alum; Thermo Fisher Scientific). Mice were exposed daily i.n., with 30 μl OVA (2 mg/ml) on day 12–15, and killed 24 h later for analysis. In experiments where ΔdblGATA mice received eosinophil transfers, ΔdblGATA mice received 1.5×10^6 eosinophils i.v. on day 12, and were then challenged with OVA 6 h later. In some experiments, ΔdblGATA or WT mice received 0.75 μg CCL11/eotaxin-1 combined with the normal dose of 30 μl (2 mg/ml) OVA on days 12–15. WT and ΔdblGATA mice primed with OVA/alum and i.n. challenged with PBS were used as controls in these experiments.

Determination of AHR and analysis of airway inflammation. AHR was determined using a custom-made mechanical ventilator (31) or a Flexivent mechanical ventilator (SciReq). Mice were anesthetized, a cannula was placed in the trachea, and mice were ventilated at 120 breaths/min, $V_T = 0.2$ ml, flow rate 1.5 ml/s at 2–3 cm H_2O PEEP. Airway pressure in response to methacholine was determined using a differential pressure transducer. Fixed and sectioned lungs were stained with HE or PAS to detect mucous (performed by the Animal Diagnostic Laboratories, Penn State University).

Adoptive transfer of eosinophils. Peritoneal eosinophils were obtained from IL-5 transgenic mice by peritoneal lavage with RPMI media, sorted by MACS negative bead selection, and washed 2 times in 1XPBS, and then 1.5×10^6 cells were resuspended in 100 μl 1XPBS and injected i.v. into ΔdblGATA mice. Typical purity was 85–90% as determined by CCR3 antibody (R&D Systems) positive flow cytometric analysis.

Adoptive transfer of neutrophils. Peripheral blood neutrophils were purified by Histopaque gradient centrifugation (1.119, 1.083, and 1.077). Cells from the 1.119/1.083 interface were harvested and washed three times. On the first day of i.n. challenge, 1.5×10^6 cells were transferred i.v. into immunized ΔdblGATA mice as outlined in the previous section.

Determination of T cell recruitment into the lungs and BAL. BAL was collected from lungs of mice in PBS. In other mice, whole lungs were dissociated using collagenase (Roche), and isolated cells from BAL or lungs were either analyzed on an Advia Blood Analyzer or stained with monoclonal antibodies to identify $CD4^+$ T cells (eBioscience), and then analyzed by flow cytometry.

Quantitative RT-PCR analysis of gene expression. RNA was isolated from lung tissue, and total RNA (1 μg) was reverse transcribed to cDNA. PCR was performed in triplicate with commercially available primers and probes as per manufacturer protocol (Applied Biosystems).

Analysis of cytokine levels. BAL or supernatants from T cell cultures were analyzed for levels of IL-4, -5, and -13 by a Luminex multiplex bead system kit (Lincoplex) on a Bioplex system (Bio-Rad Laboratories).

Data analysis. Statistical evaluation was conducted for all repetitions of each experiment using Student's *t* test with a probability value $P \leq 0.05$ considered statistically significant.

Online supplemental material. Fig. S1 demonstrates that ΔdblGATA mice have reduced lung function as assessed by plethysmograph. Fig. S2 shows the quantitative analysis of the reduced lung pathology observed in ΔdblGATA mice compared with WT mice. Fig. S3 demonstrates that ΔdblGATA mice have no defect in in vitro splenocyte proliferation or IgE production in vivo. Fig. S4 shows that transfer of IL-5 transgenic T cells to ΔdblGATA mice does not induce lung airway inflammation and T cell recruitment to the lung. Fig. S5 shows that levels of chemokine CCL17/CCL22 message in the lungs of ΔdblGATA and WT mice are not statistically different, that the $CD4^+$ T cells recruited into the lungs express CCR3, and that ΔdblGATA mice administered anti-CCL11/-CCL11/-OVA have reduced AHR compared with ΔdblGATA that receive CCL11/OVA alone. Fig. S6 shows that $CD4^+$ T cell recruitment to the lung and Th2 cytokine production are intact in BALB/c WT and ΔdblGATA mice. The online version of this article is available at http://www.jem.org/cgi/content/full/jem.20071836/DC1.

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