

The IL-3/IL-5/GM-CSF Common β Receptor Plays a Pivotal Role in the Regulation of Th2 Immunity and Allergic Airway Inflammation¹

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The eosinophil is a central effector cell in allergic asthma. Differentiation and function of eosinophils are regulated by the CD4 Th2 cytokines IL-3, IL-5, and GM-CSF, which all signal through a common β receptor subunit (β_c). Recent therapeutic approaches targeting IL-5 alone have not ablated tissue accumulation of eosinophils and have had limited effects on disease progression, suggesting important roles for IL-3 and GM-CSF. By using a mouse model of allergic airways inflammation, we show that allergen-induced expansion and accumulation of eosinophils in the lung are abolished in β_c -deficient ($\beta_c^{-/-}$) mice. Moreover, β_c deficiency resulted in inhibition of hallmark features of asthma, including airways hypersensitivity, mucus hypersecretion, and production of Ag-specific IgE. Surprisingly, we also identified a critical role for this receptor in regulating type 2 immunity. Th2 cells in the lung of allergen-challenged $\beta_c^{-/-}$ mice were limited in their ability to proliferate, produce cytokines, and migrate to effector sites, which was attributed to reduced numbers of myeloid dendritic cells in the lung compartment. Thus, the β_c plays a critical role in allergen-induced eosinophil expansion and infiltration and is pivotal in regulating molecules that promote both early and late phases of allergic inflammation, representing a novel target for therapy. *The Journal of Immunology*, 2008, 180: 1199–1206.

Allergic asthma is a chronic inflammatory disease of the airways that has increased in prevalence in developed countries over recent decades (1) and has been described as an epidemic in the New England Journal of Medicine (2). Inflammatory infiltrates in the asthmatic airways are complex, and a range of cells has been associated with disease progression. These include basophils, eosinophils, effector T lymphocytes, mast cells, macrophages, neutrophils, and, more recently, T regulatory and NK-T cells (3). Of the inflammatory cells implicated in asthma, IgE-mediated degranulation of mast cells contributes to inflammatory infiltrates and acute bronchoconstriction in the early phase of allergic inflammation, whereas recruitment of CD4⁺ Th2 cells and eosinophils is a central feature of the late-phase response (4). Th2 cytokines (e.g., IL-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF) and eosinophils are thought to play critical roles in the induction of airway hyperreactivity (AHR)³ and the development of lesions that underpin chronic airway wall remodeling (5).

Indeed, the importance of eosinophils in chronic allergic disease has been elegantly highlighted both clinically and in mouse models of asthma. Reduction in tissue levels of eosinophils (~50%) by anti-IL-5 mAb treatment in mild atopic asthmatic patients results in significantly attenuated expression of tenascin, lumican, and procollagen III in the bronchial mucosa, suggesting that eosinophils may contribute to tissue remodeling processes in chronic asthma by controlling extracellular matrix protein deposition (6, 7). These studies are supported by the temporal association between eosinophils and markers of chronic remodeling in human atopic skin, which are inhibited by i.v. infusion with anti-IL-5 mAb (8, 9). Genetic ablation of the eosinophil lineage in mice is also protective against tissue remodeling in a model of chronic asthma (10). Collectively, these studies suggest that strategies targeting eosinophils may also be effective for the resolution of at least some of the chronic manifestations of allergic asthma.

Although the above studies have identified the eosinophil as an important therapeutic target, previous attempts to inhibit accumulation of eosinophils in the airway wall of asthmatics by targeting the eosinophil-specific regulatory cytokine IL-5 have only had limited success. Notably, anti-IL-5 mAb only partially depletes tissue accumulation of eosinophils, suggesting that other molecules contribute to the regulation of this process (7, 11–13). Furthermore, we have shown that eosinophils and lesions persist in lung tissue during allergic inflammation in IL-5-deficient BALB/c mice, albeit significantly reduced (14, 15). Importantly, the elimination of eosinophils by generation of mice deficient in IL-5 and eotaxin or by genetic lineage ablation is protective against AHR or chronic tissue remodeling, respectively (10, 16). Thus, the appreciation of IL-5-independent pathways of eosinophil migration and function is

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³ Abbreviations used in this paper: AHR, airway hyperreactivity; AM, alveolar macrophage; β_c , common β receptor chain; β_{IL-3} , IL-3 β receptor chain; BALF, bron-

choalveolar lavage fluid; DC, dendritic cell; mDC, myeloid DC; MSC, mucus-secreting cell; PBLN, peribronchial lymph node; pDC, plasmacytoid DC; WT, wild type.

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of paramount importance if more complete and sustained depletion of eosinophils from the bronchial mucosa of asthmatics is to be achieved.

IL-3 and GM-CSF, which together with IL-5 are the only known mediators capable of inducing eosinophil differentiation from bone marrow progenitors, are central to the survival, migration, and activation of this granulocyte (17–19). IL-3 and GM-CSF, produced by activated T cells as well as in copious amounts by lung epithelial cells in the case of GM-CSF, share a common β receptor chain (β c) with IL-5. Indeed, signaling redundancy between these cytokines may be the critical pathway that promotes the development and recruitment of eosinophils independently of IL-5 at sites of allergic inflammation. Interestingly, limited suppression of pulmonary eosinophil numbers and AHR has been documented in a rat model of allergic asthma following partial (~60%) attenuation of the β c using antisense oligonucleotides (20). However, the role of the β c in allergic inflammation has not been unequivocally determined by genetic deletion or direct antagonism.

Signaling through the β c by IL-3 and GM-CSF is also important in the development and function of other immune cells that play central roles in allergic inflammation. This includes dendritic cells (DC) (21), which play an integral role in the immune response to inhaled allergens (22). Importantly, IL-3 is also a critical molecular switch for the differentiation, survival, and effector function of mast cells and basophils, whereas GM-CSF is a strong activator of neutrophil and macrophage function (23–25). Thus, the central role of the β c in regulating the function of a range of immune effector cells suggests that this molecule may be a pivotal regulator of allergic inflammation.

In this investigation, we determined the role of the β c in orchestrating inflammation and its potential as a therapeutic target in a mouse model of allergic asthma. Unlike human cells, mouse cells possess an additional β receptor specific for IL-3 (β_{IL-3}), which allows IL-3 signaling independently of the β c (26). To account for this, we used mice deficient in both β c and β_{IL-3} (designated as β c^{-/-}) to mimic the signaling conditions of human cells. Mice deficient in β c or in both β c and β_{IL-3} possess markedly reduced basal bone marrow and peripheral blood eosinophil numbers and fail to mount an eosinophil response to parasite infestation (27, 28). In the present study, we have used β c^{-/-} mice in an acute model of allergic airways inflammation, to show that this receptor chain is not only essential for eosinophil accumulation in tissues, but also plays a critical role in the development of hallmark features of asthma, including production of Ag-specific IgE, mucus hypersecretion, and AHR, by limiting the functional capacity of Th2 cells in the lung. Thus, β c plays a novel role in allergic disease by regulating multiple effector arms associated with the allergic inflammatory response.

Materials and Methods

Mice

Mice deficient in both β c and β_{IL-3} (29) on an SV129 background were supplied by L. Robb (Melbourne, Australia). These mice were backcrossed for >10 generations onto the BALB/c strain (genotyping performed by PCR). Mice deficient in both β c/ β_{IL-3} chain are referred to as β c^{-/-} throughout this work. Wild-type (WT) BALB/c and β c^{-/-} deficient mice were housed under specific pathogen-free conditions and experiments subject to approval by the Institutional Animal Care and Ethics Committee.

Induction of allergic airways disease

Mice (6–8 wk of age) were sensitized by i.p. injection of 50 μ g of OVA combined with 1 mg of Alhydrogel (CSL) in 0.9% sterile saline (termed allergic mice). Control mice (nonallergic) received 1 mg of Alhydrogel in 0.9% saline. On days 12, 13, 14, and 15, all groups were challenged by intranasal instillation of 10 μ g of OVA in 0.9% saline. AHR was measured 24 h after the final OVA challenge (unless otherwise stated); mice were

then sacrificed by sodium pentobarbital overdose; and immunological parameters were assessed. In experiments addressing the time course of eosinophil infiltration, mice were sacrificed on days 1, 2, 3, 7, and 14 after the final OVA challenge.

Inflammatory cells in blood and bronchoalveolar lavage fluid (BALF)

Blood was collected by cardiac puncture, and smears were stained with May-Grunwald Giemsa. Differential leukocyte counts were performed based on morphological criteria (a minimum of 200 cells counted/slide), as previously described (15). The remaining blood was centrifuged (10,000 $\times$ g for 10 min), and serum was stored at -70°C until analysis. BALF was obtained by cannulation of the trachea and flushing the airways with two 1-ml vol of HBSS. Recovered cells were treated with erythrocyte lysis buffer, and differential leukocyte counts were performed on cytospins (30).

Eosinophils and mucus-secreting cells (MSC) in lung tissue

Lung tissue representing the central (bronchi-bronchiole) and peripheral (alveoli) airways was fixed in 10% phosphate-buffered formalin, sectioned, and stained with carbol chromotrope-hematoxylin for identification of eosinophils or Alcian blue/periodic acid-Schiff for enumeration of MSC (15). The mean number of eosinophils or MSC per high-powered field ($\times$ 100) within 100 μ m basement membrane was determined following assessment of a minimum of 10 fields.

Measurement of AHR

AHR to inhaled β -methacholine (determined by alterations in resistance, R_L) was measured, as described previously (31). Responses to methacholine are expressed as the percentage change over saline control (baseline).

Determination of Ag-specific serum Ig

OVA-specific IgG1 and IgG2a were semiquantified by ELISA using reagents from BD Pharmingen. Briefly, plates were coated with either OVA (2 μ g/well) for sample wells or unlabeled anti-IgG of corresponding isotype for standard wells. After blocking, wells were incubated with serum or standards (mouse IgG1 or IgG2a), followed by detection with streptavidin-HRP anti-IgG1 or anti-IgG2a. Plates were developed with tetramethylbenzidine substrate; the reaction was stopped with 0.3 M H₂SO₄; and OD was determined at 450 nm using a Bio-Rad 680 Microplate reader. Relative levels of OVA-specific IgE were determined by ELISA. Plates were coated with unlabeled anti-IgE (BD Pharmingen) and blocked with 10% FCS. After incubation with serum samples, OVA-specific IgE was detected using biotinylated OVA (Pierce Biosciences; labeling performed according to manufacturer's instructions), followed by streptavidin-HRP. Plates were developed as above, and ODs were used to calculate ELISA units relative to standardized positive and negative control serum.

T cell proliferation and cytokine production

Peribronchial lymph node (PBLN) and spleens were dispersed through 70- μ m nylon mesh, treated with erythrocyte lysis buffer, and cultured at 37°C/5% CO₂ in 96-well plates at 1×10^6 cells/well in animal cell culture medium (0.1 mM sodium pyruvate, 2 mM L-glutamine, 20 mM HEPES, 100 U/ml penicillin/streptomycin, 50 μ M 2-ME, and 10% FBS in RPMI 1640) with 200 μ g/ml OVA. Unstimulated wells contained cells and medium only. Ag-specific proliferation was measured after 72 h using the Cell-Titer 96 kit (Promega), following the manufacturer's instructions, and calculated as percentage of proliferation in the OVA-treated compared with unstimulated cells from the same cell preparation. For cytokine analysis, cultures were incubated for 6 days and supernatants were stored at -70°C until analysis. IL-5, IL-4, IFN- γ (BD Pharmingen), and IL-13 (R&D Systems) concentrations were determined in supernatants by ELISA, according to the supplier's recommendations.

Lymphocyte and DC profiling by flow cytometry

The phenotype of lymphocytes and DCs was determined by flow cytometry. PBLN cells were prepared, as described above. Lungs were homogenized by mechanical maceration, followed by 30-min digestion in 1 mg/ml collagenase at 37°C. Tissue was filtered through nylon mesh (70 μ m) and treated with erythrocyte lysis buffer. PBLN and lung cells were plated at 1×10^6 cells/well into 96-well plates and, following 20-min incubation with Fc blocking Ab, stained with fluorochrome-conjugated Abs (BD Pharmingen) for analysis of lymphocytes (CD3, CD4, CD8, B220, and CD69) and DCs (CD11c, CD11b, GR-1, PDCA-1, MHC II, CD80, and CD86). Labeled cells were fixed in 1% paraformaldehyde and analyzed by FACS.

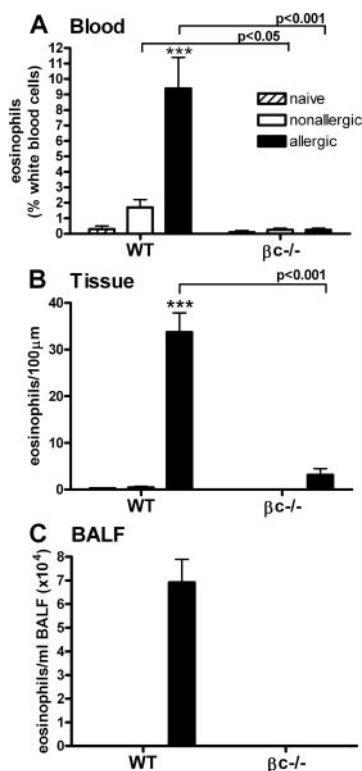


FIGURE 1. βc deficiency precludes the expansion and lung infiltration of eosinophils following Ag provocation. Groups of WT and $\beta c^{-/-}$ mice were untreated (naive), exposed to saline (nonallergic) or sensitized with OVA (allergic), and challenged intranasally with OVA. Mice were sampled 24 h after final challenge. *A*, Blood eosinophil numbers. *B*, Lung tissue eosinophil numbers within 100 μm basement membrane. *C*, BALF eosinophil numbers. Data represent the mean $\pm$ SEM for a minimum of six mice per group. ***, $p < 0.001$ compared with naive and nonallergic mice of same genotype. Significant differences between groups are indicated.

Statistical analysis

The significance of differences between experimental groups was analyzed using Student's unpaired t test. Values are reported as mean $\pm$ SEM. Differences in means were considered significant if $p \leq 0.05$.

Results

Elimination of signaling through the βc induces sustained suppression of allergen-induced eosinophils

To determine whether blockade of signaling by βc could prevent eosinophilia, eosinophil recruitment into the lungs, and induction of allergic disease, we compared immune and functional responses in allergic (sensitized) and nonallergic (control) WT and $\beta c^{-/-}$ mice. Eosinophil numbers rapidly increased in the blood, pulmonary tissue, and BALF of aeroallergen (OVA)-challenged WT mice (Fig. 1). By contrast, numbers of eosinophils in the blood of $\beta c^{-/-}$ mice were reduced to levels comparable to those observed in WT nonallergic mice. Eosinophil levels in the lung tissue of allergic $\beta c^{-/-}$ mice were also profoundly reduced compared with the WT (by 10.6-fold), and this granulocyte was entirely absent from the BALF.

In a previous study, βc -deficient mice displayed an attenuated and delayed eosinophil response to parasite infestation compared with WT controls (27). These mice were deficient only in the βc , and were capable of residual IL-3 signaling using the intact β_{IL-3} . To determine whether eosinophil expansion and mobilization were delayed in our βc and β_{IL-3} double-deficient mice, we characterized eosinophil expansion and migration over a time course of 2

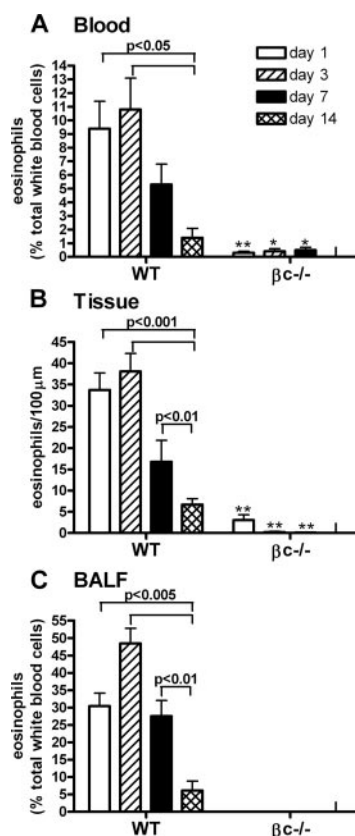


FIGURE 2. Suppression of circulating and pulmonary eosinophil numbers in $\beta c^{-/-}$ mice is sustained over time. OVA-sensitized and -challenged mice were sampled at various time points after the final challenge, and eosinophil numbers were determined in blood (*A*), lung tissue (*B*), and BALF (*C*). Eosinophil numbers remained at baseline levels in all compartments in nonallergic mice (data not shown). Data represent the mean $\pm$ SEM for a minimum of five mice per group. *, $p < 0.05$ and **, $p < 0.001$ compared with the corresponding time point in the WT group. Significant differences within groups are indicated.

wk. By contrast to the parasite study, eosinophil numbers in the peripheral blood, airway lumen, and lung tissue remained at baseline levels for the entire period of 2 wk after the final OVA challenge, at which time eosinophil levels in allergic WT mice had effectively returned to baseline levels (Fig. 2). These data demonstrate the pivotal role that βc plays in the regulation of eosinophil biology and indicate that signaling redundancy between the three eosinophilopoietic cytokines may account for the IL-5-independent pathways that regulate eosinophilic inflammation during allergic airways disease.

Elimination of signaling through the βc attenuates critical features of allergic airways disease

Next, we determined whether other critical pathophysiological features of allergic airways inflammation (mucus hypersecretion, AHR, and Ag-specific Ab production) were attenuated in the absence of βc and eosinophils. Although significantly reduced, mucus hypersecretion was still a feature in the lung of allergic $\beta c^{-/-}$ mice by comparison with allergic WT mice (Fig. 3*A*). However, whereas the pattern of expression of MSC in WT lungs commonly presented as a high number of cells within a single highly inflamed airway, causing visible obstruction of the lumen by mucus, histological examination of allergic $\beta c^{-/-}$ mice revealed that the MSC present were disseminated throughout airway tissue, and there is

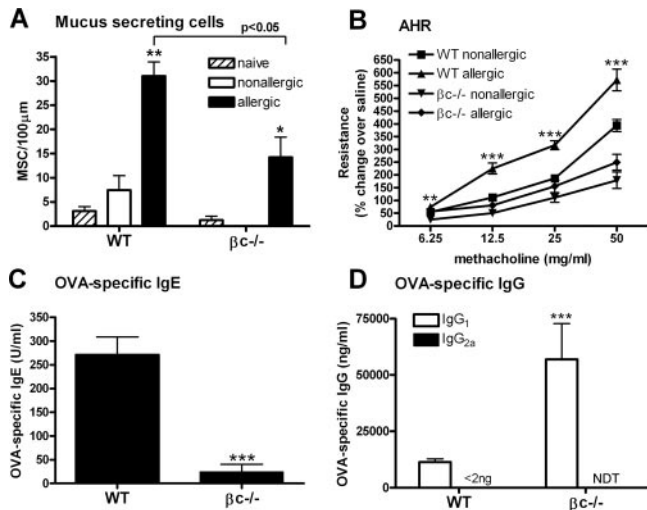


FIGURE 3. Suppression of pathophysiological features of allergic airways disease in the absence of the β c. WT and β c^{-/-} mice were exposed to saline (nonallergic) or sensitized with OVA (allergic) and challenged intranasally with OVA. **A**, Numbers of MSC in the bronchial epithelium within 100 μ m basement membrane. Asterisks indicate significant differences compared with naive and nonallergic mice of same strain. **B**, AHR. Airway resistance is expressed as a percentage of the baseline reactivity to saline in the absence of cholinergic stimuli. Asterisks indicate significant differences compared with the same methacholine dose in the nonallergic group of the same genotype. **C**, Ag-specific IgE in serum from allergic mice. **D**, Ag-specific IgG1 and IgG2a in serum from allergic mice. NDT = not detected. Asterisks indicate significant differences compared with WT. No OVA-specific Ig was detected in naive or nonallergic mice (data not shown). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. All data represent mean $\pm$ SEM for a minimum of six mice per group.

no evidence of significant luminal obstruction. Aeroallergen challenge of allergic WT mice induced AHR to β -methacholine compared with the nonallergic WT (Fig. 3B). By contrast, allergic β c^{-/-} mice did not exhibit any significant increase in airway resistance relative to their nonallergic counterparts (Fig. 3B).

Next, we observed the effects of β c deletion on the production of Ag-specific Igs during allergic inflammation. Elevated serum Ag-specific IgE and increased ratios of IgG1 to IgG2a are linked to Th2 immunity and allergic inflammation, whereas a dominant Ag-specific IgG2a response is associated with Th1-regulated inflammation. Aeroallergen challenge of allergic WT mice induced a significant increase in the serum concentration of OVA-specific IgE and IgG1. By contrast, serum levels of OVA-specific IgE were attenuated in β c^{-/-} mice (Fig. 3C). Paradoxically, despite suppression of a range of pathophysiological features of allergic disease, concentrations of OVA-specific IgG1 were significantly increased in allergic β c^{-/-} mice (Fig. 3D). Furthermore, very low levels of OVA-specific IgG2a (<2 ng/ml) were observed in the serum of allergic WT, but not in allergic β c^{-/-} mice (Fig. 3D).

Pulmonary Th2 cytokine release is impaired in the absence of β c

CD4⁺ Th2 cells play a central role in the induction of many features of allergic airways disease. Attenuated Ab secretion and AHR in β c^{-/-} mice (Fig. 3) suggest defects in the T cell response to Ag, which may contribute appreciably to the attenuated allergic inflammatory response in these mice. Indeed, the ability of T cells to proliferate in response to OVA was diminished in both PBLN and spleen cultures from allergic β c^{-/-} mice by comparison with those isolated from allergic WT mice (Fig. 4A). Notably, β c de-

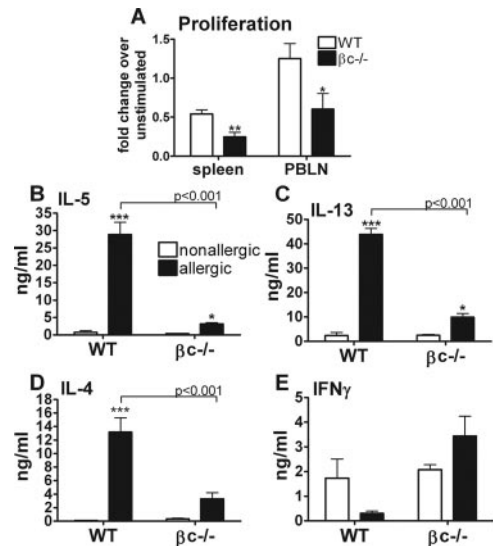


FIGURE 4. Inactivation of the β c suppresses Ag-specific cell proliferation and local type 2 cytokine production. Groups of WT and β c^{-/-} mice were exposed to saline (nonallergic) or sensitized with OVA (allergic) and aeroallergen challenged with OVA. **A**, Spleen and PBLN cells were stimulated in vitro for 3 days with OVA. Proliferation is expressed as the percentage increase over the corresponding unstimulated control. Asterisks indicate significant differences to WT. PBLN cells were cultured in vitro for 6 days with OVA, and supernatants were assayed for IL-5 (**B**), IL-13 (**C**), IL-4 (**D**), and IFN- γ (**E**). Asterisks indicate significant differences to nonallergic mice from the same strain. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Data represent mean $\pm$ SEM for a minimum of six replicate cultures.

letion was also accompanied by a striking reduction in the ability of Ag-specific T cells to produce IL-4, IL-5, and IL-13 in cultures of PBLN cells taken from allergic β c^{-/-} mice (Fig. 4, B–D). Only very low levels of the Th1-associated cytokine IFN- γ were detected, and there were no statistically significant differences between β c^{-/-} and WT in Ag-challenged mice (Fig. 4E). This effect appeared to be primarily compartmentalized to the lung, because a global reduction in Th2 cytokine responses was not observed in splenocyte cultures from β c^{-/-} mice (data not shown). Interestingly, cells from the PBLN, but not the spleen of naive β c^{-/-} mice also produce less IL-4, IL-5, and IL-13, but more IFN- γ in response to anti-CD3/anti-CD28 stimulation compared with WT controls (data not shown).

Collectively, these data demonstrate that signaling through the β c plays a pivotal role in the regulation of hallmark features in allergic inflammation, and suggest an important role in the manifestation of both the early (IgE-mediated) and late (AHR-, T cell-, and eosinophil-mediated) phases of the asthmatic response.

Activated lymphocytes fail to migrate to the lung in β c null mice

Activation of CD4⁺ Th2 cells in the PBLN and migration into the lung parenchyma play a central role in regulating the allergic inflammatory response. CD69 is an early activation marker expressed on leukocytes during inflammation (32). Thus, we characterized the profile and CD69 expression of lymphocytes in Ag-challenged β c^{-/-} and WT mice in these compartments to determine the potential contribution of T cell activation and migration to the attenuation of allergic inflammation in β c^{-/-} mice. Although levels of cytokine production from allergic β c^{-/-} PBLN cells were reduced (Fig. 4, B–E), significantly more T lymphocytes (CD3⁺ cells) occupied this compartment by comparison with WT equivalents (Fig. 5A). The increase in T lymphocytes could be

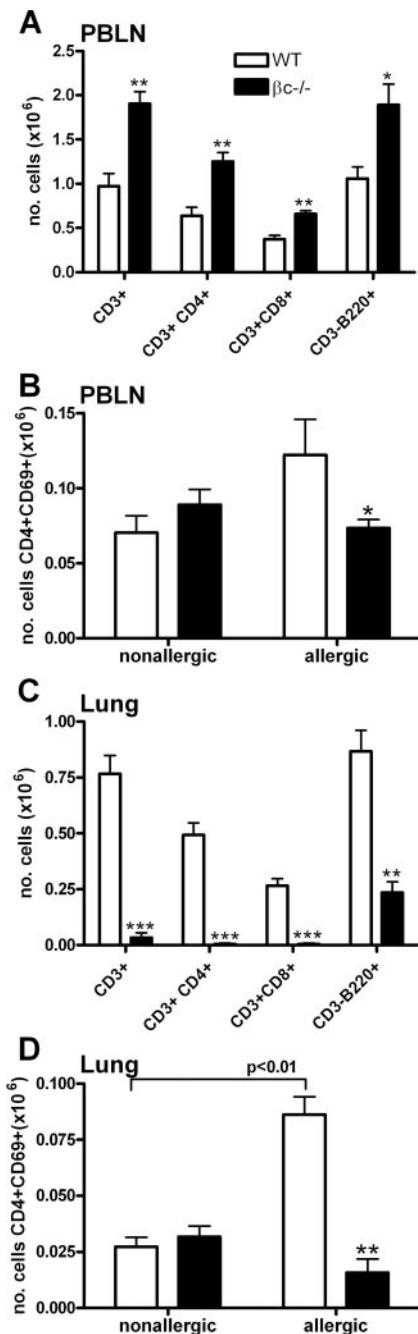


FIGURE 5. β c deficiency suppresses activation and pulmonary migration of CD4⁺ T cells. Groups of mice were exposed to saline (nonallergic) or sensitized with OVA (allergic) and aeroallergen challenged with OVA. Lymphocyte profiles were analyzed in PBLN (A) and lungs (C) of allergic mice. The cell surface marker CD69 was used to assess CD4⁺ T cell activation in PBLN (B) and lungs (D) of nonallergic and allergic mice. *, $p < 0.05$; ***, $p < 0.001$ compared with WT. Data represent mean $\pm$ SEM for a minimum of six mice per group.

accounted for by significant elevation of both CD4⁺ and CD8⁺ T cell numbers. Nevertheless, the level of activated T cells in this compartment is diminished in allergic β c^{-/-} mice by contrast to WT groups (Fig. 5B), implying that these cells were not receiving early activation signals required for migration into effector sites. Indeed, both T and B lymphocyte numbers were dramatically reduced in β c^{-/-} lung tissue, the site where they normally orchestrate allergic inflammation (Fig. 5C). The increase in CD4⁺ lymphocytes in the PBLN of β c^{-/-} mice may thus represent retention

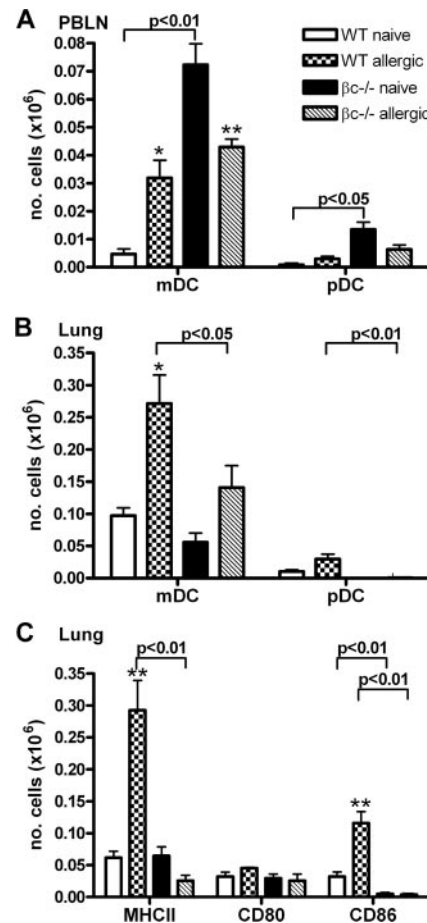


FIGURE 6. Inactivation of the β c attenuates the development and pulmonary migration of mDCs expressing MHCII and costimulatory molecules in response to Ag. Groups of WT and β c^{-/-} mice were exposed to saline (nonallergic) or sensitized with OVA (allergic) and aeroallergen challenged with OVA. Numbers of mDCs (CD11c⁺CD11b⁺) and pDCs (CD11c⁺CD11b⁻GR1⁺PDCA1⁺) were analyzed in PBLN (A) and lungs (B) of nonallergic and allergic mice. C, Activation of mDCs was determined in nonallergic and allergic mice by staining for MHCII, CD80, and CD86 in lung cell preparations. Asterisks indicate significant differences compared with naive mice of the same strain. *, $p < 0.05$; **, $p < 0.01$. Data represent mean $\pm$ SEM for a minimum of six mice per group.

of cells in the absence of specific migratory signals following Ag challenge. Moreover, the number of activated CD4⁺ T cells did not increase in response to Ag challenge in β c^{-/-} mice (Fig. 5D). These data suggest that defects in CD4⁺ T cell activation and migration occur in the absence of β c, which significantly limits the development of allergic airway disease.

βc modulates DCs in the pulmonary compartment

PBLN and airway myeloid DCs (mDCs) play pivotal roles in the activation and migration of CD4⁺ T cells in the lung and the onset of allergic inflammation through Ag presentation and costimulation (22). By contrast, plasmacytoid DCs (pDCs) promote tolerogenic responses and are protective against inflammatory responses to inhaled Ag (33). Because cytokines signaling through β c are important in the development and function of DCs, we used flow cytometry to profile subsets of mDCs (CD11c⁺CD11b⁺) and pDCs (CD11c⁺CD11b⁻GR1⁺PDCA1⁺) in the PBLN and lungs of β c^{-/-} mice. Absence of signaling through β c increased mDC numbers in the PBLN, in both naive and allergic groups, relative

to the respective WT groups (Fig. 6A). PBLN pDC numbers are also increased in naive β c^{-/-} mice (Fig. 6A). By contrast, significant Ag-induced expansion of mDC numbers in the lung tissue did not occur in β c^{-/-} mice (Fig. 6B). Moreover, a striking deficiency of pDCs in the lungs of nonallergic and allergic β c^{-/-} mice was observed, with cell numbers significantly lower than those observed in the nonallergic WT group (Fig. 6B).

Because mDCs are of particular importance in allergic inflammation, we compared the surface expression of MHCII and the costimulatory molecules CD80 and CD86 on this subset of DCs in WT and β c^{-/-} mice. Lung mDCs showed significant increases in MHCII and CD86 expression in WT allergic mice compared with the nonallergic group (Fig. 6C). However, in β c^{-/-} mice, there was no increase in mDC expressing MHCII, CD80, or CD86 following Ag provocation (Fig. 6C). Collectively, these data suggest defects in the expansion and maturation of mDCs in the lung microenvironment at baseline and during allergic inflammatory conditions in β c^{-/-} mice, which may account for the defects observed in T cell activation, proliferation, pulmonary migration, and cytokine secretion (Figs. 4 and 5). Thus, β c deficiency significantly impacts on pathways that activate T cells as well as on the generation of eosinophils (Fig. 1).

Discussion

Although the β c subunit of the GM-CSF/IL-3/IL-5 receptor complex has been shown to have an important role in eosinophil differentiation and function (27, 28), the present study is the first to determine the role of the β c in the regulation of eosinophils during allergic airways inflammation. We show that ablation of β c signaling abolishes Ag-induced accumulation of eosinophils in the peripheral blood, peribronchial tissue, and airway lumen of allergen-challenged mice. Importantly, suppression of eosinophilic inflammation was accompanied by attenuation of AHR, mucus hypersecretion, and notably the production of Ag-specific IgE. Reduced secretion of type 2 Abs in association with attenuation of late-phase pathological features suggested that Th2 cell as well as eosinophil function was impaired in β c^{-/-} mice. Indeed, characterization of CD4⁺ T cells from β c^{-/-} mice revealed defects in activation and recruitment from lymph nodes to lung tissue. Reduced activation of Th2 cells was also reflected by their limited capacity to proliferate and to secrete effector cytokines. Interestingly, this phenomenon was primarily restricted to the pulmonary compartment and correlated with reduced numbers of mDCs expressing MHCII and costimulatory molecules in the lung tissue following Ag challenge. At baseline, β c^{-/-} mice have been reported to have no hemopoietic abnormalities other than a reduction in eosinophil numbers (27, 28). However, our data show that DC and T cell function can be limited at the site of allergic inflammation, whereas systemic T cell responsiveness is maintained, because we did not observe a global decrease in the ability of these cells in the β c^{-/-} spleen to secrete cytokines in response to Ag. Thus, the β c represents a single molecule capable of regulating multiple facets of the allergic response within the lung that has significant potential as a therapeutic target for allergic diseases.

Pulmonary CD4⁺ T cell function in asthma is critically dependent on the formation of a functional immunological synapse with mDCs (22). Within the pulmonary compartment, mDCs modulate activation and polarization of naive CD4⁺ T cells to a Th2 phenotype that results in the subsequent secretion of cytokines that orchestrate inflammation (34, 35). IL-3 and GM-CSF are known to play important roles in the development of DCs in vitro (21). In this study, we demonstrate the β c signaling pathway is also critical for in vivo expansion of the pulmonary DC pool during allergic inflammation. Indeed, the significant suppression of activated

mDC numbers in the lung tissue of β c^{-/-} mice is likely to be a major mechanism underlying the defects observed in Th2 cell activation, proliferation, migration and cytokine secretion, and failure to develop allergic airway disease. Interestingly, a series of recent reports have established that mediators released by mast cells provide key instructional signals for DC maturation and migration (36, 37). Because IL-3 is important for mast cell development, defects in this cell type in the absence of IL-3 signaling may represent a further indirect mechanism for the impaired development of DCs in β c^{-/-} mice. It should be noted that alveolar macrophage (AM) can also influence T cell responses in the lung. In this regard, the increased numbers of AM at baseline in β c^{-/-} mice may be relevant because numerous studies have emphasized the potentially important negative regulatory role of AM in pulmonary immune responses. Indeed, studies have shown that depletion of AM leads to exaggerated AHR in models of allergic inflammation (reviewed in Ref. 38). However, the role of AM might be more complicated in an inflammatory environment, because pulmonary macrophages would also be influenced by the presence of proinflammatory mediators, which are able to alter the macrophage phenotype and thus their regulation of Th cell responses (39). Thus, although no definitive conclusions can be drawn, increased numbers of AM in β c^{-/-} mice may inhibit local T cell function and suppress development of allergic inflammation. Alternatively, impaired DC function due to β c deficiency may predominantly predispose to suppression of airway Th2 responses.

The potential compartmentalization of the suppressive effect on Th2 cell function is also intriguing. Production of Th2 cytokines by PBLN T cells from β c^{-/-} mice was significantly suppressed after stimulation of Ag-specific cells with OVA or naive cells with anti-CD3/anti-CD28. By contrast, β c deficiency did not lead to a global suppression of Th2 cytokine production in spleen cells from allergen-challenged mice stimulated with OVA or from naive mice stimulated polyclonally. Thus, the effect of β c deficiency appears to be compartmentalized to the lung at baseline and during induction of allergic inflammation. However, more detailed studies are required to assess whether β c-deficient mice have an intrinsic defect in pulmonary T cell function, or whether the suppression of PBLN Th2 responses is a consequence of factors within the pulmonary environment in β c^{-/-} mice, for instance DCs or macrophages.

Studies targeting individual ligands important for mature eosinophil function (e.g., IL-5) have only yielded partial ablation of tissue eosinophil numbers and shown limited improvements in disease severity, in both experimental mouse models of asthma and clinical trials (12, 13, 40, 41). Importantly, our study represents the first clinically relevant strategy, targeting a single molecule, which results in eosinophils being maintained at baseline levels following allergen provocation. The effectiveness of this approach lies in the importance of β c both for eosinophil differentiation from CD34⁺ progenitors, which may occur in the bone marrow or in situ in allergic tissue (42, 43), and for activation and survival of the mature eosinophil at sites of inflammation (17–19). Our study establishes not only that inactivation of both β c and β IL-3 prevents eosinophil expansion in allergic disease, but also that this suppression is maintained over time, distinct from the delayed parasite-induced eosinophil infiltration observed in mice in which only the β c chain is deficient (and the β IL-3 is intact) (27). This highlights the importance of eliminating signaling by all three eosinophilopoietic cytokines to effectively prevent eosinophil-driven inflammation.

Although the primary defect in T cell function in β c^{-/-} mice may be associated with the role of the β c in DC development, the involvement of this receptor in eosinophil, mast cell, and basophil

function may also contribute to the regulation of Th2 immunity. In response to pulmonary Ag provocation, eosinophils can internalize Ag, migrate to local lymph nodes, and secrete mediators that promote Th2 cell expansion and cytokine liberation (44–46). Indeed, eosinophil depletion has previously been associated with deficiencies in Th2 immunity (16). Furthermore, innate cells of hemopoietic origin (eosinophils, mast cells, and basophils) have recently emerged as the principal source of IL-4 in the early response to both parasite and allergen exposure, providing a critical molecular switch for type 2 inflammation (47–49). IL-3 is the dominant cytokine driving mast cell and basophil differentiation, and neutralization of this cytokine abolishes basophil IL-4 production and subsequent Th2 polarization in vitro (24, 50). Impaired mast cell and basophil function in the absence of IL-3 signaling in $\beta c^{-/-}$ mice may partially account for the defect in Th2 cell differentiation observed in the present study. However, because the experimental model used in the present study is primarily eosinophil and CD4⁺ Th2 cell mediated, future experiments are required using primarily mast cell-dependent models of allergic airway disease to elucidate the precise role of βc in these processes (51). Attenuation of early innate cell IL-4 production and T cell function may also explain the suppression of Ag-specific IgE production. Notably, IgE responses to parasite infestation were not suppressed in $\beta c^{-/-}$ mice, which retained IL-3 signaling via the β_{IL-3} (27), underscoring the importance of IL-3 in the generation of the type 2 inflammatory response.

It is important to note that alterations in lung homeostasis similar to human pulmonary alveolar proteinosis have previously been documented in GM-CSF ligand, GM-CSF α receptor subunit, and βc subunit ($\beta c^{-/-}$) null mice, although the phenotype of the defect in $\beta c^{-/-}$ mice appears less severe with respect to surfactant accumulation in the lung (52). Although the mice used in the present study did show accumulation of macrophages in the lung and the appearance of foamy macrophages in the BALF, the presence of pulmonary alveolar proteinosis did not appear to play a role in the development of features of acute allergic airway inflammation. This is demonstrated in naive $\beta c^{-/-}$ mice, which did not significantly differ from naive WT mice in any of the parameters assessed in our characterization of allergic airway inflammation (blood, lung tissue, and BALF eosinophils (Fig. 1)) and lung MSC (Fig. 2), with the exception of airways hyperreactivity (discussed below). Notwithstanding the presence of a background inflammatory response, we still do not see the development of functional and inflammatory lesions related to allergic airways disease in these mice. Although we cannot unequivocally rule out the possibility that altered lung homeostasis in $\beta c^{-/-}$ mice contributes to the suppression of allergic inflammation, the data obtained suggest that inhibition is due to an inability of Th2 cells to secrete cytokines. The alveolar proteinosis in $\beta c^{-/-}$ mice is likely to be a dynamic effect of gene deletion throughout life. Indeed, such abnormalities have not been documented following administration of anti-GM-CSF Abs at therapeutic doses in animal models of lung inflammation (53, 54). Optimization of treatment regimes to specifically target βc in the adult mouse lung should facilitate inhibition of allergic inflammation without altering lung homeostasis.

It is interesting to note that nonallergic $\beta c^{-/-}$ mice show significantly reduced airway resistance compared with WT BALB/c. This diminution may be related to the increased amount of surfactant present in the airways of $\beta c^{-/-}$ mice (52). Previous studies have reported that the presence of surfactant in the airway lowers surface tension, a mechanism that contributes to the distal airways remaining open (55). Indeed, inhalation of synthetic surfactant diminishes AHR in clinical studies (56). The increase in surfactant in the $\beta c^{-/-}$ airway may be a factor in the reduced response to

methacholine in nonallergic mice compared with the WT. Furthermore, the possibility that increased airway surfactant contributes to the lack of AHR in Ag-challenged $\beta c^{-/-}$ mice cannot be excluded. However, the concomitant reduction in the underlying inflammation governing AHR suggests that βc may have a specific role in regulating airway function. Future development of reagents to specifically target βc in the absence of effects on baseline lung homeostasis will facilitate further clarification of this point.

In conclusion, redundancy in cytokine signaling pathways has limited the effectiveness of targeting a single molecule for therapeutic intervention in asthma (e.g., IL-4, IL-5, and IgE) (57, 58). This report provides evidence that a wide spectrum of immune and pathophysiological features of asthma can be suppressed by targeting a single molecule that has a central role in allergic inflammation. Inhibition of βc function attenuated IgE production, tissue eosinophil numbers, and pulmonary Th2 cell effector function, which has important implications for suppression of both the early and late phases of allergic inflammation. Suppression of disease in the absence of βc was underpinned by the inability of mDCs to activate CD4⁺ T cells and their subsequent migration to effector sites in the airways. Thus, the present study enhances our understanding of the role of the βc in the regulation of molecules and cells that play pivotal roles in both early and late phases of the allergic response, and identifies this receptor chain as a clinically relevant target for suppression of disease.

Disclosures

The authors have no financial conflict of interest.

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