

Activation of c-Kit in dendritic cells regulates T helper cell differentiation and allergic asthma

Nandini Krishnamoorthy^{1,2}, Timothy B Oriss¹, Melissa Paglia¹, Mingjian Fei¹, Manohar Yarlagadda¹, Bart Vanhaesebroeck³, Anuradha Ray^{1,2} & Prabir Ray^{1,2}

Dendritic cells (DCs) are integral to the differentiation of T helper cells into T helper type 1 T_H1, T_H2 and T_H17 subsets. Interleukin-6 (IL-6) plays an important part in regulating these three arms of the immune response by limiting the T_H1 response and promoting the T_H2 and T_H17 responses. In this study, we investigated pathways in DCs that promote IL-6 production. We show that the allergen house dust mite (HDM) or the mucosal adjuvant cholera toxin promotes cell surface expression of c-Kit and its ligand, stem cell factor (SCF), on DCs. This dual upregulation of c-Kit and SCF results in sustained signaling downstream of c-Kit, promoting IL-6 secretion. Intranasal administration of antigen into c-Kit-mutant mice or neutralization of IL-6 in cultures established from the lung-draining lymph nodes of immunized wild-type mice blunted the T_H2 and T_H17 responses. DCs lacking functional c-Kit or those unable to express membrane-bound SCF secreted lower amounts of IL-6 in response to HDM or cholera toxin. DCs expressing nonfunctional c-Kit were unable to induce a robust T_H2 or T_H17 response and elicited diminished allergic airway inflammation when adoptively transferred into mice. Expression of the Notch ligand Jagged-2, which has been associated with T_H2 differentiation, was blunted in DCs from c-Kit-mutant mice. c-Kit upregulation was specifically induced by T_H2- and T_H17-skewing stimuli, as the T_H1-inducing adjuvant, CpG oligodeoxynucleotide, did not promote either c-Kit or Jagged-2 expression. DCs generated from mice expressing a catalytically inactive form of the p110δ subunit of phosphatidylinositol-3 (PI3) kinase (p110^{D910A}) secreted lower amounts of IL-6 upon stimulation with cholera toxin. Collectively, these results highlight the importance of the c-Kit-PI3 kinase-IL-6 signaling axis in DCs in regulating T cell responses.

DCs are efficient antigen-presenting cells and can prime naive CD4⁺ T cells toward a T_H1 or T_H2 response or the recently discovered T_H17 response^{1–3}. Considerable progress has been made in the last decade in our understanding of the molecular basis of T helper responses. We and others have identified the transcription factor GATA-3 as the master regulator of T_H2 differentiation^{4–7}. The transcription factors T-bet and ROR-γt are now recognized as crucial regulators of T_H1 and T_H17 differentiation, respectively^{8,9}. Studies have shown that the nature of the antigen, the antigen concentration, the pattern recognition molecules present and the cytokine milieu are some of the key regulators that determine the outcome of an immune response¹.

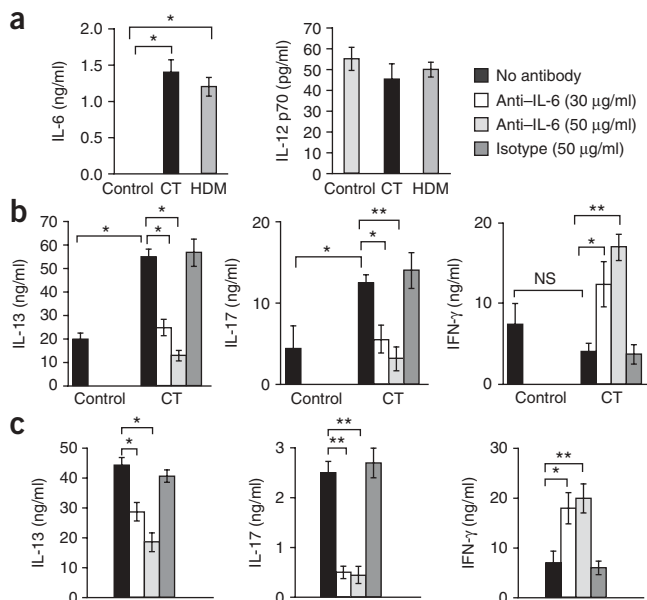
Cytokines are crucial in regulating T helper cell differentiation¹. Previous studies have shown the T_H2-skewing ability of IL-6 (refs. 10,11). More recently, IL-6 has been shown to be the quintessential cytokine regulating T_H17 development in both mice and humans^{12,13}. Notably, in certain immunological responses, such as those elicited by allergens in asthma, both T_H2 and T_H17 responses are evident¹⁴. In these situations, IL-6 would be a central regulator in not only inhibiting the T_H1 response¹¹, but also promoting T_H2 and T_H17 responses^{10,12,13}. However, mechanisms that regulate IL-6 production in DCs have not been elucidated. Additionally, there have been very

few studies¹⁵ that have addressed the role of c-Kit expressed by DCs in modulating immune responses.

Our goal was to determine molecular mechanisms by which a complex allergen such as HDM or the mucosal adjuvant cholera toxin promotes IL-6 production in DCs, which, in turn, induce T_H2 and T_H17 responses. Using microarray analysis to identify novel genes regulated by cholera toxin in bone marrow-derived dendritic cells (BMDCs), we identified marked upregulation of IL-6 and c-Kit expression in cholera toxin-treated DCs as compared to that in control DCs. Because cytokine expression is triggered by activation of cell surface receptors, we hypothesized that c-Kit expression and activation in DCs regulate the production of IL-6 and thereby the induction of a T_H2 or T_H17 response subsequent to DC–T cell interaction. To elucidate the role of c-Kit expressed by DCs in regulating IL-6 production, we used c-Kit-mutant mice (WBB6F1 *Kit*^{W/W-v}). These mice have a deletion in the transmembrane region of c-Kit and also a missense mutation in the kinase domain^{16–18}. Our *in vitro* data with cholera toxin- and HDM-stimulated BMDCs and lung DCs and our *in vivo* data with two models of allergic airways disease in mice, as well as an adoptive transfer experiment with wild-type (WT) and c-Kit-defective DCs, show that functional c-Kit

¹Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, and ²Department of Immunology, University of Pittsburgh School of Medicine, 3459 Fifth Avenue, Pittsburgh, Pennsylvania 15213, USA. ³Centre for Cell Signalling, Institute of Cancer, Barts and The London, Queen Mary's School of Medicine and Dentistry, (Queen Mary, University of London, Charterhouse Square, London EC1M 6BQ, UK. Correspondence should be addressed to P.R. (ray@pitt.edu) or A.R. (raya@pitt.edu).

Received 14 September 2007; accepted 26 March 2008; published online 4 May 2008; doi:10.1038/nm1766



signaling in DCs via upregulation of c-Kit and membrane-bound SCF is essential for the induction of T_H2 and T_H17 responses. Of note, c-Kit expression was also found to influence expression of the Notch ligand Jagged-2, which has been associated with T_H2 responses¹⁹. Using p110δ-mutant mice with defective PI3 kinase signaling, which constitutes a major signaling pathway downstream of c-Kit, we show that the c-Kit–PI3 kinase signaling axis positively regulates production of IL-6. Thus, we have identified a previously undescribed signaling axis in DCs that promotes the induction of T_H2 and T_H17 responses while markedly inhibiting the T_H1 arm of the immune response.

RESULTS

DC cytokine balance and induction of T_H2 and T_H17 responses

The mucosal adjuvant cholera toxin has been associated with IL-6 production²⁰. Recent studies have implicated IL-6 in IL-17 production^{12,13,21}, which is also involved in allergic airway inflammation¹⁴. To dissect the importance of cytokines secreted by DCs in influencing T cell response, we analyzed cytokine abundance in culture supernatants of BMDCs stimulated with cholera toxin or the allergen HDM, which causes asthma in humans²². Both cholera toxin and HDM promoted IL-6 production from BMDCs (Fig. 1a). The secretion of the T_H1 -skewing cytokine IL-12 p70 was not significantly different between HDM- or cholera toxin-treated DCs and control DCs. A similar cytokine profile of high IL-6 and low IL-12 production by lung DCs was associated with inhibition of T_H1 and promotion of T_H2 response¹¹. Neutralization of IL-6 in cocultures of BMDCs and T cells resulted in a cytokine profile that showed a switch in the T helper response to a dominant T_H1 response, with elevated levels of interferon-γ (IFN-γ) coupled with inhibition of IL-13 and IL-17 production (Fig. 1b). Furthermore, addition of IL-12 to cholera toxin-stimulated BMDCs switched the response of the T cells in coculture to a T_H1 response by promoting IFN-γ production (data not shown). These results show that altering the cytokine balance by neutralizing IL-6 or adding IL-12 diverts the dominant T_H2 - and T_H17 -skewing property of cholera toxin to a more T_H1 response. We next investigated the role of IL-6 in shaping the immune outcome *in vivo* in response to the natural allergen HDM. Mice were immunized intranasally with the allergen and the

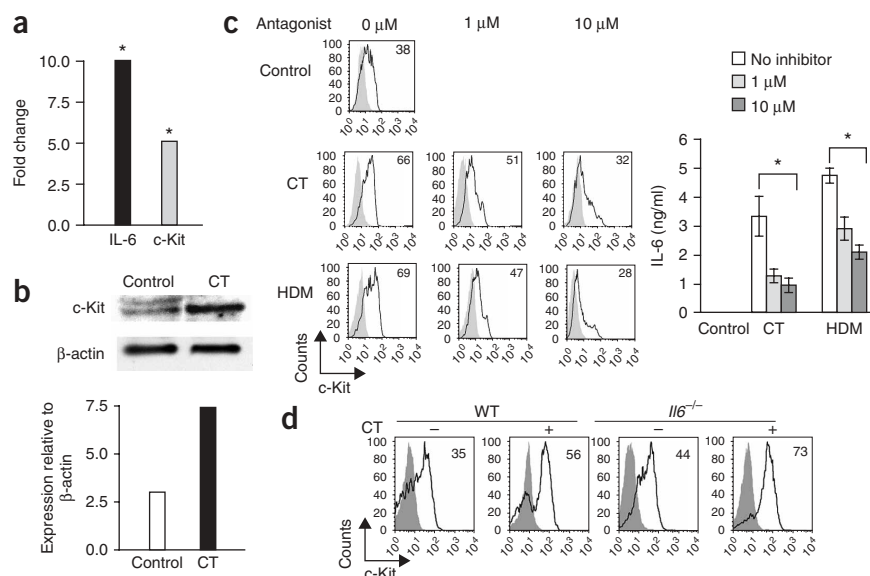
Figure 1 Cholera toxin and HDM stimulate IL-6 secretion from DCs *in vitro* and *in vivo*. **(a)** BMDCs were incubated with or without cholera toxin (CT) for 24 h. The cell supernatants were analyzed for IL-6 and IL-12 p70 abundance by ELISA. Cytokine concentrations are mean ± s.e.m. of triplicates and are representative of three independent experiments. * $P < 0.001$. **(b)** Antibody to IL-6 (anti-IL-6) inhibits T_H2 and T_H17 response but promotes T_H1 response. BMDCs were incubated with or without CT for 24 h and then cocultured with DO11.10 T cells in the presence of OVA peptide. As shown, some wells containing BMDCs and CT also contained anti-IL-6 or an isotype control. The culture supernatants were assayed for IL-13, IL-17 and IFN-γ. The values represent means of triplicates ± s.d. and are representative of two independent experiments. * $P < 0.05$ and ** $P < 0.001$. NS, not significant. **(c)** Lymph node cells derived from WT mice show diminished T_H2 and T_H17 response but increased T_H1 response upon treatment with antibody to IL-6. Lymph node cells from WT mice after HDM instillation were cultured with antibody to IL-6 or an isotype control, and IL-13 and IFN-γ concentrations in the culture supernatant were measured. Results represent means of triplicates ± s.e.m. and are representative of three independent experiments. * $P < 0.05$ and ** $P < 0.001$.

lung-draining lymph nodes were harvested. This protocol of immunization has been shown to prime the T cells from the lung-draining lymph toward a T_H2 response²³. Immunization with the allergen increased the T_H2 and T_H17 response, whereas neutralization of IL-6 crippled the dominant T_H2 and T_H17 immune response (Fig. 1c). Intranasal immunization of *Il6*^{-/-} mice with ovalbumin (OVA)–cholera toxin or HDM resulted in significant attenuation of both T_H2 and T_H17 responses (Supplementary Fig. 1 online). These results show that whether T cell responses are triggered by BMDCs or lung DCs, IL-6 has a key role in regulating the responses.

Upregulation of c-Kit in DCs by cholera toxin and HDM

To further dissect and identify molecular mechanisms underlying IL-6 upregulation by cholera toxin, we undertook a microarray approach to identify genes differentially regulated in BMDCs upon cholera toxin treatment, as this approach had previously allowed us to identify *Chi3l3* (chitinase 3 like 3, also known as Ym1), previously associated with allergic airway inflammation, as a statin-induced gene involved in T_H2 differentiation²⁴. The microarray data analysis (details to be published elsewhere) revealed the gene encoding c-Kit to be significantly upregulated along with IL-6 in BMDCs upon cholera toxin treatment (Fig. 2a). Immunoblotting of total cell lysates revealed greater c-Kit expression in cholera toxin-stimulated BMDCs compared to that in control DCs (Fig. 2b). As determined by flow cytometry, the frequency of cells expressing c-Kit on the cell surface was increased by cholera toxin treatment (Fig. 2c), and this increase was dose dependent (data not shown). We also investigated whether c-Kit is induced specifically by cholera toxin, or whether an allergen such as HDM can also do the same. Exposure of BMDCs to HDM increased the frequency of c-Kit-expressing cells comparably (Fig. 2c). Stimulation of human monocytes with cholera toxin has been shown to suppress IL-12 via a cAMP-dependent pathway²⁵. We therefore examined the role of cAMP in c-Kit upregulation by cholera toxin by using the specific cAMP antagonist Rp-cAMPS, which blocks cAMP-dependent downstream pathways such as the protein kinase A pathway²⁶. Rp-cAMPS caused a significant decrease in the number of c-Kit-expressing cells among both cholera toxin- and HDM-stimulated DCs in a dose-dependent fashion, with a concomitant decrease in IL-6 production (Fig. 2c). It is presently unclear how HDM triggers cAMP–protein kinase A-dependent mechanisms in DCs. c-Kit expression on DCs derived from *Il6*^{-/-} mice was

Figure 2 Increased expression of c-Kit in CT- and HDM-stimulated BMDCs. **(a)** Increased IL-6 and c-Kit mRNA expression in CT-stimulated BMDCs compared to that in control DCs as revealed by microarray analysis and confirmed by real-time PCR (shown). $*P < 0.005$. **(b)** c-Kit expression in CT-stimulated BMDCs, as revealed by immunoblotting of total cell lysates. The blot was stripped and reprobed for the loading control β -actin. The intensity of c-Kit bands was quantified relative to β -actin. The upper band detected by antibody to c-Kit is variably detected in different samples and is probably due to glycosylation. Data are representative of two independent experiments. **(c)** CT and HDM upregulate c-Kit expression on BMDCs in a cAMP-dependent fashion. BMDCs were pretreated with the cAMP antagonist Rp-cAMPS for 1 h and stimulated with CT (1 μ g/ml) or HDM (10 μ g/ml) for 24 h. The cells were harvested and analyzed for c-Kit expression by flow cytometry. The culture supernatants were analyzed for IL-6 production by ELISA, and the data shown are mean values of triplicates $\pm$ s.d. This experiment was performed twice with similar results. $*P < 0.05$. **(d)** BMDCs from *Il6*^{+/+} (WT) and *Il6*^{-/-} mice were incubated with or without CT for 24 h, and the expression of c-Kit was analyzed by flow cytometry. The shaded gray represents the isotype control, and the black overlay represents staining with the antibody to c-Kit. The numbers in the histograms represent the percentage of CD11c⁺c-Kit⁺ cells. This experiment was repeated twice with similar results.



comparable to that on cells derived from WT mice, suggesting that IL-6 is a downstream target of c-Kit (**Fig. 2d**).

c-Kit expression in DCs regulates T_H1 and T_H2 responses

To further understand the functional implications of c-Kit upregulation on DCs, we sorted c-Kit⁺ and c-Kit⁻ cells after stimulation with cholera toxin. The sorted populations were then cultured with T cells from naive DO11.10 mice in the presence of OVA peptide. The supernatants from the coculture were assayed for IL-13, IL-17 and IFN- γ abundance by ELISA. The cholera toxin-stimulated BMDCs that were c-Kit⁺ skewed the T cells toward production of high amounts of IL-13 and IL-17 and low amounts of IFN- γ (**Fig. 3a**). The cytokine profile was different in culture supernatants that included the c-Kit⁻ population, showing higher IFN- γ and lower IL-17 and IL-13 levels (**Fig. 3a**). In contrast to the high frequency of c-Kit⁺ cells generated in response to cholera toxin, the majority of BMDCs stimulated with CpG oligodeoxynucleotide (ODN), a T_H1-skewing adjuvant, were c-Kit⁻, and this population of DCs strongly promoted a T_H1 response (**Fig. 3b**). These results strengthened our notion that the upregulation of c-Kit on DCs elicits a strong T_H2 and T_H17 response while suppressing the T_H1 response.

c-Kit regulates IL-6 and Notch ligand expression in DCs

The ability of c-Kit⁻ cells to trigger a strong T_H1 response (**Fig. 3**) suggested that a lack of c-Kit upregulation could be a potential mechanism to subvert a T_H2 response. To

test this hypothesis, we isolated lung DCs and stimulated them with T_H2- or T_H1-skewing agents and examined the expression of different molecules associated with DC maturation and T helper differentiation. A significant increase in the percentage of c-Kit-expressing DCs was noted upon treatment with cholera toxin or HDM, but not CpG ODN (**Fig. 4a**). In contrast, CpG ODN, but not HDM or cholera toxin, promoted expression of CD40, which is associated with increased IL-12 production in DCs and with the T_H1 response²⁷(**Fig. 4a**).

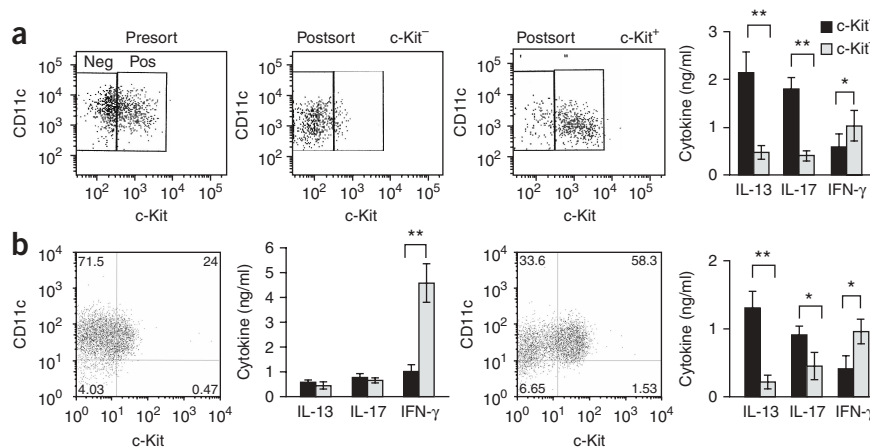


Figure 3 c-Kit⁺ but not c-Kit⁻ BMDCs resulting from CT stimulation skew T cells predominantly toward a T_H2 and T_H17 response. **(a)** BMDCs were stimulated with CT (1 μ g/ml) and sorted for expression of c-Kit. The purity of the c-Kit⁺ and c-Kit⁻ populations after sorting was $>93\%$. The supernatants from the cocultures of either c-Kit⁺ or c-Kit⁻ cells with CD4⁺ T cells were assayed for IL-13, IL-17 and IFN- γ by ELISA. **(b)** BMDCs were stimulated with either CpG ODN (1 μ M; left two graphs) or CT (1 μ g/ml; right two graphs) and sorted for c-Kit expression as in **a**, resulting in similar purities of c-Kit⁺ and c-Kit⁻ populations. The numbers denote percentage of cells in that quadrant. The sorted cells from each treatment were cocultured with CD4⁺ T cells, and the culture supernatants were assayed for cytokine concentrations by ELISA. Data are means of triplicates $\pm$ s.d. and are representative of two independent experiments. $*P < 0.05$ and $**P < 0.001$.

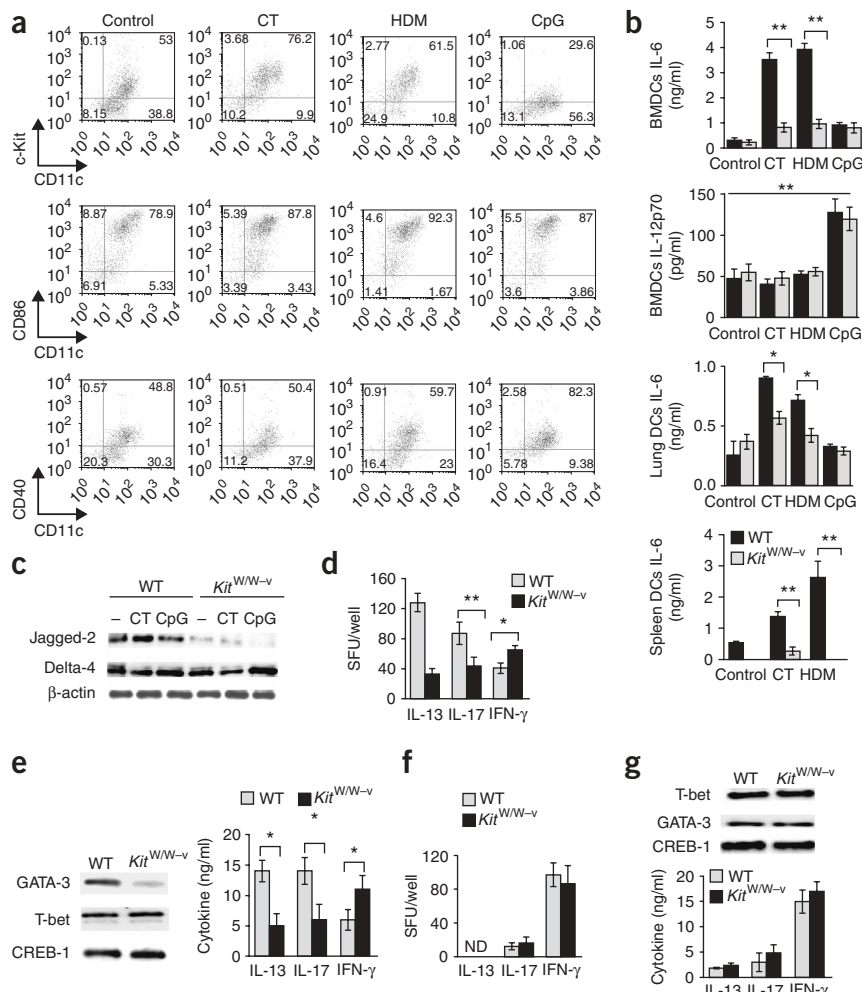


Figure 4 CT- or HDM-induced c-Kit signaling regulates IL-6 production and the expression of Notch ligands. **(a)** Lung DCs were stimulated and phenotyped for the markers as shown. The numbers denote percentage of cells in that quadrant. **(b)** Cytokine production by BMDCs, lung DCs and spleen DCs was assayed by ELISA. Data are means of triplicates $\pm$ s.d. and are representative of two independent experiments. * P < 0.05 and ** P < 0.01. **(c)** Total cell lysates of variously treated BMDCs containing equal amounts of protein were sequentially analyzed by immunoblotting for expression of the Notch ligands Jagged-2, Delta-4 and β -actin. **(d)** Cells from lung-draining lymph nodes of mice after OVA-CT immunization were stimulated with PMA (25 ng/ml) and ionomycin (500 ng/ml) for 24 h to assess T cell priming by ELISPOT. 1×10^5 lymph node cells from the individual mice were plated in each well and the SFUs for each cytokine were determined by ELISPOT. * P < 0.05 and ** P < 0.005. **(e)** Lung-draining lymph nodes were harvested after intranasal administration of OVA-CT into *Kit^{W/W-v}* and WT mice and cultured for 5 d in the presence of OVA. Nuclear extracts prepared from the cultures were analyzed sequentially by immunoblotting for GATA-3, T-bet and CREB-1 (loading control). The supernatants from the cocultures were analyzed for IL-13, IL-17 and IFN- γ by ELISA, and data are means of triplicates $\pm$ s.e.m. * P < 0.05. Data shown are representative of three independent experiments. **(f)** Cells from lung-draining lymph nodes after OVA-CpG immunization were stimulated for 24 h with PMA and ionomycin, and the SFUs were assayed by ELISPOT. ND, not detected. **(g)** The recall response was assayed by stimulating the lymph node cells with OVA for 3 d. The transcription factor profile was assayed by immunoblotting, and the cytokine abundance in the culture supernatants was assayed by ELISA.

Because we observed that treatment with cholera toxin or HDM upregulates c-Kit expression in BMDCs and that expression of this molecule is crucial for IL-6 production, we next investigated whether c-Kit signaling is an important component of IL-6 production in BMDCs and, in turn, in the regulation of T cell responses. BMDCs were cultured from WT or *Kit^{W/W-v}* mice. There was no difference between WT and *Kit^{W/W-v}* mice with respect to BMDC purity, yield and expression of major histocompatibility complex (MHC) class II and the costimulatory molecules CD40 and CD86 (Supplementary Fig. 2 online). The DCs were incubated with or without cholera toxin or HDM, and the cytokine profile was analyzed by ELISA. DCs from *Kit^{W/W-v}* mice secreted significantly lower amounts of IL-6 upon cholera toxin or HDM stimulation, indicating that c-Kit signaling in BMDCs is crucial for IL-6 production in response to these agents (Fig. 4b). The amount of IL-12 p70 in the culture supernatants was not significantly different between the mouse groups; however, treatment with CpG ODN upregulated the production of this cytokine (Fig. 4b). We observed essentially similar results in DCs isolated from the spleens or the lungs of *Kit^{W/W-v}* mice (Fig. 4b), clearly showing that mutations that impair c-Kit signaling affect IL-6 secretion from BMDCs as well as from tissue DCs.

Antigen-presenting cells have been shown to upregulate the Notch ligands Delta-4 and Jagged-2 in response to T_H1 - and T_H2 -inducing stimuli, respectively¹⁹. We therefore investigated whether the

expression pattern of these ligands was altered in DCs from WT mice compared to *Kit^{W/W-v}* mice. At the basal level, BMDCs from *Kit^{W/W-v}* mice had markedly lower Jagged-2 expression compared to DCs from WT mice, suggesting a developmental link between c-Kit and Jagged-2 (Fig. 4c). Also, cholera toxin treatment stimulated Jagged-2 expression in BMDCs from WT mice (Fig. 4c). The expression of Delta-4, on the other hand, was not compromised by c-Kit defect and was slightly elevated upon CpG ODN treatment (Fig. 4c).

To confirm that impaired c-Kit signaling has physiological relevance in T cell responses, we used an *in vivo* model of airway inflammation in which OVA, in combination with cholera toxin, was administered via the intranasal route²⁸ to WT and *Kit^{W/W-v}* mice. After 4 d of rest, the mice were killed and the lung-draining lymph nodes were harvested. The lymph node cells from the *Kit^{W/W-v}* mice showed fewer IL-13 and IL-17 spot-forming units (SFU) and a slight increase in the number of IFN- γ SFU, as determined by enzyme-linked immunosorbent spot (ELISPOT) assay (Fig. 4d). In a recall response to antigen, lower expression of the T_H2 -specific factor GATA-3 (refs. 4,5) was detected in nuclear extracts derived from *Kit^{W/W-v}* mice, whereas expression of the T_H1 -specific factor T-bet was unchanged⁹ (Fig. 4e). Correspondingly, decreased IL-13 abundance was noted in cultures containing lymph node cells from *Kit^{W/W-v}* mice as compared to those containing cells from WT mice, and the converse was noted for IFN- γ abundance (Fig. 4e). The increase in IFN- γ abundance with cells from the

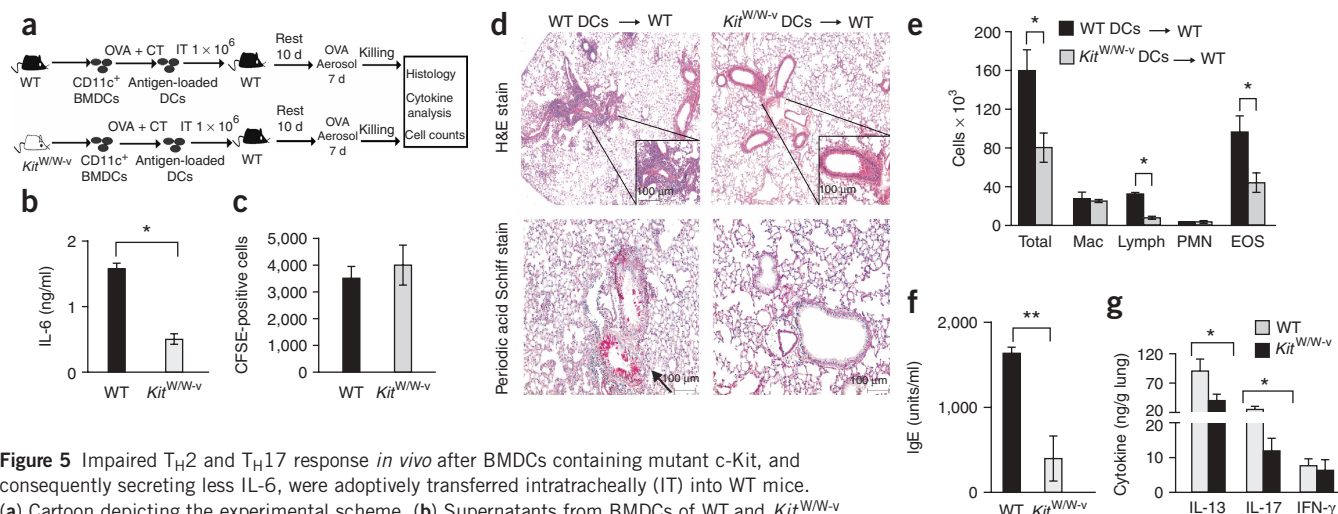


Figure 5 Impaired T_H2 and T_H17 response *in vivo* after BMDCs containing mutant c-Kit, and consequently secreting less IL-6, were adoptively transferred intratracheally (IT) into WT mice. (a) Cartoon depicting the experimental scheme. (b) Supernatants from BMDCs of WT and *Kit^{W/W-v}* mice stimulated with OVA-CT overnight were assayed for IL-6 production by ELISA. Data are means of triplicates $\pm$ s.e.m. * $P < 0.05$. Data are representative of two independent experiments. (c) The presence of CFSE-labeled CD11c⁺ BMDCs in lung-draining lymph nodes was assessed 24 h after intratracheal transfer. This experiment was performed twice and data shown are mean $\pm$ s.d. (d) Histological examination of lung sections of recipient mice stained with H&E (for assessment of inflammation) or periodic acid Schiff stain (to assess mucus production). Scale bars, 100 μ m. (e) Total and differential counts of cells recovered in the BAL fluid are shown. * $P < 0.05$. The statistical analysis was performed for four or five mice in each group. (f) Antigen-specific IgE concentrations were measured by ELISA. The results represent the means of triplicates $\pm$ s.d. * $P < 0.01$. (g) Concentrations of IL-13, IL-17 and IFN- γ in lung homogenates were measured by ELISA and are shown as mean values of triplicates $\pm$ s.d. * $P < 0.05$. Shown is a representative experiment of two.

Kit^{W/W-v} mice suggested that there would be a decrease in IL-17 production due to its known cross-regulation by IFN- γ , which we confirmed by ELISA (Fig. 4e). As expected, cultures established from lymph nodes of *Kit^{W/W-v}* mice generated less IL-6 than those established from lymph nodes of WT mice (Supplementary Fig. 3 online).

The combination of OVA and CpG ODN elicited a potent T_H1 response (Fig. 4f) that did not differ between the WT and *Kit^{W/W-v}* mice (Fig. 4g). Taken together, these data show that c-Kit signaling is essential for the elicitation of a T_H2 response, but not a T_H1 response.

Poor T_H2 response elicitation by *Kit^{W/W-v}* DCs *in vivo*

Given that *Kit^{W/W-v}* mice also have a defect in mast cells, which are associated with allergic responses, we tested whether c-Kit deficiency in DCs explained the reduced T_H2 and T_H17 priming in *Kit^{W/W-v}* mice. To address this, we adoptively transferred either WT or *Kit^{W/W-v}* DCs into WT recipients with a complete repertoire of all cell types, including mast cells, and examined development of allergic airway inflammation (Fig. 5a). BMDCs generated from *Kit^{W/W-v}* mice produced less IL-6 compared to those from WT mice after incubation with OVA and cholera toxin (Fig. 5b), although both types migrated similarly to lung-draining lymph nodes after intratracheal transfer, as confirmed by visualization of carboxyfluorescein diacetate succinimide ester (CFSE)-labeled DCs (Fig. 5c). In mice subjected to repeated antigen challenge after DC transfer, assessment of lung histology (Fig. 5d), differential cell counts in bronchoalveolar lavage (BAL) fluid (Fig. 5e), antigen-specific IgE abundance (Fig. 5f) and cytokine production (Fig. 5g) showed that BMDCs from *Kit^{W/W-v}* mice induce less allergic airway inflammation compared to those from WT mice.

We also addressed the role of c-Kit in a different model associated with a T_H17 response, the *Klebsiella pneumoniae* model of lung infection²⁹. WT and *Kit^{W/W-v}* mice were infected with the bacteria, and the mice were killed on day 4 after the infection (Supplementary Methods online). Analysis of bacterial counts in the mice showed that the c-Kit mutants had significantly fewer colony-forming units in the spleen, liver and lungs (Supplementary Fig. 4 online). Notably, the

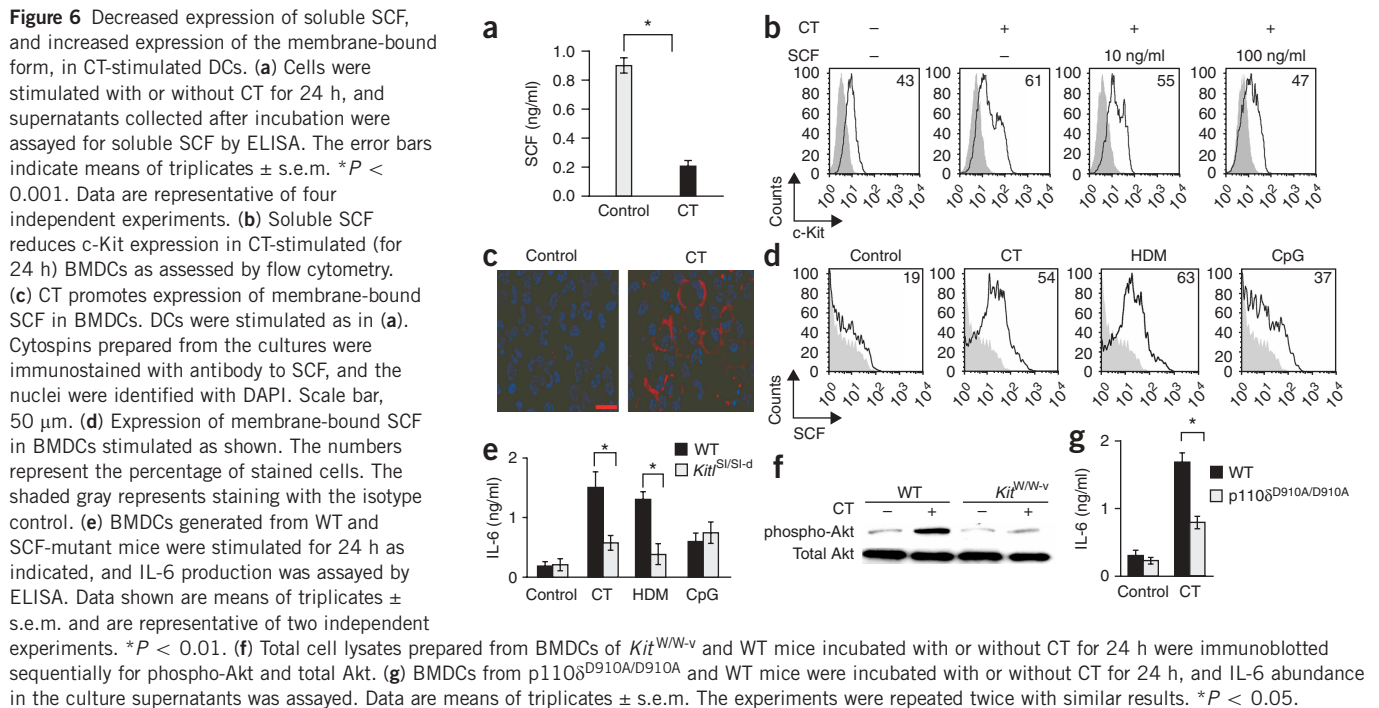
c-Kit mutants also had a higher IFN- γ response in the lung-draining lymph nodes, as assayed by ELISPOT technique (Supplementary Fig. 4). This was similar to the increased IFN- γ secretion observed upon neutralization of IL-6 (Fig. 1) and in response to OVA–cholera toxin in c-Kit mutant mice (Fig. 4d). Thus, in the *Klebsiella* infection model, too, compromised c-Kit signaling in the c-Kit mutant mice impairs the T_H17 response and increases the IFN- γ response, and the mice show less bacterial burden in several organs.

Allergen-induced membrane-bound stem cell factor in BMDCs

We next asked how c-Kit is stimulated to induce IL-6 production in DCs. Previous studies have shown that c-Kit is internalized upon binding its ligand, stem cell factor (SCF, encoded by *Kitl*), indicating a key role for SCF in regulating the cell surface expression of c-Kit³⁰. Analysis of SCF abundance in culture supernatants of control versus cholera toxin–stimulated BMDCs showed lower amounts in the latter cells (Fig. 6a), and the expression of c-Kit was reduced upon treatment with soluble SCF (Fig. 6b).

Because SCF is also expressed in a membrane-bound form owing to alternative splicing³¹, we analyzed the expression of membrane-bound SCF by different techniques. The results of immunostaining showed that cholera toxin treatment of BMDCs promoted expression of membrane-bound SCF, rather than the soluble form that is favored in control cells (Fig. 6c). Cholera toxin or HDM stimulation induced a greater frequency of SCF-expressing BMDCs than did stimulation by CpG ODN, as analyzed by flow cytometry (Fig. 6d). To further elucidate the functional significance of this differential expression, we used DCs from *Kit^{sl/sl-d}* mice, in which expression of the membrane-bound form of SCF is selectively compromised^{31,32}. IL-6 expression was significantly compromised after cholera toxin or HDM stimulation of DCs generated from *Kit^{sl/sl-d}* mice (Fig. 6e). DCs generated from *Kit^{sl/sl-d}* mice showed negligible cell surface staining for SCF (Supplementary Fig. 5 online).

Among the signaling pathways induced by c-Kit, that of PI3 kinase has a crucial role³³. The sustained expression of c-Kit after 24 h,



coupled with the high expression of membrane-bound SCF, suggested persistent signaling through c-Kit via PI3 kinase in the DCs. Phosphorylated Akt (phospho-Akt), triggered by activated PI3 kinase³⁴, was readily detectable in cell extracts prepared from cholera toxin-stimulated BMDCs derived from WT but not *Kit*^{W/W-v} mice (Fig. 6f). We next investigated whether cholera toxin-stimulated IL-6 production was compromised in PI3 kinase 110 δ -mutant mice (p110 δ ^{D910A/D910A} mice). These mice lack the 110 δ subunit of PI3 kinase and have impaired B and T cell signaling and mounting of allergic airways disease^{35,36}. Cholera toxin-stimulated BMDCs from p110 δ ^{D910A/D910A} mice secreted less IL-6 as compared to cells from WT mice, indicating the involvement of the PI3 kinase pathway in IL-6 production (Fig. 6g). Thus, c-Kit expressed by BMDCs in conjunction with membrane-bound SCF causes prolonged activation of the PI3 kinase–Akt pathway in DCs that promotes a higher IL-6 and lower IL-12 expression profile in DCs, which, in turn, promotes T cell differentiation toward the T_H2 and T_H17 lineage.

DISCUSSION

The discovery of DCs has enhanced our knowledge of the divergent pathways of the T_H1 and T_H2 responses, as well as the recently discovered T_H17 response. In this study, we show that dual upregulation of c-Kit and membrane-bound SCF on DCs, causing sustained activation of the c-Kit–PI3 kinase signaling axis, is crucial for promoting IL-6 production. Defective c-Kit signaling, associated with reduced IL-6 production, compromises the ability of DCs to mount a robust T_H2 and T_H17 response and allergic airway inflammation in mice.

Our results show that exposure of DCs to selective agents such as the mucosal adjuvant cholera toxin or the allergen HDM, both associated with allergic immune responses^{1,22,28}, induces expression of both c-Kit and its ligand SCF in the DCs stimulating IL-6 production, which promotes both T_H2 and T_H17 responses. However, upregulation of c-Kit and its ligand was not simply a result of DC maturation, as the T_H1 (IFN- γ)-inducing adjuvant CpG ODN did not elicit the same responses. IL-12 production in DCs by microbial

stimuli has been tightly linked to CD40 ligation²⁷, and IL-12 promotes T_H1 differentiation. Although IL-6 production by DCs has been associated with T_H2 and T_H17 differentiation^{10,11,20}, no cell surface event in DCs has heretofore been identified that promotes IL-6 and limits IL-12 production. Of note, in mice lacking Rab guanine nucleotide exchange factor-1 (RABGEF1), which is a negative regulator of c-Kit, the abundance of IL-6 was found to be elevated³⁷. Collectively, our data show that the activation of the c-Kit–PI3 kinase axis in DCs, stimulating IL-6 production, is a key regulatory mechanism that promotes T_H2 and T_H17 responses and limits the T_H1 response.

Not only did this study unravel a link between c-Kit and IL-6 production in DCs, but also the expression of a Notch ligand, Jagged-2, which is specifically aligned with T_H2 response¹⁹, was found to be dependent on functional c-Kit. Essential regulatory roles for Notch have been recently identified in the immune system. For example, Notch has been implicated in T cell polarization, which has been associated with upregulation of specific Notch ligands in response to T_H2 or T_H1 stimuli on antigen-presenting cells¹⁹. We have recently demonstrated cross-talk between Notch- and transforming growth factor- β -induced pathways in antigen-induced tolerance in the airways³⁸. Here, we show that DCs expressing functionally inactive c-Kit express low amounts of Jagged-2 compared to DCs from WT mice, thus identifying a potentially crucial role for c-Kit in the expression of a specific Notch ligand during development. The relationship between Jagged-2 and c-Kit is also in line with previous observations showing that the Notch–Jagged-2 axis promotes IL-6 secretion in different cell types³⁹. Although Notch activation in CD4⁺ T cells is important for T_H2 differentiation^{19,40,41}, bidirectional Notch–Notch ligand signaling has been documented in T helper cell–DC conjugates, with evidence of nuclear translocation of Hes1 and STAT3 in both cell types, Hes1 being a Notch-induced transcription factor which, in turn, promotes STAT3 activation⁴². Thus, the Notch pathway may be necessary for promoting *Il6* gene expression in DCs, with c-Kit playing an essential regulatory part in Jagged-2 expression.

As suggested by our data, the differential SCF expression seen in cholera toxin- or HDM-stimulated DCs could be a mechanism by which prolonged activation of c-Kit and its downstream targets is achieved. It should be noted that soluble SCF can transiently cause Akt phosphorylation, and phosphorylated Akt can no longer be detected 15 min after addition of SCF³⁷. Our data showing persistent phospho-Akt expression in cholera toxin-stimulated DCs and the results obtained in cells from *Kit^{sl/sl-d}* mice reiterate the fact that the absence of membrane-bound SCF markedly reduces signaling via c-Kit, impairing IL-6 production. Thus, dual upregulation of the c-Kit receptor and the membrane-bound form of SCF contributes to increased IL-6 production from allergen-stimulated DCs.

Although c-Kit has been best studied in the context of mast cells, administration of SCF *in vivo* has been shown to have mast cell-independent effects⁴³. c-Kit-expressing DCs have been shown to modulate NK cell function by increasing the cytolytic and IFN- γ -secreting ability of NK cells, thereby defining the consequence of this receptor in at least one aspect of DC function¹⁵. Another noteworthy aspect of this study is that the authors clearly show that DCs are the pharmacological target of Gleevec administration *in vivo*, indicating that there are mast cell-independent effects of this tyrosine kinase inhibitor. Although mast cells are known to finely tune T cell responses because of their ability to secrete a variety of cytokines, DCs, being professional antigen-presenting cells, have a quintessential role in the orchestration of the adaptive immune response. Our experiment of adoptive transfer of DCs demonstrated that using the intact mast cell c-Kit signaling that is present in the recipients to cripple c-Kit signaling only in adoptively transferred DCs blunts the T_H2 response, which prevents induction of full-blown allergic airway inflammation. Thus, although mast cells can modulate T cell responses, they are unable to supplant DCs in eliciting a strong T_H2 response. On the basis of these results, it seems probable that defective DC functions may be responsible for the reported lower susceptibility of *Kit^{W/W-v}* mice to allergic airways disease⁴⁴.

The importance of c-Kit signaling via PI3 kinase has also been explored in our study. Notably, *p110^{8D910A/D910A}* mice have been shown to be resistant to allergic responses, indicating a positive role for PI3 kinase in mediating T_H2 responses³⁵. A previous study has shown a negative role for PI3 kinase signaling in IL-12 p70 production from DCs⁴⁵. Our study shows a positive role for this pathway in IL-6 production from DCs, and our data suggest that sustained phosphorylation of Akt in DCs is required for the expression of specific cytokines, such as IL-6, that can influence the outcome of an immune response. Prolonged c-Kit expression coupled with the expression of membrane-bound SCF ensures sustained Akt activation, as is evident from our study and from previous studies, in which soluble SCF was shown to impede signaling via c-Kit³⁰, whereas membrane-bound SCF prolonged c-Kit signaling⁴⁶.

The cytokine balance in the microenvironment is a key regulator of the net immune response. Our study highlights the importance of this balance in regulating T cell responses by demonstrating a central role for c-Kit expressed by DCs in the fine-tuning of the IL-6–IL-12 p70 expression profile. Our findings also have ramifications for DC-based vaccines in cancer therapy, where inhibition of c-Kit may promote the efficacy of the vaccine by augmenting a T_H1 response.

METHODS

Mice. We purchased BALB/c ByJ, C57BL/6, *Il6^{-/-}* (Stock number 002254), *Kit^{sl/sl-d}* (Stock number 100401), c-Kit mutant WBB6F1^{W/W-v} (*Kit^{W/W-v}*; Stock number 100410) and the WT control (*Kit^{+/+}*) from The Jackson Laboratory.

DO11.10 transgenic mice were originally provided by K. Murphy at Washington University, St. Louis and were bred at the animal facility at the University of Pittsburgh. We housed all mice under pathogen-free conditions and used them between 6 and 12 weeks of age. Within experiments, the mice were age and sex matched. All studies with mice were approved by the Animal Care and Use Committee at the University of Pittsburgh.

Cell culture. For cell culture, we used RPMI 1640 (Gibco) supplemented with 10% heat-inactivated FBS (Gemini), 100 U/ml of penicillin and 100 μ g/ml of streptomycin sulfate (Gibco), 1 mM sodium pyruvate (Gibco) and 50 μ M 2-mercaptoethanol (Sigma). All reagents used had <0.6 U/ml of LPS. For DC cultures, we removed the femur and tibia of mice and flushed out the bone marrow using a 27-gauge needle. We washed the cells and cultured them with 10 ng/ml recombinant murine granulocyte macrophage colony-stimulating factor (Peprotech) at 1×10^6 cells/ml. On the third day, we refed the cells with fresh medium and cytokine. On day 6, we harvested the loosely adherent cells. Prior to harvesting, the flask was gently shaken at 7 Hz/s for 5 min on a MicroMix shaker (DPC Instruments). We then subjected the cells to purification with magnetically labeled beads specific for mouse CD11c (Miltenyi Biotech). The labeled cells were passed through the magnetized column twice, and CD11c expression of the resulting cells was >98%. The contaminating population of CD3⁺ and CD19⁺ cells was negligible. The CD11c⁺ cells were considered immature DCs, as ascertained by low expression of CD40, CD86 and MHC class II (data not shown). We plated the immature DCs at 1×10^6 cells/ml in 12-well plates in medium containing 10 ng/ml GM-CSF with or without cholera toxin (List Biologicals Laboratories) or HDM (Greer Laboratories) for 24 h. We used cholera toxin at 1 μ g/ml and HDM at 10 μ g/ml, both dissolved in PBS, for stimulating the DCs unless specified otherwise. We stripped both the OVA and the HDM preparations of lipopolysaccharide (LPS) with an EndoTrap column (Profos AG). We estimated the residual LPS in the solutions by the Limulus Amebocyte Lysate assay (Cambrex). The concentration of LPS in the cell cultures containing OVA or HDM was less than 0.1 ng/ml. The cholera toxin contained undetectable LPS as purchased. We isolated the lung and spleen DCs as described previously⁴⁷, although the lung DC protocol was slightly modified. We performed a high-volume lavage to isolate alveolar macrophages. After CD11c separation, we sorted the lung cells on a FACS ARIA machine on the basis of the autofluorescence. We sorted the low autofluorescence cells, which we considered to be dendritic cells, from the high autofluorescence cells, the macrophages, and used them in our experiments. For the inhibition of cAMP, we preincubated the BMDCs with the cAMP antagonist Rp-cAMPS, triethylammonium salt (Calbiochem) for 1 h before stimulation of cells. We harvested the cells and used them in various experiments. We obtained the CD4⁺ T cells from splenocytes as previously described²⁸.

Antibodies. We purchased antibodies for either flow cytometry or blocking experiments from BD Pharmingen unless otherwise stated. We used antibody to CD11c (allophycocyanin-labeled; clone HL3), antibody to CD40 (phycoerythrin (PE)-labeled; clone 3/23), antibody to CD86 (PE-labeled; clone GL1), antibody to CD117 (c-Kit; PE-labeled; clone 2B8) and antibody to MHC class II (Southern Biotech; PE-labeled clone NIMR-4), along with the appropriate isotype controls for flow cytometry. We used antibodies to GATA-3, T-bet, Jagged-2, Delta-4, CREB-1 (Santa Cruz Biotechnology), c-Kit, phospho-Akt (on Thr308) and Akt (Cell Signaling Technology) for western blot analysis. We used antibody to SCF from Chemicon for immunofluorescence studies and for detection of SCF by flow cytometry; we carried out indirect staining with antibody to SCF from Abcam together with PE-conjugated goat-antibody to rat IgG (Serotec). Antibody to IL-6 (clone MP5-20F3) and the isotype control were purchased from BD Pharmingen.

Intranasal antigen instillation. We performed intranasal administration of OVA–cholera toxin or OVA–CpG and *ex vivo* stimulation of single-cell suspensions of cells from lung-draining lymph nodes with OVA as described previously^{28,47}. We performed the experiments with HDM (Greer Laboratories) as described previously²³ with minor modifications. We anesthetized mice with isoflurane before intranasal administration of any antigen. In the experiments involving immunization of mice, 25 μ l of the instilled solution contained 10 ng

of residual LPS. We carried out intranasal administration of 25 µg of HDM for 7 consecutive d, and we rested the mice for 3 d. We stimulated single-cell suspensions of the lung-draining lymph nodes *ex vivo* with HDM (10 µg/ml). We prepared nuclear extracts from OVA- or HDM-stimulated cells after 5 d. The CpG ODN 1826 (5-TCCATGACGTTCTGACGTT-3), which is known to be optimal for stimulation of mouse cells, and the control ODN 1911 (5-TCCAGGACTTTCCTCAGGTT-3) were used at a concentration of 1 µM to stimulate DCs. The control ODN did not influence cytokine production or expression of any molecules in DCs in initial experiments, as previously reported by us, and was not used in all experiments⁴⁷. The LPS concentration in the ODN preparations was low (<0.1 ng/mg DNA). We purchased the oligonucleotides from Oligos Etc.

Adoptive transfer of DCs into mice and induction of allergic airway inflammation. We isolated CD11c⁺ BMDCs from *Kit*^{+/+} and *Kit*^{W/W^{-v}} mice and incubated them overnight with OVA (100 µg/ml) and cholera toxin (1 µg/ml). We harvested the cells, washed them and adoptively transferred 1×10^6 cells intratracheally into C57BL/6 recipients. After transfer, we rested the mice 10 d and then challenged them by exposure to aerosolized 1% OVA for 7 consecutive d with an ultrasonic nebulizer (Omron Healthcare). We killed the mice and examined the lungs for inflammation 24 h after the last aerosol. We performed cell counting in the BAL fluid and lung histology as previously described^{6,28,48}, and we performed PAS staining according to the manufacturer's instructions (Richard Allan Scientific)⁴⁸. For tracking of the cells *in vivo*, we labeled them with CFSE dye after stimulation with OVA and cholera toxin. We labeled 10×10^6 BMDCs with CFSE (1 µM, Invitrogen) for 15 min at 37 °C. We stopped the labeling by washing the cells twice with PBS containing 2% FBS. We then counted the cells again and suspended them at a concentration of 1×10^6 BMDCs in 50 µl of PBS.

Flow cytometry. The staining procedures were essentially as described previously²⁸. We gated the live cells using propidium iodide staining and collected at least 10,000 events within the live gate with a FACSCalibur flow cytometer. We performed staining for SCF by first adding the unconjugated primary antibody (1 µg) and incubating on ice for 1 h. We washed the tubes and added the secondary antibody (PE-conjugated goat antibody to rat IgG) at the same concentration and incubated for 0.5 h. We analyzed the data with FlowJo software (Tree Star).

Cell extracts and immunoblotting. We prepared nuclear extracts and total cell lysates as stated previously^{24,49}. We prepared total cell lysates from DCs at 24 h after stimulation using $1 \times$ cell lysis buffer (Cell Signaling).

Microarray analysis. We performed the isolation of RNA and microarray analysis essentially as described previously²⁴. We isolated RNA from BMDCs incubated with or without cholera toxin (1 µg/ml) for 24 h. We assessed total RNA quality before cRNA target preparation and labeling. We synthesized cRNA from the total RNA samples with the CodeLink Expression Assay Reagent Kit (GE Healthcare). We fragmented the labeled cRNA and hybridized it to CodeLink UniSet Mouse I Bioarrays (GE Healthcare), which identify 10,012 unique mouse genes. We scanned the hybridized bioarrays with the GenePix 4000B Array Scanner (Axon Instruments). The normalized intensities of all slides were then imported into Spotfire to create one file, which included annotations supplied by CodeLink. Only the 'discovery' genes and genes with good- or low-quality flags were selected with the Spotfire query devices. These selected genes were then exported into Excel. Then the data was formatted into the acceptable form for the DOS-based data analysis program Scoregene. Scoregene globally normalized the data by calculating the geometric mean across the controls and experimentals and then log-transformed the data to base 2. *t*-test *P* values were also calculated by Scoregene. Genes with *t*-test *P* values <0.05 and a fold change of 1.5 (SLR signal log ratio of 0.5849625) were determined to be statistically significant and selected for hierarchical clustering in Spotfire. The clustering method used was UPGMA (unweighted pair-group method average) with a Euclidean distance similarity measurement. Genes were considered to be significantly upregulated or downregulated if the fold change was greater than 1.5 or less than -1.5. We isolated RNA in three independent experiments from control and cholera toxin-treated BMDCs and pooled bone marrows from at least four mice in each experiment.

Immunofluorescence. We cytospun 1×10^5 cells onto slides and fixed them with 95% ethanol and 5% glacial acetic acid. We blocked the slides with 2% goat serum diluted in PBS and incubated them overnight at 4 °C with the primary antibody to SCF. We washed the slides with PBS and incubated them with the secondary antibody tagged with Cy3 for visualization. We mounted the slides with mounting medium Vectashield containing DAPI stain (Vector Laboratories) to identify the nuclei. We ensured the specificity of staining by using control slides in which the incubation with the primary antibody was skipped.

Enzyme-linked immunosorbent assay. We performed ELISAs for IL-12 p70, IL-6, IL-4, IL-13 and IFN-γ with kits from R&D Systems per the manufacturer's instructions. We performed ELISA for soluble SCF with a kit from Peprotech. Unless otherwise indicated, we carried out all statistical analyses using mean values of triplicate wells, and the results shown are representative of two to four independent experiments. We measured OVA-specific IgE abundance as previously described²⁸. We derived the concentration of IgE from standard curves generated with pooled sera from mice immunized with OVA-alum and repeatedly challenged with aerosolized OVA, and we assigned the standard an arbitrary unit of 10,000 U/ml.

Enzyme-linked immunosorbent spot. We performed the IL-13, IL-17 and IFN-γ ELISPOT assays with kits from eBioscience per the manufacturer's specifications. Briefly, we precoated ELISPOT plates (Millipore 96-well Multi-Screen HTS) with the primary antibody at 4 °C overnight. We plated lymph node cells at a concentration of 1×10^5 or 1×10^4 per well and stimulated them with PMA (25 ng/ml) and ionomycin (500 ng/ml) for 24 h. We used biotin-conjugated secondary antibody to detect the secreted cytokine. We developed the plates with Avidin-horseradish peroxidase and peroxidase substrate (Vectastatin). We quantified the SFU with an automated ELISPOT plate reader (ImmunoSpot; Cellular Technology).

Statistical analyses. We used Student's unpaired two-tailed *t*-test for all statistical analyses. Differences between groups were considered significant when *P* < 0.05. All statistical analyses (except those performed for microarray data) were performed with GraphPad Prism software.

Accession codes. Gene Expression Omnibus microarray accession code, GSE10815.

Note: Supplementary information is available on the Nature Medicine website.

ACKNOWLEDGMENTS

We dedicate this study to the memory of Professor Ray Wu, who inspired us to tread the untrodden path. We would like to thank K. Murphy (Washington University, St. Louis) for DO11.10 transgenic mice, A. Henry and B. Dixon-McCarthy for technical assistance with the adoptive transfer experiment and assessment of airway inflammation, M. Zheng and J.K. Kolls for help with the *Klebsiella* infection model, S. Plevy for providing us with breeding pairs of p110δ^{D910A/D910A} mice, and S. Shapiro for critically reading the manuscript. This work was supported by US National Institutes of Health grants HL 060207 and HL 069810 (to P.R.), HL 077430 and AI 048927 (to A.R.) and HL 084932 (to P.R. and A.R.).

AUTHOR CONTRIBUTIONS

N.K., T.B.O. and M.E. performed *in vitro* and *in vivo* experiments. M.P. and M.Y. performed microarray and PCR experiments. B.V. provided PI3 kinase-mutant mice and critically read the manuscript. N.K. and T.B.O. also participated in study design and N.K. prepared the manuscript. P.R. and A.R. conceived and designed the study, analyzed the data and wrote the manuscript.

Published online at <http://www.nature.com/naturemedicine>
Reprints and permissions information is available online at <http://npg.nature.com/reprintsandpermissions>

1. Kapsenberg, M.L. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat. Rev. Immunol.* **3**, 984–993 (2003).
2. Park, H. *et al.* A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* **6**, 1133–1141 (2005).
3. Harrington, L.E. *et al.* Interleukin 17-producing CD4⁺ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* **6**, 1123–1132 (2005).

4. Zhang, D.H., Cohn, L., Ray, P., Bottomly, K. & Ray, A. Transcription factor GATA-3 is differentially expressed in murine T_H1 and T_H2 cells and controls T_H2-specific expression of the interleukin-5 gene. *J. Biol. Chem.* **272**, 21597–21603 (1997).
5. Zheng, W. & Flavell, R.A. The transcription factor GATA-3 is necessary and sufficient for T_H2 cytokine gene expression in CD4 T cells. *Cell* **89**, 587–596 (1997).
6. Das, J. *et al.* A critical role for NF κ B in GATA3 expression and T_H2 differentiation in allergic airway inflammation. *Nat. Immunol.* **2**, 45–50 (2001).
7. Zhu, J. *et al.* Conditional deletion of *Gata3* shows its essential function in T_H1–T_H2 responses. *Nat. Immunol.* **5**, 1157–1165 (2004).
8. Ivanov, I.I. *et al.* The orphan nuclear receptor ROR γ t directs the differentiation program of proinflammatory IL-17⁺ T helper cells. *Cell* **126**, 1121–1133 (2006).
9. Szabo, S.J. *et al.* A novel transcription factor, T-bet, directs T_H1 lineage commitment. *Cell* **100**, 655–669 (2000).
10. Rincon, M., Anguita, J., Nakamura, T., Fikrig, E. & Flavell, R.A. Interleukin (IL)-6 directs the differentiation of IL-4-producing CD4⁺ T cells. *J. Exp. Med.* **185**, 461–469 (1997).
11. Dodge, I.L., Carr, M.W., Cernadas, M. & Brenner, M.B. IL-6 production by pulmonary dendritic cells impedes T_H1 immune responses. *J. Immunol.* **170**, 4457–4464 (2003).
12. Bettelli, E. *et al.* Reciprocal developmental pathways for the generation of pathogenic effector T_H17 and regulatory T cells. *Nature* **441**, 235–238 (2006).
13. Acosta-Rodriguez, E.V., Napolitani, G., Lanzavecchia, A. & Sallusto, F. Interleukins 1 β and 6 but not transforming growth factor- β are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat. Immunol.* **8**, 942–949 (2007).
14. Kolls, J.K. & Linden, A. Interleukin-17 family members and inflammation. *Immunity* **21**, 467–476 (2004).
15. Borg, C. *et al.* Novel mode of action of c-Kit tyrosine kinase inhibitors leading to NK cell-dependent antitumor effects. *J. Clin. Invest.* **114**, 379–388 (2004).
16. Chabot, B., Stephenson, D.A., Chapman, V.M., Besmer, P. & Bernstein, A. The proto-oncogene c-Kit encoding a transmembrane tyrosine kinase receptor maps to the mouse *W* locus. *Nature* **335**, 88–89 (1988).
17. Geissler, E.N., Ryan, M.A. & Housman, D.E. The dominant-white spotting (*W*) locus of the mouse encodes the c-Kit proto-oncogene. *Cell* **55**, 185–192 (1988).
18. Qiu, F.H. *et al.* Primary structure of c-Kit: relationship with the CSF-1/PDGF receptor kinase family—oncogenic activation of v-Kit involves deletion of extracellular domain and C terminus. *EMBO J.* **7**, 1003–1011 (1988).
19. Amsen, D. *et al.* Instruction of distinct CD4 T helper cell fates by different Notch ligands on antigen-presenting cells. *Cell* **117**, 515–526 (2004).
20. Braun, M.C., He, J., Wu, C.Y. & Kelsall, B.L. Cholera toxin suppresses interleukin (IL)-12 production and IL-12 receptor β 1 and β 2 chain expression. *J. Exp. Med.* **189**, 541–552 (1999).
21. Veldhoen, M., Hocking, R.J., Atkins, C.J., Locksley, R.M. & Stockinger, B. TGF- β in the context of an inflammatory cytokine milieu supports *de novo* differentiation of IL-17-producing T cells. *Immunity* **24**, 179–189 (2006).
22. Illi, S. *et al.* Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* **368**, 763–770 (2006).
23. Cates, E.C. *et al.* Intranasal exposure of mice to house dust mite elicits allergic airway inflammation via a GM-CSF-mediated mechanism. *J. Immunol.* **173**, 6384–6392 (2004).
24. Arora, M. *et al.* Simvastatin promotes T_H2-type responses through the induction of the chitinase family member Ym1 in dendritic cells. *Proc. Natl. Acad. Sci. USA* **103**, 7777–7782 (2006).
25. Bagley, K.C., Abdelwahab, S.F., Tuskan, R.G., Fouts, T.R. & Lewis, G.K. Cholera toxin and heat-labile enterotoxin activate human monocyte-derived dendritic cells and dominantly inhibit cytokine production through a cyclic AMP-dependent pathway. *Infect. Immun.* **70**, 5533–5539 (2002).
26. Crackower, M.A. *et al.* Regulation of myocardial contractility and cell size by distinct PI3K–PTEN signaling pathways. *Cell* **110**, 737–749 (2002).
27. Schulz, O. *et al.* CD40 triggering of heterodimeric IL-12 p70 production by dendritic cells *in vivo* requires a microbial priming signal. *Immunity* **13**, 453–462 (2000).
28. Oriss, T.B. *et al.* Dynamics of dendritic cell phenotype and interactions with CD4⁺ T cells in airway inflammation and tolerance. *J. Immunol.* **174**, 854–863 (2005).
29. Happel, K.I. *et al.* Divergent roles of IL-23 and IL-12 in host defense against *Klebsiella pneumoniae*. *J. Exp. Med.* **202**, 761–769 (2005).
30. Yee, N.S., Langen, H. & Besmer, P. Mechanism of Kit ligand, phorbol ester, and calcium-induced down-regulation of c-Kit receptors in mast cells. *J. Biol. Chem.* **268**, 14189–14201 (1993).
31. Flanagan, J.G., Chan, D.C. & Leder, P. Transmembrane form of the Kit ligand growth factor is determined by alternative splicing and is missing in the *Sld* mutant. *Cell* **64**, 1025–1035 (1991).
32. Brannan, C.I. *et al.* Steel-Dickie mutation encodes a c-Kit ligand lacking transmembrane and cytoplasmic domains. *Proc. Natl. Acad. Sci. USA* **88**, 4671–4674 (1991).
33. Serve, H. *et al.* Differential roles of PI3-kinase and Kit tyrosine 821 in Kit receptor-mediated proliferation, survival and cell adhesion in mast cells. *EMBO J.* **14**, 473–483 (1995).
34. Franke, T.F. *et al.* The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. *Cell* **81**, 727–736 (1995).
35. Ali, K. *et al.* Essential role for the p110 δ phosphoinositide 3-kinase in the allergic response. *Nature* **431**, 1007–1011 (2004).
36. Okkenhaug, K. *et al.* Impaired B and T cell antigen receptor signaling in p110 δ PI 3-kinase mutant mice. *Science* **297**, 1031–1034 (2002).
37. Kalesnikoff, J. *et al.* RabGEF1 regulates stem cell factor/c-Kit-mediated signaling events and biological responses in mast cells. *Proc. Natl. Acad. Sci. USA* **103**, 2659–2664 (2006).
38. Ostroukhova, M. *et al.* T_{reg}-mediated immunosuppression involves activation of the Notch-HES1 axis by membrane-bound TGF- β . *J. Clin. Invest.* **116**, 996–1004 (2006).
39. Houde, C. *et al.* Overexpression of the NOTCH ligand JAG2 in malignant plasma cells from multiple myeloma patients and cell lines. *Blood* **104**, 3697–3704 (2004).
40. Amsen, D. *et al.* Direct regulation of Gata3 expression determines the T helper differentiation potential of Notch. *Immunity* **27**, 89–99 (2007).
41. Fang, T.C. *et al.* Notch directly regulates Gata3 expression during T helper 2 cell differentiation. *Immunity* **27**, 100–110 (2007).
42. Luty, W.H., Rodeberg, D., Parness, J. & Vyas, Y.M. Antiparallel segregation of notch components in the immunological synapse directs reciprocal signaling in allogeneic T_H1:DC conjugates. *J. Immunol.* **179**, 819–829 (2007).
43. Chatterjea, D. *et al.* Adoptive transfer of mast cells does not enhance the impaired survival of *Kit^W/Kit^{W-v}* mice in a model of low dose intraperitoneal infection with bioluminescent *Salmonella typhimurium*. *Immunol. Lett.* **99**, 122–129 (2005).
44. Lukacs, N.W. *et al.* Stem cell factor (c-Kit ligand) influences eosinophil recruitment and histamine levels in allergic airway inflammation. *J. Immunol.* **156**, 3945–3951 (1996).
45. Fukao, T. *et al.* PI3K-mediated negative feedback regulation of IL-12 production in DCs. *Nat. Immunol.* **3**, 875–881 (2002).
46. Miyazawa, K. *et al.* Membrane-bound Steel factor induces more persistent tyrosine kinase activation and longer life span of c-Kit gene-encoded protein than its soluble form. *Blood* **85**, 641–649 (1995).
47. Chen, L. *et al.* Distinct responses of lung and spleen dendritic cells to the TLR9 agonist CpG oligodeoxynucleotide. *J. Immunol.* **177**, 2373–2383 (2006).
48. Zhang, D.H. *et al.* Inhibition of allergic inflammation in a murine model of asthma by expression of a dominant-negative mutant of GATA-3. *Immunity* **11**, 473–482 (1999).
49. Ostroukhova, M. *et al.* Tolerance induced by inhaled antigen involves CD4⁺ T cells expressing membrane-bound TGF- β and FOXP3. *J. Clin. Invest.* **114**, 28–38 (2004).