Published in final edited form as:

Clin Immunol. 2010 December ; 137(3): 406–414. doi:10.1016/j.clim.2010.09.001.

Altered cytokine production by dendritic cells from infants with atopic dermatitis

Weiguo Yao^{a,b}, JiHoon Chang^e, Sarita Sehra^{a,b}, Jeffrey B. Travers^{b,c}, Cheong-Hee Chang^e, Robert S. Tepper^{a,b}, and Mark H. Kaplan^{a,b,d}

^a Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN 46202

^b Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN 46202

^c Department of Dermatology, Indiana University School of Medicine, Indianapolis, IN 46202

^d Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN 46202

^e Department of Microbiology and Immunology, University of Michigan, Ann Arbor MI

Abstract

Dendritic cells (DC) are potent initiators of immune responses, compared to other professional antigen-presenting cells, based on their ability to capture antigen, express high amounts of MHC and co-stimulatory molecules, and to secrete immunostimulatory cytokines. Altered functions of DC in atopic individuals have been observed, though it is not clear if this is a cause or a result of the development of allergic disease. In this report we demonstrate altered cytokine production by DC isolated from infants with atopic dermatitis but without a diagnosis of asthma, compared to infants with non-atopic dermatitis. Increased production of IL-6, IL-10 and IFN α from DC isolated from atopic infants is less apparent when DC from infants were examined one year later. An increase in the same cytokines was observed in neonatal mice that are genetically predisposed towards allergic inflammation. These results suggest that an atopic environment promotes altered cytokine production by DC from infants.

Introduction

The link between atopic immune responses (high serum IgE, allergen-specific IgE, production of Th2 cytokines such as IL-4 and IL-13) and the development of asthma is clear and has been extensively evaluated in the literature [1;2;3]. However, the cells responsible for initiating the development of allergic responses are still not clear. Recent work has supported an important role for basophils as antigen presenting cells in initiating allergic inflammation [4]. However, it is well established that dendritic cells (DC) also contribute to the development of allergic inflammation [5;6;7]. DC capture allergen in target organs and activate T cells to initiate the immune response, and can largely be divided in two populations, myeloid or conventional DC

© 2010 Elsevier Inc. All rights reserved.

Address Correspondence to: Mark H. Kaplan, Ph.D. Department of Pediatrics Indiana University School of Medicine HB Wells Center for Pediatric Research 1044 West Walnut St. Room 202 Indianapolis, IN 46202 317-278-3696 mkaplan2@iupui.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

(cDC) and lymphoid or plasmacytoid DC (pDC). While cDC predominate as a percentage in adult blood, pDC predominate in cord blood [8], suggesting that there are age-dependent changes in DC homeostasis.

Previous studies have examined DC numbers and function in atopic patients. No differences were observed in cDC or pDC numbers in cord blood from infants with high or low risk of atopy [9], but pDC numbers were decreased in asthmatic children or in children that had previous respiratory syncytial virus infection [9;10] and numbers of pDC are inversely correlated with the development of subsequent wheezing and asthma [11]. Atopic children did have lower numbers of cDC than non-atopic children, although these differences were not significant when corrected for age, race, skin prick test positivity and environmental factors [10]. Functionally, pDC in atopic children or adults produce less IFN α in response to virus infection than healthy controls [12;13]. Moreover, TLR9 agonist-stimulated IFN α production is decreased from adult allergic patients, compared to controls, though decreases were not associated with changes in pDC numbers or in TLR9 expression [14]. Adults with atopic dermatitis had cDC that produced lower IL-12 and pDC that produced lower IFN α than DC from healthy controls [15]. Together these data suggest that atopic individuals may have decreased pDC following the onset of atopic disease, and that both cDC and pDC may have decreased production of cytokines such as IL-12 and IFN α that promote Th1 development and anti-viral immunity.

Importantly, previous studies have examined children 6 years of age or older, following a diagnosis of asthma, and no studies have examined the function of DC in infants pre-disposed to atopic disease. This may be important since there are age-related changes in DC numbers and function [8]. In a population of infants with atopic or non-atopic dermatitis, we have shown that increasing percentages of cDC are associated with a protective effect from the development of wheezing episodes in subsequent years [16]. In this report we have examined DC function in infants upon entry into the study and one year later. We demonstrate higher numbers of pDC than cDC in peripheral blood, similar to previous observations with cord blood. DC numbers were not significantly different between atopic and non-atopic infants upon initial evaluation. However, DC from atopic infants had increased production of several cytokines. Similar observations were made in cytokine production from DC from neonatal mice prone to allergic inflammation, suggesting that an atopic environment initially alters DC cytokine production.

Methods

Patient samples

The patient population in this study has been described in detail elsewhere [16;17]. Briefly, infants were enrolled in the study between the ages of 2 and 19 months when they presented in the clinic with dermatitis. There were 116 subjects recruited into the study, and 98 remained in the study at the second visit one year later. Table I summarizes the populations studied at the initial and follow-up visit. There were no significant differences between the atopic and non-atopic group in sex, gender or age. Total IgE levels were significantly higher in atopic infants versus non-atopic infants ($p < 0.005$) at both visits, though there was no difference in the family history of asthma between atopic and non-atopic infants. Peripheral blood mononuclear cells were isolated from blood using Ficoll-paqueTM PLUS (GE Healthcare, Sweden) and collected from infants at both visits before cryopreservation at 10^7 cells/ml/vial using Opti-Freeze DMSO Cryopreservation Medium (Fisher) in liquid nitrogen for 6 to 12 months. Because of limited sample volume, only 69 subjects were examined by flow cytometry at the first visit, and 92 subjects at the second visit. There were no significant demographic differences between the total population and the subset used for flow cytometry. Atopy was defined as positivity for IgE titers above 0.35 IU/ml for at least one of 10 allergens (egg white, milk, wheat, cat, house dust mite, timothy grass, Bermuda grass, ragweed, alternaria, and cedar)

as tested by ImmunoCap. All patient studies were approved by the Indiana University School of Medicine Institutional Review Board.

Human DC purification and culture

Dendritic cell subsets were analyzed and purified using reagents from Miltenyi Biotec. The percentages of blood dendritic cells was detected using the Human Blood Dendritic Cell Enumeration Kit to identify cells that are CD19-, CD14-, and positive for either BDCA-1 (cDC) or BDCA-2 (pDC) using flow cytometry. To purify cDC and pDC, the CD1c Dendritic Cell Isolation Kit and CD304 Microbead Kit were used, respectively, to purify cells that are positive for BDCA-1 antigen positive (cDC) or BDCA-4/Neuropilin-1 positive (pDC) according to the manufacturer's instructions (Miltenyi Biotec). The yield of DC was 1-1.5% of the total PBMC, and purity of DC after selection was greater than 90%. Total PBMC or purified DC subsets were stimulated with control or CpG-ODN (1 μ M; Invivogen) or poly (I:C) (50ug/ml; Invivogen) for 24 hours. Amounts of cytokines in cell-free supernatants were tested using Multiplex bead assay as described [18]. Each Multiplex reagent is non-crossreactive to other cytokines and cytokine concentrations across assays were similar. Sensitivity of the assay was <3 pg/ml for each cytokine, and there were no samples in which cytokines were not detected. Statistics were performed using a student's T test.

Stat6VT transgenic mice

The generation of Stat6VT transgenic mice was previously described [19]. Transgene positive founders (Stat6VT (78) line), where the human Stat6 gene with V546 and T547 mutated to alanine is under transcriptional control of the CD2 locus control region, were backcrossed to C57BL/6 mice (Harlan Laboratories). Controls for experiments were transgene-negative littermates, or C57BL/6 mice (Harlan). All mice were maintained in specific pathogen-free conditions and experiments were approved by the Indiana University Institutional Animal Care and Use Committee.

Mouse DC isolation and culture

Flow cytometry was performed using standard protocols with the antibodies indicated in the Figure. Mouse splenic DCs were isolated by positive selection with anti-CD11c magnetic beads following manufacturer's instructions (Miltenyi Biotec). Purity of CD11c+ cells was 65-75%. Isolated splenic DCs were stimulated with control or CpG-ODN (Invivogen) for 24 hours. Cell free supernatants were collected. IFN α level was detected using a mouse IFN α ELISA Kit from PBL Biomedical Laboratories. Amounts of IL-6 and IL-10 were determined using ELISA (reagents from BD Biosciences, San Jose CA).

Western analysis for transgene expression

Total cell extracts (50 μ g) were prepared from the spleen, thymus and lymph nodes of STAT6VT transgenic mice, and splenic DCs isolated as described above from WT and STAT6VT spleen cells. Cells were homogenized in ice-cold lysis buffer containing 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 2 mM EDTA, 10% glycerol, 150 mM NaCl, 50 mM Tris (pH 7.4), and protein inhibitors (1 mM phenylmethanesulphonylfluoride, aprotinin at 1 mg/ml, and leupeptin at 1 mg/ml). Western blot analysis was performed as previously described [20] using specific Abs against FLAG (Sigma-Aldrich, St. Louis, MO) and GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA) as loading control.

Results

Altered DC subsets in atopic infants

To examine DC subsets in the peripheral blood of infants with atopic and non-atopic dermatitis, we examined the percentages of cells that are CD19-, CD14-, and positive for either BDCA-1 (cDC) or BDCA-2 (pDC) using flow cytometry. At an initial visit, with infants aged 2-19 months, there were no significant differences between the atopic (n=48) and non-atopic (n=21) groups in either cDC or pDC (Fig. 1). Consistent with previous reports there were higher percentages of pDC than cDC [8]. We then analyzed the same patient group one year later. The percentages of pDC were increased, but were not significantly different between the atopic (n=57) and non-atopic (n=35) groups (Fig. 1). The percentages of cDC did not increase significantly in the non-atopic group, but there was a significant increase of cDC in the atopic group compared to the non-atopic group ($p=0.016$) (Fig. 1).

Altered cytokine production in DC from atopic infants

We then tested the responses of total PBMCs to TLR ligand stimulation from a subset of patient samples where there were sufficient numbers of cells available for further characterization. There were no significant differences in demographics or total serum IgE among subjects used for TLR ligand stimulation compared to the entire population. PBMCs from non-atopic (n=7) or atopic (n=13) infants were stimulated with control or CpG-oligodeoxynucleotides (CpG-ODN), a ligand for the endosomal TLR9 receptor expressed predominantly by B cells and pDC [21;22;23;24], and cytokine production was assessed using multiplex reagents. IFN α and IL-10 production was increased following stimulation with CpG-ODN, and production was significantly increased in cultures from atopic infants compared to those from non-atopic infants (Fig. 2A). CpGODN induction of IL-6 was modest in cultures from non-atopic infants, but was significantly induced in cultures from atopic infants (Fig. 2A). In contrast there were no significant differences in TNF α , IL-1 β or IL-12 production (data not shown). The significant increases in IFN α and IL-6 were consistent in cultures from atopic infants at the follow-up visit one year later (Fig. 2B). There were similar trends in IL-10, although the differences were not significant.

Since multiple cell types can respond to CpG-ODN [21;22;23;24], we then purified DC subsets from PBMCs before stimulation. Purified cDC demonstrated minimal responses to CpG-ODN (data not shown) consistent with their lack of TLR9 expression [21;22;24;25]. To test responses of these cells, we stimulated cDC from non-atopic (n=8) and atopic (n=9) infants with poly I:C, a ligand for TLR3, another endosomal receptor expressed in cDC [21;22;25;26]. At the initial visit, cDC from atopic infants secreted significantly more IFN α , IL-6 and IL-10 ($p<0.05$) than cDC from non-atopic infants (Fig. 3A). At the follow up visit, cDC from atopic infants still secreted more IFN α and IL-10 ($p<0.05$) than non-atopic cDC (Fig. 3B). However, the trend towards increased IL-6 in the cDC cultures from atopic infants was not significantly different from the cultures from non-atopic infants (Fig. 3B).

Stimulation of purified pDCs with CpG-ODN demonstrated similar results. At the initial visit, pDC from atopic infants (n=9) secreted significantly more IFN α and IL-10 ($p<0.05$), although IL-6 was not significantly increased, compared to non-atopic (n=8) cultures (Fig. 4A). At the follow up visit, only the increase in IFN α was still significant (Fig. 4B). The pDC secreted 5-10 fold more IFN α than cDC, consistent with the role of these cells as primary type I IFN producers [27].

DC function in STAT6VT transgenic mice

Data from the previous experiments suggested that an atopic environment might alter the cytokine production from DC, perhaps by affecting TLR signaling. To test if an atopic

environment alters DC cytokine production, we used a model wherein mice express a constitutively active STAT6 in T cells but not in dendritic cells (Fig. 5A) [19]. These mice have increased Th2 development and are predisposed towards the development of allergic inflammation [19;28;29]. To test if the development of DC is altered by the expression of active STAT6 in T cells, we examined the percentages of splenic DC subsets in wild type and STAT6VT transgenic mice before the development of any atopic disease. We observed that the percentages of pDC (B220+, F4/80-, CD11c+) and cDC (B220-, F4/80-, CD11c+) were similar in spleens from wild type and STAT6VT transgenic mice (Fig. 5B). Both populations contained similar percentages of MHC II+ cells. Thus, the development of DC subsets is not altered in STAT6VT transgenic mice.

Since our initial observations were in infants, we examined DC in STAT6VT transgenic mice 2 weeks after birth, at the time of weaning (4 weeks) and in adult mice. CD11c+ cells isolated from 2-week old STAT6VT transgenic neonates demonstrated the same pattern of increases in cytokines (IFN α , IL-6, IL-10), compared to non-transgenic controls (Fig. 6A), that was observed from infant DC. However, this was not a stable phenotype since there was no significant difference between DC from control and STAT6VT transgenic mice at 4 weeks or 28 weeks of age (Fig. 6B and C). Thus, these results suggest that the presence of a pro-allergic environment at a young age promotes an increase in the production of particular cytokines.

Discussion

Dendritic cells scan the environment at mucosal interfaces to acquire and present foreign antigen. This DC function is critical for the initiation of both protective anti-pathogen immune responses and deleterious responses including allergies. The subsequent initiation of adaptive responses is directed by the cytokine secretion profile of the APC. Several reports have suggested that DC from atopic patients have altered cytokine production. In this report we have examined cytokine production by DC isolated from infants with atopic or non-atopic dermatitis. We demonstrate increased production of several cytokines by DC subsets in response to TLR ligand stimulation. However, these changes are likely specific to the early time point examined because differences in cytokine production were less apparent in the population one-year later, and published reports suggest diminished production of some of these cytokines by older atopic patient DC [12;13]. In parallel studies we show that transgenic mice predisposed to atopic disease also have increases in the same cytokines increased in infant samples at a time point before weaning. Thus, this altered cytokine production may be a feature of DC early in the development of an atopic environment.

The development of allergic diseases in mice expressing a constitutively active STAT6 in T cells has been previously described [28;29]. However, DC development or function in these mice has not been extensively examined. We observe that cDC and pDC development is normal in transgenic mice, but that there is altered cytokine production in DC from neonatal mice. At 2 weeks of age TLR9 ligands stimulated increased IL-6, IL-10 and IFN α production by CD11c+ DC isolated from transgenic mice, compared to control mice. However, increased cytokine production is not a stable phenotype since cytokine production by DC from transgenic mice is indistinguishable from control DC by the age of weaning (4 weeks) or in older mice (~28 weeks). Importantly, this is an effect of the atopic environment in transgenic mice since the transgene is expressed in T cells but not DC [19;28](Figure 5A), and cytokine production by bone-marrow-derived DC from control and STAT6VT transgenic mice was indistinguishable (data not shown). This is consistent with the observation that monocyte-derived DC from atopic individuals functioned similar to controls in response to TLR ligands [30]. One limitation of the parallels between the analysis of human and mouse cells is that differences could arise from factors that are unique to the mouse or human systems, such as the presence of specific DC subsets in each sample, and their responsiveness to TLR ligands which differ between species.

Thus, our interpretations could be overly simplistic. However, our results suggest that an atopic environment promoted by Th2 polarized cells results in altered cytokine production from DC in neonates.

Previous work has suggested that percentages of DC in the blood change during development. In cord blood, pDC predominate over cDC [8]. The percentages of both subsets expand over time [11], but at some point during development cDC become the predominant subset in adulthood [8]. Our results confirm the observations that pDC predominate in infants and that there are increases in percentages with age, although we are examining a more limited age range than previous studies [11]. Developmental stage-dependent changes in DC function might also explain the early increases in cytokine production from DC in atopic individuals. It is known that IL-4 can alter cytokine production by DC [31]. It is possible that in a sustained environment of IL-4, as would be present in an atopic individual, DC may become refractory to further stimulation. Alternatively, there may be a limited period for DC to respond to this environment during development because of changes in cytokine receptor expression, or the atopic environment might promote faster maturation of DC, although DC in non-atopic children eventually mature such that there are not differences between the groups as they age. Further analysis of this cohort as they age will explore these possibilities.

The age of the children examined in this study, as well as the methodology used in this study, likely account for some differences with other published studies. We observed an increase in the cDC population, using BDCA1 as a marker, at the second visit (age 15-30 months). Lebre et al did not observe any difference in cDC using the same marker, although the population examined were adults (age 20-50 years) and the number of subjects was smaller than our study [15]. Upham et al observed no increase in cDC percentages in infants similar in age to our study, though that study used CD11c as the primary marker [11]. Thus, both the age of the subjects and the antibodies used to identify cDC likely impact the conclusions of the study. Lebre et al also observed decreased IFN α from DC isolated from AD patients [15], opposite to our observations. As noted above, the previous study examined adults, and since we noted age-related changes in cytokine production during infancy, further changes in cytokine production may occur as the atopic subjects age. The continued study of our population through childhood will allow us to examine some of these questions.

There are several limitations to this study. The foremost is that the number and cytokine production of DC isolated from the blood may not reflect the number or function of DC in tissues of environmental exposure, such as airways and skin. It is difficult, in infants, to isolate DC from those tissues both technically, due to the small sample size, and ethically, due to increased risk to the subjects. Thus cells isolated from the peripheral blood represent an approximation of tissue DC function, similar to many other published studies [12;13;14;15]. Another limitation is in the comparison of different ligands for the isolated DC subsets. This is necessary due to the restricted expression of specific TLR on DC subsets [21;22;25;26]. However, the similar patterns of cytokine production among PBMCs stimulated with CpG-ODN or LPS (Fig. 2 and data not shown), pDC with CpG (Fig. 4) and cDC stimulated with poly(I:C) (Fig. 3) suggests that the effects observed are common to multiple Toll-like receptors.

There are obvious differences between the cytokines produced by stimulated PBMC and purified DC subsets as there are other CpG-responsive cells in total PBMC cultures, and DC represent a minor population of all PBMC. While IL-6, IL-10 and IFN α were all significantly increased in PBMC cultures from atopic infants, IFN α was the cytokine predominantly increased in pDC cultures. IFN α production was also increased in poly(I:C)-stimulated cDC cultures from atopic infants, though quantities were less than those produced by pDC. IL-6 was produced in greater concentrations from both DC subsets than from PBMC, though the differences between cultures from atopic or non-atopic infants were less dramatic from purified

DC. IL-10 was also increased in cDC cultures from atopic infants, and in concentrations considerably higher than from PBMC or pDC cultures. These differences among in purified DC versus total PBMC likely reflect the specialization of the cells; pDC characterized as potent IFN α producers for anti-viral activity [32], and cDC producing cytokines that effect multiple cell types by secreting IL-6 and IL-10 [5;6]. Moreover, altered cytokine production in cultures from atopic infants may reflect changes in TLR ligand responses in cells other than DC.

It is unclear whether the altered DC cytokine production is simply a result of an atopic environment, or whether the increases in IL-6, IL-10 and IFN α facilitate further allergic responses. The function of type I IFNs, particularly from pDCs, is thought to be anti-viral, and increases might be expected to aid in clearance of viral infections, which might decrease virus-induced wheezing. Indeed, we, and others have observed that increasing percentages of pDC and cDC in infants are negatively correlated with the development of subsequent wheezy illness or asthma [11;16]. However, IL-10, produced in the highest amounts by cDC, can inhibit Th1 cytokine production, and IL-6 can promote Th2 and Th17 development. Thus it is possible that the increases in cytokine production by DC may contribute to promoting the atopic environment. It is likely that the balance between the concentrations of these cytokines, and where they are locally expressed, will determine their effects on viral immunity and promoting allergic disease.

In this report we have examined the function of dendritic cell subsets in infants and mice prone to the development of atopic disease. We observed early increases in cytokine production from DC that suggests altered DC function during a limited time frame in vivo. These observations, and further exploring how they impact the atopic environment in vivo, will be important in determining the mechanisms promoting allergic disease.

Acknowledgments

This work was supported by grants from the NIH (U19 AI070448 to MHK, CHC, JBT and RST, RO1 HL080071 to RST).

References

1. Finkelman FD, Boyce JA, Vercelli D, Rothenberg ME. Key advances in mechanisms of asthma, allergy, and immunology in 2009. *J Allergy Clin Immunol* 2010;125:312–8. [PubMed: 20159240]
2. Finkelman FD, Hogan SP, Hershey GK, Rothenberg ME, Wills-Karp M. Importance of cytokines in murine allergic airway disease and human asthma. *J Immunol* 2010;184:1663–74. [PubMed: 20130218]
3. Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008;454:445–54. [PubMed: 18650915]
4. Sokol CL, Medzhitov R. Role of basophils in the initiation of Th2 responses. *Curr Opin Immunol* 2010;22:73–7. [PubMed: 20144855]
5. Hammad H, Lambrecht BN. Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma. *Nat Rev Immunol* 2008;8:193–204. [PubMed: 18301423]
6. Holt PG, Strickland DH, Wikstrom ME, Jahnsen FL. Regulation of immunological homeostasis in the respiratory tract. *Nat Rev Immunol* 2008;8:142–52. [PubMed: 18204469]
7. Lambrecht BN, Hammad H. Biology of lung dendritic cells at the origin of asthma. *Immunity* 2009;31:412–24. [PubMed: 19766084]
8. Borrás FE, Matthews NC, Lowdell MW, Navarrete CV. Identification of both myeloid CD11c+ and lymphoid CD11c- dendritic cell subsets in cord blood. *Br J Haematol* 2001;113:925–31. [PubMed: 11442485]
9. Hagendorens MM, Ebo DG, Schuerwegh AJ, Huybrechts A, Van Bever HP, Bridts CH, De Clerck LS, Stevens WJ. Differences in circulating dendritic cell subtypes in cord blood and peripheral blood of healthy and allergic children. *Clin Exp Allergy* 2003;33:633–9. [PubMed: 12752592]

10. Silver E, Yin-DeClue H, Schechtman KB, Grayson MH, Bacharier LB, Castro M. Lower levels of plasmacytoid dendritic cells in peripheral blood are associated with a diagnosis of asthma 6 yr after severe respiratory syncytial virus bronchiolitis. *Pediatr Allergy Immunol* 2009;20:471–6. [PubMed: 19140903]
11. Upham JW, Zhang G, Rate A, Yerkovich ST, Kusel M, Sly PD, Holt PG. Plasmacytoid dendritic cells during infancy are inversely associated with childhood respiratory tract infections and wheezing. *J Allergy Clin Immunol* 2009;124:707–13. e2. [PubMed: 19733903]
12. Bufer A, Gehlhar K, Grage-Griebenow E, Ernst M. Atopic phenotype in children is associated with decreased virus-induced interferon-alpha release. *Int Arch Allergy Immunol* 2002;127:82–8. [PubMed: 11893858]
13. Gehlhar K, Bilitewski C, Reinitz-Rademacher K, Rohde G, Bufer A. Impaired virus-induced interferon-alpha2 release in adult asthmatic patients. *Clin Exp Allergy* 2006;36:331–7. [PubMed: 16499644]
14. Tversky JR, Le TV, Bieneman AP, Chichester KL, Hamilton RG, Schroeder JT. Human blood dendritic cells from allergic subjects have impaired capacity to produce interferon-alpha via Toll-like receptor 9. *Clin Exp Allergy* 2008;38:781–8. [PubMed: 18318750]
15. Lebre MC, van Capel TM, Bos JD, Knol EF, Kapsenberg ML, de Jong EC. Aberrant function of peripheral blood myeloid and plasmacytoid dendritic cells in atopic dermatitis patients. *J Allergy Clin Immunol* 2008;122:969–976. e5. [PubMed: 18845325]
16. Yao W, Barbe-Tuana FM, Llapur CJ, Jones MH, Tiller C, Kimmel R, Kisling J, Nguyen ET, Nguyen J, Yu Z, Kaplan MH, Tepper RS. Evaluation of airway reactivity and immune characteristics as risk factors for wheezing early in life. *J Allergy Clin Immunol*. 2010 in press.
17. Tepper RS, Llapur CJ, Jones MH, Tiller C, Coates C, Kimmel R, Kisling J, Katz B, Ding Y, Swigonski N. Expired nitric oxide and airway reactivity in infants at risk for asthma. *J Allergy Clin Immunol* 2008;122:760–5. [PubMed: 18760452]
18. Travers JB, Kozman A, Mousdicas N, Saha C, Landis M, Al-Hassani M, Yao W, Yao Y, Hyatt AM, Sheehan MP, Haggstrom AN, Kaplan MH. Infected atopic dermatitis lesions contain pharmacologic amounts of lipoteichoic acid. *J Allergy Clin Immunol* 2010;125:146–52. e1–2. [PubMed: 19962742]
19. Bruns HA, Schindler U, Kaplan MH. Expression of a constitutively active Stat6 in vivo alters lymphocyte homeostasis with distinct effects in T and B cells. *J Immunol* 2003;170:3478–87. [PubMed: 12646608]
20. Chang HC, Zhang S, Thieu VT, Slee RB, Bruns HA, Laribee RN, Klemsz MJ, Kaplan MH. PU.1 expression delineates heterogeneity in primary Th2 cells. *Immunity* 2005;22:693–703. [PubMed: 15963784]
21. Hornung V, Rothenfusser S, Britsch S, Krug A, Jahrsdorfer B, Giese T, Endres S, Hartmann G. Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J Immunol* 2002;168:4531–7. [PubMed: 11970999]
22. Kadowaki N, Antonenko S, Liu YJ. Distinct CpG DNA and polyinosinic-polycytidylic acid double-stranded RNA, respectively, stimulate CD11c- type 2 dendritic cell precursors and CD11c+ dendritic cells to produce type I IFN. *J Immunol* 2001;166:2291–5. [PubMed: 11160284]
23. Krug A, Rothenfusser S, Hornung V, Jahrsdorfer B, Blackwell S, Ballas ZK, Endres S, Krieg AM, Hartmann G. Identification of CpG oligonucleotide sequences with high induction of IFN-alpha/beta in plasmacytoid dendritic cells. *Eur J Immunol* 2001;31:2154–63. [PubMed: 11449369]
24. Wagner H. Toll meets bacterial CpG-DNA. *Immunity* 2001;14:499–502. [PubMed: 11371352]
25. Jarrossay D, Napolitani G, Colonna M, Sallusto F, Lanzavecchia A. Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. *Eur J Immunol* 2001;31:3388–93. [PubMed: 11745357]
26. Kadowaki N, Ho S, Antonenko S, Malefyt RW, Kastelein RA, Bazan F, Liu YJ. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med* 2001;194:863–9. [PubMed: 11561001]
27. Ito T, Wang YH, Liu YJ. Plasmacytoid dendritic cell precursors/type I interferon-producing cells sense viral infection by Toll-like receptor (TLR) 7 and TLR9. *Springer Semin Immunopathol* 2005;26:221–9. [PubMed: 15592841]

28. Sehra S, Bruns HA, Ahyi AN, Nguyen ET, Schmidt NW, Michels EG, von Bulow GU, Kaplan MH. IL-4 is a critical determinant in the generation of allergic inflammation initiated by a constitutively active Stat6. *J Immunol* 2008;180:3551–9. [PubMed: 18292582]
29. Sehra S, Yao Y, Howell MD, Nguyen ET, Kansas GS, Leung DY, Travers JB, Kaplan MH. IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation. *J Immunol* 2010;184:3186–90. [PubMed: 20147633]
30. Terhorst D, Kalali BN, Weidinger S, Illig T, Novak N, Ring J, Ollert M, Mempel M. Monocyte-derived dendritic cells from highly atopic individuals are not impaired in their pro-inflammatory response to toll-like receptor ligands. *Clin Exp Allergy* 2007;37:381–90. [PubMed: 17359388]
31. Yao Y, Li W, Kaplan MH, Chang CH. Interleukin (IL)-4 inhibits IL-10 to promote IL-12 production by dendritic cells. *J Exp Med* 2005;201:1899–903. [PubMed: 15967820]
32. Asselin-Paturel C, Boonstra A, Dalod M, Durand I, Yessaad N, Dezutter-Dambuyant C, Vicari A, O'Garra A, Biron C, Briere F, Trinchieri G. Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. *Nat Immunol* 2001;2:1144–50. [PubMed: 11713464]

Figure 1. Dendritic cells in atopic and non-atopic infants

PBMCs from atopic (n=57) and non-atopic (n=35) infants at initial visit (age range 2-19 months) and one year later were examined for the percentages of cDCs (BDCA-1+) or pDCs (BDCA-2+) using flow cytometry. Cells shown are gated to eliminate CD14+, CD19+ and dead cells. Numbers indicate the mean percentages $\pm$ SEM of all patients analyzed. *, p=0.016.

Figure 2. Cytokine production by infant PBMCs

A, PBMCs from atopic (n=13) and non-atopic (n=7) infants at initial visit were stimulated with control or CpG-containing oligonucleotides for 24 hours before cell-free supernatants were tested for the concentration of the indicated cytokines using Multiplex assay. B, PBMCs from atopic (n=11) and non-atopic (n=10) infants at follow-up visit were stimulated with control or CpG-containing oligonucleotides for 24 hours before cell-free supernatants were tested for the concentration of the indicated cytokines using Multiplex assay. *, $p < 0.05$.

Figure 3. Cytokine production by infant cDC

Conventional DCs from atopic and non-atopic infants at initial visit (A) or follow-up visit (B) were stimulated with poly I:C for 24 hours before cell-free supernatants were tested for the concentration of the indicated cytokines using Multiplex assay. *, $p < 0.05$.

Figure 4. Cytokine production by infant pDC

Plasmacytoid DCs from atopic and non-atopic infants at initial visit (A) or follow-up visit (B) were stimulated with CpG-containing oligonucleotides for 24 hours before cell-free supernatants were tested for the concentration of the indicated cytokines using Multiplex assay.

*, $p < 0.05$.

Figure 5. Dendritic cells in atopic mice

A, Immunoblot analysis of STAT6VT transgene expression in the indicated organs and in purified CD11c+ dendritic cells from wild type (WT) or STAT6VT transgenic mice with antibodies specific for FLAG peptide tag of STAT6VT or GAPDH as a control. B, Flow cytometric analysis of splenic DC populations in wild type and STAT6VT transgenic mice. Analysis shows CD11b and CD11c expression on cDCs (left, B220-, F4/80-) and pDCs (right, B220+, F4/80-). Numbers represent the mean $\pm$ SEM of three mice of each genotype.

Figure 6. Cytokine production by DC in atopic mice

Splenic CD11c+ DC were isolated from wild type or STAT6VT Tg mice aged 2 weeks (B), 4 weeks (C) or 28 weeks (D) and stimulated with control or CpG-containing oligonucleotides for 24 hours before concentrations of the indicated cytokines were assayed using ELISA. Data are the mean $\pm$ SEM of 7-10 mice in each group. *, $p < 0.05$.

Table I

	Initial Evaluation	1-Year Follow-up	Initial (Dropped-out)	Initial (Retained)	P value*
Number of Subjects	116	98	18	98	
Age: months (mean $\pm$ SE)	10.7 $\pm$ 0.4	24.2 $\pm$ 0.5	9.57 $\pm$ 5.0	10.95 $\pm$ 4.6	0.252
Gender (% Males)	56/116 (48%)	49/98 (50%)	7/18 (39%)	49/98 (50%)	0.386
Race (% Caucasian)	53/116 (46%)	48/98 (49%)	5/18 (28%)	48/98 (49%)	0.097
Family History - Asthma/Allergy (% Yes)	89/115 (77%)	82/97 (85%)	11/18 (61%)	78/97 (80%)	0.120