Instruction of effector T cell programs by flexible dendritic cells

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CHAPTER 4

How to deal with polarized Th2 cells: exploring the Achilles' heel.

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Abstract
The central effector cells in the pathogenesis of atopic allergic diseases are Th2 cells, that display an aberrant cytokine profile, dominated by type 2 cytokines. Initial reports from mouse studies indicated that established and committed Th2 cells are stable and unsusceptible to modulation. However, there is a growing awareness that in humans, established effector Th2 cells are more flexible and can be reverted to predominant Th1 phenotypes. In fact, the Th1-driving cytokine IL-12 is the crucial factor in this respect. IL-12 is mainly produced by dendritic cells (DC), that can be primed for high or low IL-12 production, dependent on inflammatory and/or microbial signals they encounter during their residence in the peripheral tissues. Accordingly, both the regulation of and the priming for IL-12 production in DC form ideal targets for therapeutic intervention. The development of new therapies for atopic allergy now focuses on local IL-12-promoting substances to target both the development of new Th2 cells and the persistent population of established allergen-specific Th2 cells.

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**Introduction**

Atopic allergic disorders, like allergic asthma, allergic rhinitis and atopic dermatitis, are characterized by inflammatory responses to normally harmless antigens, accompanied by local tissue infiltration with mast cells, eosinophils and T helper (Th) lymphocytes (1). The immunological hallmark of this response is the predominant generation of type 2 T helper (Th2) cells and the consequent production of the type 2 cytokines interleukin (IL)-4, IL-5, IL-9 and IL-13, but not the Th1 cytokine interferon-γ (IFN-γ) (2). The type 2 cytokines are directly involved in the effector phase of atopic allergy. For example, high levels of IL-4 and/or IL-13 will induce IgE production in B cells. Allergen-specific IgE is essential for the immediate hypersensitivity response, leading to mast cell degranulation and histamine release (1). Furthermore, IL-13 also contributes to airway hyperreactivity in asthma by the induction of smooth muscle cell contraction and mucus overproduction, and to the chronic stage of asthma, by promoting airway remodeling (3). The inflammatory late-phase reaction in allergic rhinitis and asthma is associated with high expression of IL-5 (4). This cytokine promotes the maturation, recruitment and activation of eosinophils, which in turn will cause local tissue damage. Finally, IL-9 participates in local inflammatory responses and airway hyperreactivity in asthma by the induction of mast cell hyperplasia and mucus overproduction (5;6). Since aberrant Th2 cytokine production is central to the manifestations of allergic diseases, important issues are how these polarized Th2 cells can develop and whether this polarized phenotype, once established in atopic allergic individuals, is susceptible to modulation. Here we will summarize the data on the cellular and molecular mechanisms that control Th2 differentiation and discuss the unstable feature of this phenotype. Subsequently we will discuss possibilities for therapy based on modulation of the Th2 cytokine profile.

**Generation of Th2 cells: Th1/Th2 paradigm**

Memory Th cells can be divided into different subsets, based on distinct functional properties which are conferred by the cytokines they secrete. In addition to cytokines that Th1 and Th2 cells produce in common, such as GM-CSF and IL-3, Th1 cells produce IFN-γ, instrumental in cell-mediated immunity against intracellular pathogens, and Th2 cells produce IL-4, IL-5, IL-9 and IL-13, and drive humoral immune responses against extracellular pathogens (7;8). Polarized Th1 and Th2 cells may develop from naïve precursor Th cells during activation in the lymph nodes. Th cell activation is initiated by T cell receptor (TCR) triggering by antigen-derived peptides presented in the context of MHC-II molecules expressed by antigen presenting cells (APC). Naive Th cells respond only to specialized APC, i.e. dendritic cells (DC), that express high levels of costimulatory molecules (reviewed in 9). DC reside in peripheral tissues as immature sentinel cells, sampling the environment for possible 'danger'-signals. At this stage, immature DC are extremely well equipped for antigen uptake and processing, but are poor stimulators of naïve Th cells (10). Exposure to microbial
and/or inflammatory products induces the maturation of the DC and the cells will migrate to the draining lymph nodes loaded with antigen (11). During this process, the DC lose their antigen uptake capacity, but strongly upregulate their expression of costimulatory molecules, making the cells qualified to activate naive Th cells and drive their development into memory/effector Th cells (reviewed in 9). Upon activation by DC, naive Th cells may develop into either Th1 or Th2 cells, directed by various soluble or membrane-bound molecules present in the microenvironment (12). Most prominent in this respect are the cytokines IL-12 and IL-4, that drive Th1- and Th2 cell development, respectively (13). IL-4 initiates the Th2-specific transcription program, by the phosphorylation of signal transducer and activator of transcription (STAT)6, and thereby increasing the expression of the Th2-specific transcription factor GATA-3 (14). GATA-3 opens the IL-4/IL-13 locus and enhances transcription of these Th2 cytokine genes (15). IL-12, instead, induces Th1 cell development by the STAT4-dependent upregulation of the Th1-specific transcription factor T-bet, which opens the IFN-γ locus and allows for transcription of the IFN-γ gene. This Th1 program is stabilized by the parallel inhibition of GATA-3 by STAT4 (16) and the shut down of type 2 cytokine gene transcription by T-bet (17). Likewise, GATA-3 suppresses IL-12 responsiveness (discussed below), thereby stabilizing the IL-4-induced Th2 development (18). Thus, it can be concluded that IL-12 and IL-4 not only drive selective Th1- or Th2 cell development, but also, counteract the opposite programs, both with a central role for the subset-specific transcription factors GATA-3 and T-bet (figure 1). Based on this knowledge, it is important to evaluate what cell types produce IL-12 or IL-4 and under which circumstances. Although IL-4 can be produced by various cell types, including mast cells and eosinophils, the major source of IL-4 is probably the Th cell population itself (19;20). However, naive Th cells must first undergo several cell divisions to be able to produce IL-4 and upon primary stimulation do not produce sufficient amounts of IL-4 to contribute to Th2 polarization in an autocrine fashion (21;22). Therefore the level of IL-4 production is highly subject to regulation by factors that are present in the early stage of Th cell activation. During primary response the most crucial factor in this respect is IL-12, produced by DC (23). The IL-12 producing capacity of mature DC in the lymph nodes is pre-established in their tissue of origin and is strongly influenced by tissue-environmental factors, such as pathogens, during their initial activation as immature DC. Upon maturation, the capacity to produce IL-12 is imprinted and is not susceptible to modulation anymore (24). If IL-12 production is low, the priming for high IL-4 production in the developing naive Th cells will not be blocked by STAT4 and T-bet. Upon subsequent stimulation, IL-4 can act in an auto- and paracrine fashion and strongly drive Th2 development.

Various other soluble or membrane-bound molecules, in part derived of DC, have been identified that can contribute to Th cell polarization (25). However, for most of the soluble factors it is evident that their influence on Th cell development is mediated mainly via the regulation of IL-12 production. Furthermore, the physiological significance of most
surface molecules that have been described to promote Th1- or Th2 cell development is less well established or function only under low cytokines conditions, leaving IL-12 as one of the key players in Th cell development. The importance of IL-12 and its receptor as determining factor in Th cell polarization is further underlined in various studies in mice and humans using models for infectious diseases, auto-immunity or allergy (26;27). It is therefore fundamental to understand how IL-12 production and IL-12 responsiveness is regulated.

IL-12 responsiveness is subject to extensive regulation

Th cell responsiveness to IL-12 depends on the expression of the high affinity IL-12 receptor (IL-12R), which is composed of two subunits, referred to as $\beta_1$ and $\beta_2$ (28;29). The IL-12R$\beta_2$ chain in particular is the major signaling chain, as the intracellular domain contains three tyrosine residues, that, upon phosphorylation by JAK2, can act as docking sites for STAT4 (28). The IL-12R$\beta_2$ chain is not expressed on naive Th cells, but is rapidly induced to moderate levels after antigen stimulation (30). The initial TCR-induced expression of the IL-12R$\beta_2$ chain is strongly upregulated and perpetuated by IL-12 itself. In the absence of IL-12, the TCR-induced $\beta_2$ chain expression is not further boosted and becomes hardly detectable within a few days after stimulation. Re-exposure of quiescent Th1 cells to IL-12 three weeks after stimulation leads to a rapid increase of IL-12R$\beta_2$ expression, even in the absence of TCR-triggering, underlining the importance of IL-12 for the upregulation and maintenance of its own receptor (31). In contrast, during Th2 development from naive Th cells, IL-12R$\beta_2$ expression is suppressed by IL-4, leading to IL-12 unresponsive Th2 cells (30;32). Indeed, both Th2 cell lines (30;33) and allergen-specific Th2 cell clones (34) lack STAT4 phosphorylation and IFN-$\gamma$ production in response to IL-12. Mouse studies (17;18) demonstrated that the reciprocal effects of IL-4 and IL-12 on IL-12R$\beta_2$ expression are mediated by GATA-3 and T-bet, respectively. GATA-3, induced by IL-4 and augmented by auto-induction (15), inhibits IL-12R$\beta_2$ gene transcription, whereas T-bet, induced by IL-12 and also augmented by auto-induction (17), upregulates IL-12R$\beta_2$ gene expression (figure 1). Several other molecules have been described to affect IL-12R$\beta_2$ chain expression. Type I interferons ($\alpha$ and $\beta$) (30;35) and to a lesser extent IL-18 (36), enhance the IL-12R$\beta_2$ expression, whereas IL-10 (37;38), transforming growth factor $\beta$ (TGF$\beta$) (37;38), PGE2 (39), Cholera toxin (CT) (40) and dexamethasone (DEX) (39) all suppress IL-12R$\beta_2$ expression. Although for some of these factors the mechanism of action is well studied, the physiological relevance of these alternative regulatory mechanisms is not always entirely clear.

The in vitro data on IL-12R$\beta_2$ expression in Th1 and Th2 cells is supported by in vivo studies of human Th1- or Th2-mediated diseases. IL-12R$\beta_2$ protein expression could hardly be detected on lung T cells of allergic asthma patients (31) and the in situ IL-12R$\beta_2$ mRNA expression was even further downregulated on broncho-alveolar lavage (BAL) T cells after allergen challenge (41). In contrast, lung T cells isolated from patients suffering from Th1-mediated lung diseases, such as tuberculosis (37) or sarcoidosis (31), expressed high levels
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of both IL-12Rβ2 and IFN-γ. Given the dominant role of IL-12 in IFN-γ production and Th1 development, the question is to what extent the loss of IL-12Rβ2 expression on polarized Th2 cells has bearing on the stability or sensitivity to modulation of the phenotype.

**Stability of established Th2 cells**

Several studies in the mouse have shown that the phenotype of early and newly committed Th1 and Th2 cells is not stable. Only once polarization is completed by repeated stimulation under polarizing conditions, Th1 and Th2 cells are resistant to reversal (42;43). Mouse Th2 cells, in particular, rapidly become stably resistant to IL-12-mediated signaling by the IL-4-induced loss of the IL-12Rβ2 chain. In contrast, human Th2 populations, both newly generated and long-term memory Th2 cells, remain susceptible to modulation. Paradoxically, although human Th2 cells lack expression of the signaling IL-12Rβ2 subunit, several reports have suggested the involvement of IL-12 in the reversal of Th2 cells to IFN-γ-producing phenotypes (41;44-46). We have recently shown that IL-12-induced Th2 reversal is characterized by stably restored IL-12Rβ2 expression, IL-12-induced signaling and IFN-γ production, concomitant with increased T-bet expression and strongly decreased GATA-3 expression (33). The paradox of how IL-12 is able to promote the expression of its own receptor on IL-12-unresponsive Th2 cells, can probably be explained by the transient nature of the suppression of the IL-12Rβ2 chain. Indeed, TCR-stimulation alone, in the absence of IL-4, re-induced low levels of IL-12Rβ2 expression, that in turn allowed IL-12 to signal and upregulate its own receptor (33). Without TCR-triggering, IL-12 was not capable of restoring IL-12Rβ2 chain expression in Th2 cells, indicating the need for TCR-triggering for basal production (31). Once accomplished, the Th2 reversal was stable, and a subsequent exposure to IL-4 was unable to undo the reversal, leaving IFN-γ production (45) and the reduced GATA-3/T-bet ratio unchanged (33). The stable character of phenotype reversal in human IL-12-treated Th2 cells may be explained by the high and unaffected level of T-bet

**figure 1:** Schematic overview of the (cross-)regulation of human Th1- and Th2 cell development by the transcription factors T-bet and GATA-3.
expression upon restimulation in IL-4 (33). That T-bet can function as a masterswitch for Th1 development is underlined by mouse studies with retrovirally transfected T-bet into polarized Th2 cells, demonstrating a full redirection of the polarized phenotype, including high levels of IFN-\(\gamma\) production, expression of the IL-12R\(\beta_2\) subunit and suppression of the Th2 cytokines IL-4 and IL-5 (16;17). However, when instead of T-bet expression, IL-12 responsiveness was restored in developing or committed mouse Th2 cells, either by transgenic (47) or by ectopic IL-12R\(\beta_2\) expression (48), stimulation in the presence of IL-12 could not revert established mouse Th2 cells. Only when endogenous IL-4 was blocked in these experiments, a substantial production of IFN-\(\gamma\) production was found. A possible explanation for the discrepancies between (1) mouse and human Th2 cell reversibility and (2) the effects of ectopic T-bet and ectopic IL-12R\(\beta_2\) expression in mouse Th2 cells may find its basis in differences in the regulation of T-bet expression. In the mouse, T-bet expression is strongly inhibited by IL-4 (17), explaining that Th2 reversal can be forced by IL-4-insensitive ectopic T-bet expression but not by ectopic IL-12R\(\beta_2\) expression, since endogenous IL-4 will inhibit IL-12-induced T-bet expression, preventing T-bet-mediated Th2 reversal. In humans, there are no indications that (endogenous) IL-4 can block T-bet expression, and thus IL-12-induced T-bet expression can easily induce Th2 reversal, even in the presence of IL-4 (41).

Since several \textit{in vitro} studies (33;45;46) demonstrate that IL-12 responsiveness can be restored in established human allergen-specific Th2 cells by a single stimulation in the presence of IL-12, the question can be raised whether the suppression of the IL-12R\(\beta_2\) chain on human Th2 cells, actively contributes to the persistence of the Th2 response or should be regarded as an early but transient Th2 cell feature, mainly important for initial polarization of naive Th cells. Especially so, as micro-environmental conditions, rather than intrinsic Th cell features, determine the expression of the IL-12R\(\beta_2\) chain and consequently, IL-12 responsiveness. Maintenance of Th2-mediated responses in atopic allergy therefore more likely results from suppression of Th1-driving factors, such as IL-12 itself (49), than from the initial lack of IL-12 responsiveness. As IL-12 can readily revert human polarized Th2 cells, these cells may be an excellent target for therapy of atopic diseases. It is therefore of great relevance to understand how IL-12 production is regulated and how DC, the main producers of IL-12, can be motivated to enhance its production.

**Regulation of IL-12 production**

IL-12 is almost exclusively produced by APC, although the levels vary for different APC types. IL-12 production can be induced by bacterial or viral infection or by incubation with microbial compounds, such as lipopolysaccharide (LPS), DNA, or double-stranded RNA (dsRNA) (27;50;51). Alternatively, IL-12 production by APC can be induced by Th cells, through ligation of CD40 by CD40-ligand (CD40L), expressed on Th cells after initial activation by antigen presentation. The level of IL-12 production is extensively regulated:
CD40L-induced IL-12 expression is strongly upregulated by IFN-γ, IL-4 and bacterial compounds (46;52-54), but is downregulated by IL-10, TGF-β (51), vitamin D₃ (55), corticosteroids (56) and by cAMP-inducing agents such as PGE₂ (39;57), cholera toxin (40), β agonists and histamine (40;58). More importantly, DC can be committed to produce high or low levels of IL-12 (12). The level of IL-12 produced by mature DC upon cognate interaction with Th cells, is a critical determinant in the degree and direction of Th cell polarization. According to their Th cell polarizing effector function, the mature DC are classified as DC0, DC1 or DC2 (12). The IL-12 production capacity of mature DC is dictated by compounds that are present during the initial activation of the DC in their immature stage. For example, exposure of immature DC to IFN-γ (24) or to (compounds of) intracellular pathogens, like M. tuberculosis (59) or pertussis toxin (E.C. de Jong et al., unpublished observations, 2001), induces maturation into DC that produce high levels of IL-12 and have a strong ability to induce Th1 development (DC1). In contrast, PGE₂ (60), cholera toxin (61) and certain helminth compounds (E.C. de Jong et al. unpublished observations, 2001) lead to the generation of mature DC with very low levels of IL-12 and the potential to induce Th2 cell development. Thus, on the basis of their experience and signals picked up during their immature stage, mature DC will produce variable amounts of IL-12 and mount different Th cell responses dependent on the type of infectious agent and the type of affected tissue. Effector Th cells may be further or additionally modulated under the control of IL-12 production locally induced by these Th cells in various APC types in non-lymphoid tissues. The outcome then will depend on the type of APC encountered and the type of peripheral tissue. For example, the phenotype of polarized Th2 cells is likely to be maintained by interaction with IL-12-deficient APC, such as B-cells, or in tissues rich in IL-12suppressing factors, such as IL-10, TGF-β or PGE₂, in the mucosa of the respiratory tract or the intestinal tract. Indeed, DC isolated from the airways or from gut-associated lymphoid tissue have been shown to be IL-12 deficient (62;63). In contrast, the skin is an example of a tissue type that can accommodate DC that allow for local IL-12 production and can induce local, potentially dangerous and harmful Th1 responses. When polarized Th2 cells migrate to the skin, local IL-12 production can provide the optimal setting for modulation of polarized Th2 cells into IFN-γ-producing phenotypes. In fact, although not completely understood, this phenomenon has been observed in the pathological conditions of atopic dermatitis patients. Activation of allergen-specific Th2 cells in the skin results in an early wave of Th2 cytokine production, followed by a late phase of IL-12 and IFN-γ (64). We have recently shown, that under certain conditions in vitro, Th2 cell-derived IL-4 can enhance CD40L-induced IL-12 production in immature DC, and that IL-4 is as potent as IFN-γ in this respect (46). This mechanism may explain the late wave of IL-12 and IFN-γ production in the skin of atopic dermatitis patients, suggesting that reversal of polarized Th2 cells can occur in vivo as well, if allowed by the specific micro-environmental tissue conditions. Since atopic diseases are characterized by a persistent population of polarized Th2 cells, that both in vitro and in vivo
can be modulated by IL-12, it may be hypothesized that the onset and persistence of the Th2 phenotype are results from aberrant IL-12 production.

**Aberrant APC-function in Atopy**

In patients with atopic allergy, Th2 cell development and the persistence of the Th2 phenotype may indeed be related to insufficient APC-derived IL-12 production, as the levels of IL-12 production in whole blood cultures of patients with allergic asthma (49) and in peripheral blood monocytes of patients with atopic dermatitis (65) are significantly lower as compared to the same cultures of control subjects, and is associated with decreased IFN-\(\gamma\) production in the T cells (49). In addition, the number of IL-12p40/p35 mRNA expressing cells was decreased in bronchial biopsies from allergen-challenged allergic asthma patients as compared to similar biopsies from control subjects (66). Moreover, apart from decreased IL-12 production, PGE\(_2\) production was increased in monocytes from atopic dermatitis patients (65;67). These data suggest that atopic allergy is associated with the occurrence of type 2-biased APC with decreased IL-12 and increased PGE\(_2\) production and the ability to induce Th2 cell development. Although the cited studies in the human are limited to peripheral blood APC, animal models confirm the data and point towards a crucial role for DC, both in the sensitization and inflammatory phases of atopic allergy. For example, adoptive transfer of ovalbumin (OVA)-pulsed DC in a mice model of asthma, induced sensitization to inhaled antigen (62;68;69). Furthermore, the contribution of DC to the inflammatory phases of asthma was pointed out in transgenic mice with the suicide gene thymidine kinase that preferentially was expressed in cells of the DC-lineage (70). Treatment with the antiviral drug ganciclovir selectively depleted DC, but not other APC like macrophages or B-cells, from the airways of OVA-sensitized animals. In addition, treatment with ganciclovir, resulted in a complete disappearance of OVA-induced eosinophilic airway inflammation and goblet cell hyperplasia. These findings, together with suppressed IL-4 and IL-5 expression in the lungs, suggest that not only naive Th cells, but also memory Th2 cells are normally activated by DC, and that antigen-presentation by other APC, such as macrophages or B-cells, apparently cannot adopt to this function. If so, DC are the critical APC to induce and support Th2 activation, mediating airway inflammation and airway hyperresponsiveness. A possible explanation for the poor APC function of B cells and macrophages in ongoing Th2 responses may be that memory Th cells in vivo are more dependent on costimulatory signals than was previously concluded from in vitro data, and rely on DC, that can provide these signals. Indeed, also other studies point towards a role for DC as provider of reliable costimulation in the activation of human allergen-specific Th2 cells. For example, when DC were adoptively transferred into PBMC-reconstituted humanized severe combined immuno-deficiency mice (hu-SCID mice), using PBMC from house dust mite (HDM)-allergic donors, the production of HDM-specific IgE was boosted, suggesting that the DC had activated memory Th2 cells (71). Furthermore, allergen-induced IL-4 and IL-13 production in bronchial explants and in peripheral blood from asthma patients was abrogated.
by antibodies against CD80 and CD86 (72;73). Finally, also animal studies have demonstrated the abrogation of eosinophilic airway inflammation, by blocking the B7.1/B7.2-CD28 pathway (74). Taken together, it can be concluded that DC, by virtue of their costimulatory potential and by varying the production of IL-12, play a substantial role in the initiation of primary allergen-specific Th2 responses. Unexpectedly, DC may also be important as costimulators of memory Th2 cells and thus, in maintaining and potentiating established Th2 responses in allergic diseases. An important question is whether DC or IL-12 production can be targeted in such a way that this will not only prevent the development of new Th2 cells but will also lead to modulation of established Th2 phenotypes and relieve symptoms of allergic diseases.

**Intervention leading to modified cytokine profiles**

In general, immune responses to biologically inactive or weakly active antigens can be cross-regulated by simultaneous infection with certain bacteria or viruses. This may also apply for atopic disease, as suggested by the finding that infection by Varicella zoster virus in atopic dermatitis skin lesions induced local IL-12 production and converts a Th2 cytokine profile towards a Th1 cytokine profile. Both locally in the lesions and systemically in the peripheral blood, specific responses against mite allergens were shifted towards a Th1 phenotype (75). This fits the concept of the recently proposed hygiene hypothesis, which states that the increased prevalence of atopic disorders is due to a decrease in the exposure to certain infectious micro-organisms, as a result of a changing lifestyle and increased personal hygiene (76). A mouse model of allergic asthma demonstrated that intranasal infection of OVA-sensitized mice with Bacillus Calmette-Guerin (BCG), strongly reduced local IL-5 production and inflammation in the lung after OVA-challenge (77). The suppressive effect was associated with elevated levels of IFN-γ, probably the consequence of increased IL-12 production, induced by M. bovis (78). This study supports the concept that bacterial infections have the potential to suppress the development of allergic diseases. The beneficial factor in the microbial infection model has been narrowed down to the induction of IL-12 production and several mouse studies have demonstrated similar effects of direct local delivery of exogenous IL-12 (gene), resulting in reduced local Th2 cytokine production and reduced airway hyperresponsiveness (79-82). However, the first results of a clinical study applying subcutaneous IL-12 therapy in allergic asthma patients do not seem very promising. No changes in airway hyperreactivity or late phase reaction after allergen-challenge were observed and several patients had to withdraw from the study prematurely, due to moderate to severe side-effects (83), so far giving little hope for a high therapeutic potential of this approach. Instead, vaccination with IL-12-promoting substances may be more successful. Promising candidates in this respect may be synthetic oligodeoxynucleotides (ODN), i.e. synthetic bacterial DNA sequences containing a high frequency of unmethylated CpG dinucleotides (adjacent cytosine and guanine residues) that have been shown to induce type 1 associated cytokines, like IFN-γ, IL-12, IL-18, TNF-α and IFN-α/β in B-cells, NK-cells,
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macrophages and DC (84-86). CpG containing oligonucleotides administered to mice after antigen sensitization resulted in reduced airway hyperresponsiveness and airway eosinophils. These effects correlated with increased IFN-$\gamma$ and reduced IL-4 production (87-90) and these effects proved to be long lasting, since the suppression of airway eosinophilia and airway hyperresponsiveness persisted during repeated exposures to antigen (89). Furthermore, specific targeting of allergen-specific Th cells in BALB/c mice by co-administration of allergen coupled to CpG ODN, a condition that mimics the clinical situation of a patient receiving allergen immuno-therapy, gave rise to a stronger immunogenicity and less allergenicity (91). This suggests that future immunotherapy with CpG ODN-allergen conjugates may provide enhanced efficacy while minimizing allergenicity and the risk of anaphylactic reactions. Recent in vitro studies of human responses to CpG ODN show similar results, including the induction of the type 1 cytokines IL-12, IFN-$\alpha$ and IL-18 and in allergen-specific Th2 cells of atopic patients an in vitro shift towards a Th1 cytokine profile (91-94). Taken together, the data derived from mouse allergy models and human in vitro studies suggest that CpG ODN may be effective for the treatment of allergic diseases, in part because of their strong potential to modulate ongoing allergen-specific Th2 cell responses. The first clinical trials examining the combination of CpG ODN and allergen for the treatment of allergic rhinitis are under way. These studies will reveal the efficacy of this novel approach and whether the accomplished improvements are long lasting.

Concluding remarks

The above reviewed studies indicate a weak stability of human established allergen-specific Th2 cells. In contrast to their mouse equivalents, these cells appear to be flexible and upon exposure to IL-12, revert their cytokine profile to a predominant type 1 response. Dendritic cells are not only the main endogenous source of IL-12, these cells also appear to be the orchestrator of the activation and maintenance of persistent allergen-specific Th2 responses, presumably, at least in part, by the provision of costimulatory signals. Therefore, new therapeutic strategies that focus on modulation of IL-12 production by DC, are highly promising as they not only target local IL-12 production, but also the principal cell type that can induce the local activation of effector Th2 cells in affected tissues. In this respect, the modulatory capacity of CpG ODN is extremely potent, directly targeting DC both during the onset and during the established phase of atopic diseases. Therefore, expectations for the use of these CpG ODN for future therapy are high.

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