

REVIEW

Is Th1 the solution for Th2 in asthma?

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Introduction

The increasing awareness that inflamed airways represent the key factor in the pathogenesis of asthma has important therapeutic implications [1]. The most effective therapy currently available aims at controlling the airway inflammation. The current guidelines for asthma treatment propose 'anti-inflammatory' drugs in any patient using β_2 -agonist inhalers more than once a week [2]. The introduction of inhaled corticosteroids not only revolutionized the treatment of chronic asthma but also urged researchers to unravel the inflammatory nature of allergic airway disease. Over the years it became obvious that the allergen-induced airway inflammation is orchestrated by activated Th2 cells. These cells produce Th2 cytokines which in turn activate a series of inflammatory cells infiltrating the asthmatic airways. Control of this Th2-mediated airway inflammation is currently believed to be a possibility, allowing a more specific asthma treatment. Switching off the Th2-driven allergic airway inflammation can be accomplished either indirectly by stimulation of Th1 cells with the ensuing IFN- γ and IL-12 production or directly by interfering with the Th2 cell function. In this review, we argue that, based on the currently available literature, stimulation of Th1 cells is unlikely to be a successful approach to dissolve the allergen-induced airway inflammation. The main reason is that overactivation of Th1 cells in its own right could also induce pulmonary pathology. This is not observed with strategies which selectively down-regulate Th2 cell activity. The development of newer therapeutic strategies for asthma should therefore be focused on the selective inactivation of the Th2 cells driving the allergen-induced airway inflammation and not on the stimulation of Th1 cells.

The activated Th2 cell orchestrates the airway inflammation in asthma

The current paradigm for allergic airway inflammation regards the activated T lymphocyte as the choirmaster of the inflammatory response [3]. There is compelling evidence that the T cells in the airways of asthma patients, but not of healthy individuals, produce a restricted but characteristic array of cytokines [4]. The description of the T cell dichotomy is based on the cytokine repertoire with Th1 cells producing IFN- γ , TNF- β and IL-2 while the Th2 subclass produces preferentially IL-4, IL-5,

IL-9 and IL-13 [5]. The finding that these T cell subsets function as mutual antagonist seems to be highly relevant for the pathogenesis of asthma. Biopsies and bronchoalveolar lavage fluids (BALF)s from patients with asthma demonstrate not only increased numbers of activated T cells, eosinophils and mast cells, but also increased amounts of IL-4 and IL-5, suggesting that asthma is characterized by a Th2-dominated immune response [6–8]. A large series of animal models underscore the importance of the Th2 cells and cytokines for the generation of allergen-induced airway hyper-responsiveness. The strongest evidence is provided by experiments in which passive transfer of Th2 cell clones is able to induce airway hyper-responsiveness [9–14]. However, to consider asthma only as a Th2-mediated inflammatory process is probably an oversimplification and not in keeping with current data. Although Th2 cells might largely orchestrate this entire process, other cells such as the macrophages, neutrophils, dendritic cells, other lymphocytes, epithelial cells and fibroblasts are all claiming their position in the pathogenesis of the inflammatory processes involved.

Numerous factors influence the Th1/Th2 balance

Several events in an immune response influence the ensuing Th1/Th2 balance [15]. These events are dictated by factors such as the type of antigen/micro-organism, the dose of antigen, the route of sensitization, the function of the antigen presenting cells (APC), the selective activation of different types of co-stimulatory molecules, the local cytokine milieu, the genetic background of the subject and the presence of a concomitant infection.

Since the naïve Th cells become activated when they recognize antigen-derived peptides in the context of class II major histocompatibility complexes expressed on APC, it is reasonable that these APC play a potentially important role in determining the cytokine profile of the activated Th cell. The APC provide the naïve T cells not only with an antigen-specific stimulatory signals (signal 1) and a series of co-stimulatory signals (signal 2), but probably also with signals polarizing the Th cell cytokine profile towards Th1 or Th2 (signal 3) [16–18].

The nature and dose of the antigen influence the Th1/Th2 balance

A number of allergens have enzymatic activity that indirectly supports Th2 cell differentiation [19]. Der p 1 from *Dermaphagoides pteronyssinus* is a proteolytic enzyme cleaving the α subunit of the IL-2R [20]. A decrease in lymphocyte proliferation and IFN- γ production follows. In addition, Der p 1 cleaves CD23 on B cells [21]. As CD23 normally suppresses

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immunoglobulin (Ig)E synthesis, cleavage of CD23 is associated with an up-regulation of IgE synthesis [19].

The dose of the antigen also influences the Th1/Th2 balance [22,23]. In a murine model, it was shown that higher doses of antigen favoured the development of Th1 cells producing IFN- γ [22]. Similar findings were made with CD4⁺ T cells from atopic patients [23]. These human cells also produced high levels of IL-4 when stimulated with low doses of allergen, but little IL-4 when stimulated with higher doses of allergen [23]. At very high (> 10 μ M) concentrations of antigen, a dramatic switch to the development of Th2 cells, producing increasing amounts of IL-4 and decreasing amounts of IFN- γ , was documented [22].

The mechanism underlying the effect of very high antigen dose exposure on the Th2 shift in the murine models remains unclear. One possibility is that the production of IL-12 and IL-18 (formerly IFN- γ inducing factor) by the dendritic cells and macrophages is inversely correlated with the amount of receptor bound antigen. Another possibility is that when the antigen is present in high concentrations, other cells that do not produce these Th1-favouring cytokines such as B cells take over the antigen presenting process.

Co-stimulatory molecules can influence the Th1/Th2 balance

Next to the type of antigen (signal 1), the presence of different co-stimulatory molecules (signal 2) is also important in determining the type of immune response. Especially the members of the Ig superfamily CD80 (B7.1) and CD86 (B7.2) both expressed on the APC (dendritic cells, macrophages and B cells) but also on activated T and NK cells seem to have a differential effect on Th1/Th2 cell development. Optimal activation of T cells implies an interaction of either CD80 or CD86 with the CD28 molecule, which is a receptor present on the T cells [24]. Some *in vitro* studies suggested that interaction of CD28 with CD80 preferentially induces Th1 cell development while interaction of CD28 with CD86 preferentially induces Th2 cell development [25,26]. Another co-stimulatory molecule, OX40, has also a role in enhancing the Th2 cell development [27,28]. Recently, a new member of the B7/CD28 family has been identified, namely B7h. This protein resembles both B7-1 and B7-2, but does not bind to CD28. Instead, it recognizes the CD28 homologue, inducible co-stimulator (ICOS) which is expressed on a subset of activated T cells [29]. To date, there are some indications that the B7h–ICOS interaction might play a role in the Th2 development [30].

Another intriguing T cell factor is T1/ST2. This molecule is expressed exclusively on Th2 cells and plays probably a role in Th2 cell development and cytokine responses, as suggested by experiments with T1/ST2-deficient mice [31]. The mechanisms remain however, unclear.

CD40, a molecule also expressed on APC and an important co-stimulatory factor through ligation of CD40L expressed on T cells, has also not been reported to have an effect on the differentiation of Th cell subsets [32].

Dendritic cell-derived signals influence the Th1/Th2 balance

The molecular constitution of signal 3 as direct polarizing signal remains obscure. Elucidation is important since this could reveal the factors causing the Th2 bias observed in allergic

disorders. A number of candidate molecules have been identified [3,15,16]. Especially IL-12, which is produced during antigen presentation by the APC, but also IL-18 and PGE₂ are of special interest [16]. The important role of IL-12 in the differentiation of Th1 cells is based on a large series of experiments. Interaction of IL-12 with its receptor activates signal transducer and activator of transcription factors (STAT)-2, -3 and -4, from which only STAT-4 is specific for IL-12-mediated signal transduction [33]. IL-12 acts directly on T cells and primes for strong IFN- γ responses [34]. This pathway is not mediated through IFN- γ since anti-IFN- γ does not inhibit Th1 differentiation in the presence of IL-12 [34]. In accordance, STAT-4-deficient mice [35] and IL-12 knock-out (KO) [36] mice show impaired Th1 responses underscoring the pivotal role of IL-12 for Th1 differentiation. It has been suggested that naïve APC have a commitment to produce upon antigen encounter a certain amount of IL-12 and that in atopic patients, an aberrant (possibly genetically determined) function of the APC with low IL-12 production is present. A number of factors can in turn influence this baseline production hereby modulating the type of primary immune response. Factors such as IFN- γ , PGE₂ and IL-10 can also influence the APC function indirectly by modulating the APC-derived IL-12 synthesis [16]. In addition to IL-12, IL-18 is also considered to play a role in the controlling of the Th1/Th2 balance [37]. As is IL-12, IL-18 is produced by APC and co-operates, probably through different pathways, with IL-12 in the induction of IFN- γ synthesis.

T cell-derived cytokines influence the Th1/Th2 balance

Together with the antigen presentation process, Th cell-derived cytokines and transcription factors are dominant regulators of the Th cell differentiation [15]. Until now, IL-4 is the only identified Th2 cell-derived cytokine that further pushes the Th1/Th2 balance towards the Th2 side via an autocrine positive feedback loop [38]. *In vivo* and *in vitro* experiments have conclusively demonstrated that IL-4 is the critical cytokine for the normal development of Th2 cells [39,40]. The strongest evidence for the critical role of IL-4 comes from experiments with IL-4-deficient mice. Infection of these mice with nematodes such as *Nippostrongylus brasiliensis* induced a significantly lower Th2 cell differentiation as measured by eosinophilia and IL-4 and IL-5 when compared to the wild type littermates [41]. Animal models of allergen-induced airway inflammation based on immunization with ovalbumin supported these findings [42,43]. STAT-6-deficient mice, which are deficient in a tyrosine kinase that is phosphorylated following IL-4 receptor binding, also present with impaired Th2 cell differentiation [44–47]. In the mouse, two specific Th2 cell transcription factors have also been identified, namely c-Maf and GATA-3 [48,49]. The former induces IL-4 production through activation of the IL-4 promoter gene [50], while the latter allows, through a down-regulation of the IL-12R β 2 unit expression, an up-regulation of several Th2 cytokines in both developing and committed Th1 cells [51,52]. While the role of c-Maf in humans remains to be established, it was shown that increased GATA-3 is present in Th2-committed human cells and in bronchial biopsy specimens from asthmatic patients [53].

IL-13, another T cell-derived cytokine, shares many biologic activities with IL-4. However, T cells do not express functional IL-13 receptors. Therefore, IL-13 fails to induce directly Th2 cell differentiation, although indirect mechanisms exist [54,55].

In contrast to IL-4, Th1 cell-derived IFN- γ acts as a heavy-weight to the other side of the Th cell balance. The role of IFN- γ in Th1 cell differentiation has been a matter of intense debate since IFN- γ -deficient mice as well as IFN- γ receptor KO mice are still able to mount efficient Th1 responses [56]. It was shown that IFN- γ on its own is unable to drive Th1 cell development [57]. The role of IFN- γ in changing the Th1/Th2 balance is more subtle but therefore not less important. Mice lacking the IFN- γ receptor have an impaired ability to resolve lung eosinophilic inflammatory responses and produce more Th2 cytokines [58]. IFN- γ has been shown to render naïve mouse T cells more responsive to the Th1-inducing effects of IL-12 through an up-regulation of the IL-12R β_2 chain [57,59]. IFN- γ also enhances IL-12 production by APC, providing a positive feedback loop on the Th1 cell differentiation [60]. IL-12 and IFN- γ therefore act together for shifting the Th1/Th2 balance towards the Th1 side. IL-18 induces IFN- γ synthesis and synergizes with IL-12 in Th1 development. The IL-18R is selectively expressed on Th1 cells and transmits signals that support a further Th1 development through the IL-12R complex [61]. Just as for the Th2 cell lineage, a transcription factor has recently been identified as relevant for Th1 cell development, namely T-bet [62]. It is selectively expressed in Th1 cells and causes not only Th1 cell development, but is also able to redirect already committed Th2 cells towards a Th1 phenotype [62].

Infectious agents can influence the Th1/Th2 balance

Another issue of potential relevance is that the cytokine response to an antigen/allergen can be influenced by the prevalence of childhood infections. It has been suggested that a lack of microbial stimulation of sufficient intensity in early life may affect the maturation of the immune system. This inadequate maturation could then be the substrate for Th2-biased responses upon allergen encounter later in life [63,64]. However, no direct evidence has yet been provided for this possible interaction between allergen exposure and concomitant infection.

The genetic background influences the Th1/Th2 balance

It has to be stressed that the contributions of the mechanisms outlined above all occur in the context of a particular genetic predisposition. Since numerous molecules are involved in the pathogenesis of asthma and atopic diseases, the elucidation of the predisposing genetic factors is very complex. It has been suggested that people who are genetically predisposed for the development of atopy, are characterized by a slower postnatal immune deviation from a Th2 towards a Th1 type. This could give rise to a window of vulnerability during which high-dose of allergen exposure might consolidate the Th2 phenotype [65]. Much attention has been given to the genes on the human chromosome 5 (with its murine homologue chromosome 11) encoding IL-4, IL-5, IL-13, IL-12 p40 chain, and IFN- γ response factor-1 (IRF-1) although many other genetic loci seem to be relevant for an increased risk for asthma [66].

Despite missing links in the immunology model of asthma, much progress has been made in the understanding of the importance of Th2 cells along with the Th2 cytokine profile. That knowledge now opens numerous opportunities towards the development of novel therapeutic approaches. The latter should be characterized by a very specific activity critically

interfering with one or more pathways involved in the pathogenesis, resulting in a full resolution of the allergen-induced airway inflammation. Since Th2 cells and cytokines play a pivotal role in the pathogenesis of asthma and since Th1 cells are the endogenous functional antagonists of the Th2 cells, it can be argued that effective treatment could be accomplished by counterbalancing Th2 cell activity through an up-regulation of Th1 cytokines. The other possibility is a selective down-regulation of the Th2 cell activity.

Counterbalancing Th2-driven allergic airway inflammation

The first possible approach for interfering with established Th2-driven allergic airway inflammation is to stimulate the development of Th1 cells and to promote IFN- γ production. The rationale is that high levels of IFN- γ antagonize Th2 cells so that the Th2-driven allergic airway inflammation could be counterbalanced. This can be achieved with different protocols such as injecting recombinant IFN- γ , transferring Th1 cell clones or providing substances or infectious agents that promote Th1 development such as IL-12, heat-killed *Listeria monocytogenes*, *Mycobacterium tuberculosis*, CpG motifs or synthetic compounds such as M50367.

IFN- γ

One method is to use purified IFN- γ . Promising results arose from murine asthma models in which IFN- γ , given either during the primary sensitization or during the secondary immune response, decreased IgE production and airway inflammation and normalized the airway function [67,68]. An effective counterbalancing in the cytokine profiles was accomplished since a decreased capacity of T cells to produce Th2 cytokines was accompanied by an up-regulation of the Th1 cytokine profile [68]. Unfortunately, those experiments only evaluated the capacity of IFN- γ to prevent allergen-induced airway changes. They did not evaluate the effects on already established airway eosinophilia and hyper-responsiveness. In contrast, systemic as well as pulmonary IFN- γ gene delivery was proven to be effective not only for preventing but also for suppressing already established allergen-induced airway hyper-responsiveness in a mouse model [69,70]. A two-centre randomized, double-blind, placebo-controlled trial with subcutaneous INF- γ in asthma patients followed. In this study with steroid-dependent asthmatic patients, no beneficial effects were observed [71]. Elevating the doses was impossible because of toxicity reasons [71]. In another approach with aerosolized IFN- γ , it was shown that the molecule could be safely delivered to the lungs of healthy volunteers. Interestingly, this was accompanied with an increase in BALF lymphocyte counts [72]. Despite a significant reduction of eosinophils in the BALF of asthma patients exposed to aerosolized IFN- γ , the symptom scores and pulmonary function did not change at all after IFN- γ treatment [73].

Another way of counterbalancing Th2-driven allergic inflammation is to transfer IFN- γ -secreting Th1 cells. In an animal model, a passive transfer of ovalbumin (OVA)-specific Th1 cells was performed either before sensitization or after allergen-induced airway inflammation was established. The results

showed that an increase of airway inflammation was observed together with an augmented recruitment of host Th1 and Th2 cells [14]. Accordingly, transfer of OVA-specific Th1 cells into severe combined immune deficient (SCID) mice did not attenuate Th2 cell-induced airway hyper-responsiveness, but rather caused severe airway inflammation [74]. Taken together, these results all suggest that antigen-specific Th1 cells do not prevent or protect against Th2-mediated allergic disease, but rather initiate an additional Th1-driven acute lung pathology.

IL-12

One can also achieve an effective Th1 up-regulation by administering IL-12 as an immunoregulatory cytokine. Assessment of the IL-12 mRNA expression in bronchial biopsies from asthmatic subjects and healthy persons suggested a defective IL-12 synthesis in asthma [75]. IL-12 has therefore also been proposed as a potential tool for modulation of the Th2 inflammatory response in asthma [76,77]. The ability of IL-12 to stimulate Th1 immune responses has been demonstrated both *in vitro* [78–81] and *in vivo* animal models [82,83]. IL-12 cytokine therapy suppressed the allergen-induced airway eosinophilia and hyper-responsiveness. Moreover, a complete inhibition of allergen-specific IgE synthesis was obtained only when IL-12 was given during the sensitization but not during a secondary challenge [82]. The protection was accompanied not only by a suppression of IL-4 but also by an induction of the IFN- γ levels in the BALF [83]. Despite the efficient reduction of airway hyper-responsiveness after IL-12 therapy in these mouse models, which is in contrast with the results of Th1 cell clone transfer, there are numerous reasons why IL-12 will probably never reach the status of asthma therapeutic. A clinical trial in which recombinant human IL-12 was administered subcutaneously in mild asthmatics showed a reduction in blood and sputum eosinophils whilst the airway hyper-responsiveness and the late asthmatic reaction following allergen challenge were not altered [84]. A major limitation of IL-12 as possible therapeutic agent is its toxicity [85,86]. Severe toxicity, including two deaths, has been associated with treatment of renal carcinoma patients receiving intravenously 0.5 $\mu\text{g/kg/day}$ of recombinant human (rh)-IL-12 [86]. Although the pathogenesis of IL-12-induced toxicity is not fully known, it is well documented that the induction of secondary Th1 immune responses with high IFN- γ production is pivotal [87]. This is elegantly proven in experiments using IFN- γ receptor-deficient mice in which the IL-12 toxicity was greatly reduced [88]. Giving one priming dose of IL-12 a few days before the daily therapy has shown to reduce the toxic effects to a certain extent [89]. Another potentially related criticism against IL-12 cytokine therapy arises from the histopathology findings. As with Th1 cell transfer, it is clear from studies on BALF in murine models of asthma, that IL-12 cytokine therapy for allergen-induced airway inflammation does not result in a full normalization of the BALF cellular composition [82]. This is underscored with findings in IL-10 KO mice which are characterized by a more severe allergen induced airway inflammation when compared to wild-type littermates [90–92]. This can be explained by the function of IL-10 as an endogenous dampener of the immune responses [93–95]. Studying whether the anti-allergic effects of IL-12 were mediated through an up-regulation of the immunosuppressive cytokine IL-10, showed that IL-12 remains capable

of counterbalancing Th2 responses towards Th1, even in IL-10 KO mice. However, despite a full down-regulation of the airway hyper-responsiveness and airway eosinophilia, a marked Th1-like peribronchial inflammation was observed in all mice treated with IL-12 [90].

Infection or vaccination

Counterbalancing Th2 inflammatory reactions can also be achieved through infection or vaccination which stimulate the Th1 development [96–98]. Since infections with intracellular pathogens stimulate Th1-driven inflammatory responses, it has been suggested that atopic disorders represent the default inflammatory pathway in the absence of these infections [63]. Mouse experiments suggested a protective effect of *Bacillus Calmette-Guérin* (BCG) vaccination [97,99]. Although these findings do not necessarily implicate a role for BCG vaccination in preventing allergy in humans, a limited study indicated that the vaccination of mild asthmatics with *Mycobacterium Vaccae* reduced the airway hyper-responsiveness along with an *in vitro* suppression of the IL-5 production [100]. Although this remarkable result, obtained in asthmatic patients in which the T cell were already committed to the Th2 subset, needs to be evaluated further in larger studies, it could indicate the *in vivo* flexibility of the human T cell subsets to vaccination.

CpG motifs

Oligodeoxynucleotides containing CpG motifs found in bacterial but not vertebrate DNA constitute an infectious danger signal and have a strong Th1-promoting capacity. The recognition of these motifs activates macrophages and dendritic cells to secrete IL-12 hereby inducing IFN- γ -secreting Th1 cells [101–104]. Administration of these CpG motifs in murine models of asthma resulted in an inhibition of both allergen-induced airway eosinophilia and hyper-responsiveness [105–109]. These CpG motifs even showed to have synergistic effects on immunotherapy based on allergen plasmid DNA vaccination in a mouse model of pollen allergy [110]. The non-specific Th1 activation and toxic effects (via TNF- α induction) associated with CpG therapy hamper its usefulness as a therapeutic immunomodulator for human allergic diseases [111,112]. Although a critical role for IFN- γ was shown using IFN- γ -deficient mice [105], others suggested a direct suppressive effects of the CpG motifs on Th2 cells based on the findings in IL-12/IFN- γ double KO mice. Those mice required 10 times as much CpG for protection against airway eosinophilia than wild-type animals. However, these findings might point at the possibility of a CpG-induced Th2 suppression without a concomitant Th1 up-regulation [113]. In addition, the local administration of CpG motifs appears to be less toxic, but it is obvious that further evaluation is necessary before clinical testing can be done [114,115]. The same holds for some recently described oral compounds that modulate the Th1/Th2 balance [116].

These data all show that numerous possibilities exist for counterbalancing the Th2-driven allergic airway inflammation through induction of Th1 cytokines. A major drawback of this approach is that the Th1 cells and cytokines stimulate the recruitment and activation of mononuclear phagocytes and more lymphocytes and can cause severe pulmonary disease [117]. However, the normal state is characterized by the absence of any inflammatory response to allergens. Therefore, therapy

for asthma based on a replacement of allergen-specific Th2 cells with activated Th1 cells for counterbalancing the effects of the Th2 cells is probably not beneficial for asthma.

Down-regulating Th2-driven allergic airway inflammation

Interfering with the Th2-driven allergic airway inflammation can also aim at the protagonists of the Th2 response itself. A subtle regulation of the cytokine milieu rather than a conversion from a prevalent Th2 to a Th1 cytokine production profile can be achieved using different approaches. These could lead to a state of tolerance of the Th2 cells to allergens [117].

Immunotherapy

Allergen immunotherapy is based on the administration of gradually increasing quantities of an allergen extract to an allergic subject. The mechanisms involved in the therapeutic effects of immunotherapy are very complex and may depend on the type of allergen (venoms or inhalation allergens) and on the route of allergen administration. The hypothesis that the beneficial effects of allergen immunotherapy are dependent on allergen-specific IgG intercepting the allergens before they can bind to IgE for triggering the allergic response [118,119] is unlikely since clinical effects precede the increase in IgG [120]. The same reasoning holds for immunotherapy-induced decreases in serum IgE [121]. A far more likely mechanism is that allergen-specific T cells become tolerant for the allergens with a selective down-regulation of the Th2 cytokine production process [122–128]. The effects of immunotherapy on Th1 cytokine secretion are less consistent [121]. Some studies reported the concomitant up-regulation of IFN- γ cytokines [129,130] while others found no effect or even a down-regulation [127,128]. Overall, the induction of non-responsiveness of the allergen-specific Th2 cells emerges to be the underlying mechanism for successful immunotherapy [121,126,131,132]. Allergen immunotherapy is not recommended medical practice for treating asthma for several reasons. The moderate efficacy, the relatively high cost, the risk for adverse effects and the availability of safer and more effective pharmacological therapies, particularly inhaled corticosteroids are the most important arguments [120]. Other approaches modulating the ongoing immune responses to allergens and inducing an inactivation of Th2 cells by applying altered peptide ligands represent promising concepts for future immunotherapy [133].

Interference with co-stimulatory molecules

Inducing a state of Th2 cell non-responsiveness upon allergen encounter can also be realized by disrupting a normal antigen presentation process at the level of the co-stimulatory molecules. Initial studies based on the use of monoclonal antibodies reported the capability of the anti-B7-2 (CD86), but not of anti-B7-1 (CD80) to down-regulate allergen-induced airway eosinophilia and hyper-responsiveness in conjunction with a suppression of the IL-4 and IL-5 levels without a change in IFN- γ production [134,135]. Studies with a special mutant of anti-B7-1 (namely Y100F) which blocks the CD80 molecule without any intrinsic activity (in contrast with the classical anti-CD80 antibodies [136]), proved actually to prevent eosinophil

and lymphocyte accumulation in the lungs of immunized mice without evidence of Th1 induction [137]. It is not surprising that a full block of the CD28/B-7 pathway using CTLA-4-Ig, which is a soluble form of CTLA-4 binding both CD80 and CD86, inhibits airway hyper-responsiveness, inflammatory infiltration, expansion of thoracic lymphocytes, and allergen-specific responsiveness of thoracic T cells in murine models of allergic asthma [138–140]. Others reported in this respect a concomitant up-regulation of IFN- γ [141,142]. Recently, a third member of the CD28/CTLA4 family, namely inducible co-stimulator protein (ICOS) has also been demonstrated to play a critical role, since ICOS inhibition attenuated Th2-driven mucosal inflammation [143]. In this respect, ICOS inhibition is worthwhile for further evaluation.

The same holds for the Toll-like receptors (TLR), which are evolutionarily conserved membrane proteins involved in the antigen recognition by macrophages. Activation of those TLR induces the synthesis of pro-inflammatory molecules and leads to the up-regulation of co-stimulatory molecules representing a link between innate and acquired immune system. In the mouse, it was shown that tolerance was associated with a down-regulation of TLR-4 expression [144]. Interference with TLR is therefore also a potentially interesting pathway to be further investigated.

Accordingly, blocking the ICAM/LFA-1 interaction showed to inhibit IL-5-driven eosinophil accumulation and IL-4-driven IgE production [145].

Despite the promising results of interfering with co-stimulation obtained in murine asthma models, no data on clinical results of these substances are currently available.

Anti-T cell therapy

As outlined above, the intrapulmonary delivery of allergen evokes in the patient with allergic asthma a Th2 cell-mediated immune response. However, the normal situation is characterized by a state of tolerance against those otherwise harmless molecules. It is suggested that allergy and asthma result from a breakdown in the normal regulatory mechanisms that operate at the mucosal surfaces. The state of tolerance appears to be critically mediated by T regulatory cells (Tr cells). Those Tr cells favour both the 'linked immunosuppression' (implicating that tolerance against one epitope of an allergen induces a linked tolerance against the other epitopes) and the 'bystander immunosuppression' (meaning that the action of one type of Tr cells also controls the activity of Th cells with a related epitope specificity). Thus, the linked and bystander immunosuppression are a tool of the immune system to control the immune reactions against harmless antigens. Whether naïve T cells develop into allergen-specific Th1 or Th2 cells on the one side or into Tr cells on the other side appears to be critically mediated by the NOTCH signalling pathway. The reception of signals through T cell receptor (TCR) and costimulatory molecules together with ligation of NOTCH results in tolerance through the differentiation of T cells in Tr cells. In contrast, signalling through TCR in the absence of NOTCH favours an active immune response.

By consequence, the expansion and activity of the Th2 cells in asthma could be inhibited by means of a stimulation of the Tr cell population. Transgenic mice over-expressing serrate-1, which is the DC ligand of the NOTCH receptor, are characterized by a profound T cell tolerance which is mediated by Tr cells

[146]. Much further work in this area is necessary to evaluate a potential role in asthma therapy.

One can also target the activated Th cells with anti-CD4 monoclonal antibodies. It was shown that these induce beneficial effects not only in mouse models of asthma [147] but also in a clinical study with intravenous keliximab [148,149]. Although no serious adverse effects such as opportunistic infections or neoplasms related to the treatment were noted, anti-CD4 T cell therapy is quite aspecific and no further studies have been reported [148].

In agreement, T cell activity can also be inhibited by means of Cyclosporin A (CsA). Following the findings in murine models, it was shown that CsA treatment improves lung function, decreases exacerbations and oral corticoid requirements in patients with severe asthma [150]. Treatment with CsA is associated with an inhibition of bronchial eosinophils, IL-5 and eotaxin resulting in a significant inhibition of the late (T cell-mediated) but not the early asthmatic reaction [151].

In a murine model, the administration of a monoclonal antibody directed against the Th2-specific marker T1/ST2 showed a selective inhibition of Th2 cell activation with an inhibition of both IL-4- and IL-5-driven IgE synthesis and eosinophilia [152]. This approach is more specific than treatment with keliximab and the results obtained so far justify further research about this approach.

Anti-Th2 cytokine therapy

Th2 proinflammatory cytokines such as IL-4, IL-5, IL-13 and their receptors are considered to be possible candidates for a critical intervention in Th2-driven inflammatory processes and thus represent valuable therapeutic options in asthma [153]. Cytokine antagonism can be realized by monoclonal antibodies against the particular cytokine or cytokine receptor and by receptor antagonists or soluble receptors capturing the circulating cytokine. Numerous studies based on the use of KO and transgenic mice have proven the critical role of IL-4, IL-5 and IL-13 not only for eosinophilic airway inflammation but also for airway hyper-responsiveness.

Inhibition of IL-5 in allergic diseases is one possibility for paralyzing the Th2 inflammation. IL-5 synthesis inhibitors, IL-5 antagonists and monoclonal antibodies against IL-5 represent the different options currently available [154]. Non-steroid, non-specific inhibition of IL-5 synthesis can be achieved by a range of synthetic compounds of which the most familiar are macrolide antibiotics [154]. However, since all of these substances appear to be broad acting agents that affect not only IL-5 gene transcription but also the transcription of several other cytokines, the clinical usefulness is limited. In contrast, anti-sense IL-5 oligonucleotide works specifically and has proved to reduce both the eosinophilic airway inflammation and hyper-responsiveness in a mouse asthma model [155]. Accordingly, the transgenic expression of a dominant-negative mutant of GATA-3, a transcription factor controlling the expression of Th2 cytokines, led to a reduction in Th2 cytokines, airway eosinophilia, mucus production, and IgE synthesis [156]. Similar results were found with a specific anti-sense phosphorothioate oligonucleotide overlapping the translation start site of GATA-3 [157]. *In vitro* data suggest that IL-5 effects can also be down-regulated by preventing the cytokine from binding to its receptor using human soluble receptor analogues or modified receptors [158–160]. A variant of the α subunit of the IL-5R has

been described [158]. This analogue does not bind to the membrane β subunit so that no intracellular signalling takes place upon IL-5 engagement on the α subunit. Covalent modification of the IL-5R by isothiazolones also prevents IL-5 from binding to the receptor [161]. Unfortunately, no clinical data on these substances are available. The most advanced of all methods for inhibiting IL-5 effects is simply removing it by means of monoclonal antibodies. The capacity of anti-IL-5 monoclonal therapy for shutting down Th2 inflammatory responses has been demonstrated in numerous mouse [162–166], guinea-pig [167,168], and human-SCID mouse chimera [169] and primate models [170]. For patients with asthma, the tolerability, safety and activity of humanized anti-IL-5 (SB-240563 or SCH55700) has been confirmed [171,172]. In a small study, a single dose of anti-IL-5 resulted in a significant and long-lasting down-regulation of blood and sputum eosinophilia, while there was no marked effect on the allergen-induced late asthmatic reaction and airway responsiveness in mild asthmatics [171]. In accordance, a single dose of anti-IL-5 in steroid treated severe asthmatics was also well tolerated, but failed to affect the baseline FEV₁ [172]. The effects of repeated anti-IL-5 dosing have yet to be explored.

Mouse anti-IL-4 monoclonal antibodies have been shown to be capable of preventing both eosinophilia and IgE production when given during the allergen sensitization (but not when given during the secondary immune responses) [163,173]. This effect was confirmed with human anti-IL-4 monoclonals in Hu-PBL-SCID mouse models [174–176]. Accordingly, IL-4 receptor blockade with a monoclonal antibody inhibits allergen-induced airway responses in a mouse model [177]. No changes in IFN- γ concentrations and thus in Th1 activity were observed, suggesting that a selective inactivation of the Th2-driven inflammatory response was achieved [177]. In another mouse model of asthma, aerosolized soluble IL-4 receptors (sIL-4R) capturing IL-4 not only reduced the IgE synthesis, but also prevented the development of airway hyper-responsiveness in some studies [178,179] although this was not invariably confirmed [180]. A clinical trial in which sIL-4R was nebulized showed a decrease in the metacholine airway responsiveness and asthma scores as well as a beneficial anti-inflammatory effect without significant toxicity [181,182]. Both for mouse and for human, mutant IL-4 molecules have been developed with a functional antagonism of IL-4-induced Th2 cell proliferation, differentiation and signal transduction [183–186]. Although the mouse IL-4 mutant abrogates the immune responses to allergens [183], and although the human IL-4 mutant is effective in preventing the development of allergen-induced airway hyper-responsiveness in a human-SCID mouse chimera model of asthma [169], no clinical data are currently available about the effects on allergen-induced airway changes in humans. The IL-4 signal transduction can also be blocked by members of the SOCS (suppressor of cytokine signalling) family of proteins. These can inhibit JAK-STAT signalling [187]. However, no animal, nor clinical data on the effects of SOCS-1 are currently available.

Antagonization of IL-13 is also of interest. This Th2 cell-derived cytokine has a marked redundancy with IL-4 and synergies in the IgE synthesis [55,188–190]. The effects of IL-13 on Th2 cell development are very complex and regulated through a suppression of IFN- γ and IL-12 [54]. IL-13 KO mice present with a suppressed Th2 development [191], while anti-IL-13

therapy in wild-type mice actively suppresses the allergen-induced airway inflammation and hyper-responsiveness [192,193]. No major effects on the IgE synthesis or eosinophilia were documented (despite a known suppressive effect on the eotaxin levels [194]). The importance of IL-13 in the development of an asthmatic phenotype is further underscored by the findings that IL-13 administration in naïve mice induces airway hyper-responsiveness [192]. Overall, IL-13 is thought to play a critical role in the secondary immune responses, while IL-4 has its main effects on the initial Th2 cell development [55].

Since patients with asthma are already Th2-committed when they seek medical help, the blocking of IL-13 might be more logic than just blocking IL-4. This could also explain why the anti-IL-4 antibodies are not effective during the secondary immune responses in the murine models [173]. Importantly, the IL-4 mutants mentioned above inhibit the effects of both IL-4 and IL-13 on their respective receptors by interfering with the binding to the common IL-4R α chain. Until now, only *in vitro* data exist on the effects of anti-IL-13 on human cells [190].

Anti-IgE therapy

The direct neutralization of IgE with monoclonal antibodies such as Rhu-Mab-E25 or CGP-51901 could also be beneficial. Mouse models of allergen-induced airway inflammation suggested that anti-IgE therapy not only suppresses the circulating levels of IgE but also inhibits the T cell production of IL-4 and airway eosinophilia [195]. These findings were followed by clinical studies with anti-IgE therapy in asthma patients. The results suggested that intravenous, but not aerosolized, Rhu-Mab-E25 has a beneficial clinical effect in asthma patients [196,197]. Since anti-IgE therapy represents a breakthrough in the concepts of asthma therapy, further evaluation is warranted. Although some evidence exists that IgE is not essential for normal immune functions, even for protection against parasites, more data are still needed to ascertain the safety [198,199]. A more recent study on more than 300 patients reported no adverse effects and demonstrated the efficacy of anti-IgE therapy for asthma treatment in patients with moderate to severe allergic asthma [200]. More clinical studies with anti-IgE are currently ongoing or in publication. The exact place of this novel anti-asthma treatment needs further evaluation in clinical studies comparing anti-IgE therapy with the current standard treatment of asthma.

IL-10

Diminishing the Th2 activity can finally also be achieved by increasing the production of IL-10. IL-10 is an anti-inflammatory cytokine with a therapeutic potential for asthma [201]. It does not redirect Th2 into Th1 responses, but acts as a general inhibitor of proliferative and cytokine responses in T cells [95,202]. Due to its ability to down-regulate APC function, to induce Th2 cell tolerance, to inhibit IL-5 and chemokine synthesis, and to block certain functions of eosinophils and mast cells, IL-10 represents a potentially interesting therapeutic agent [203–205]. Clinical data suggested a significant role for IL-10 in asthma pathogenesis. Alveolar macrophages from asthma patients produce significantly lower amounts of IL-10 compared to non-asthmatic healthy volunteers [206], while a genotype associated with low IL-10 production was found in patients with severe asthma [207]. In addition, corticosteroids

up-regulate the IL-10 production along with the clinical improvement in the asthma patient [208]. *In vitro* experiments suggested that IL-10 induces tolerance in Th2 cells from patients with allergic asthma [203]. IL-10, concurrently delivered with OVA also protects immunized mice from developing allergen-induced eosinophilic lung inflammation [209,210]. In another mouse model, IL-10 treatment has proven to be efficient in reducing the corn dust extract-induced airway inflammation and hyper-responsiveness [211]. Transfer of the IL-10 gene into sensitized mice even inhibited a Th2 cell development during allergen sensitization and thus inhibited subsequent allergen-induced airway changes [212]. On the other hand, IL-10^{-/-} mice have more allergen-induced airway inflammation than their wild-type littermates [92]. Administration of IL-10 in normal volunteers and in transplant patients is well tolerated with only the induction of flu-like symptoms at high doses [213–215]. Whether IL-10 is beneficial for asthma patients remains to be determined. Determining the optimum timing of administration and dose of IL-10 will be one of the important issues to be addressed.

Conclusion

Evidence suggesting that a Th2 lymphocyte-driven allergic airway inflammation is pivotal for inducing the clinical features of asthma directs us to develop new therapeutic strategies, critically interfering with the development and maintenance of this airway inflammation. Two strategies for diminishing the Th2-driven allergic airway inflammation can be applied. Since Th1 and Th2 cells suppress the development and cytokine synthesis of one another, it could be argued that protocols in which Th1 cells become activated might be beneficial for asthma. However, from the studies summarized above, it is clear that activation of Th1 cells not only is accompanied with cytokine production (INF- γ) suitable for suppressing activated Th2 cells, but also with unwanted immune responses. Indeed, counterbalancing Th2 inflammatory responses through Th1 cell activation implicates that those Th1 cells will stimulate the recruitment and activation of other inflammatory cells. Moreover, Th1 stimulation can be very dangerous in patients with ongoing Th1-mediated diseases such as autoimmune disorders. The normal state is characterized by a tolerance to inhaled or ingested allergens without any type of inflammatory reaction.

Therefore, instead of counterbalancing the Th2-orchestrated inflammatory reaction in asthma, it might be more rational to induce Th2 cell tolerance. The subtle regulation at the level of the APC or of the cytokine milieu rather than a vigorous redirection from a Th2 to a Th1 inflammatory pattern should therefore be the aim of the newer therapeutic strategies. Different possibilities such as selective immunotherapy, anti-IL-4/IL-13, anti-IL-5, anti-IgE or IL-10 cytokine therapy could be efficient in resolving the Th2-driven allergen-induced airway inflammation. We are now coming into the exciting phase of clinical testing of these substances. Especially, the more long-term effects of these various treatments on the underlying Th2-driven immune reaction will be most interesting. It remains however, important to recognize that asthma is a very complex pathology involving a whole range of cell types. Therefore, one has to take into account that inhibition of the Th2 cell function may not be sufficient to completely control the disease.

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