

Review article

T cell activation, from atopy to asthma: more a paradox than a paradigm

During the last 15 years, it was largely shown that allergic inflammation was orchestrated by activated Th2 lymphocytes, leading to IgE production and eosinophil activation. Indeed, Th2 activation was shown to be necessary to induce allergic sensitization in animal models. In humans, a Th2 skewing was shown in atopic children soon after birth. In asthma, descriptive studies showed that Th2 cells were more numerous in patients than in controls. In addition, during specific allergen stimulation, an increase of Th2 cells was described in most cases. According to this Th2 paradigm, it was proposed that early avoidance of microbial exposure could explain the increase of atopic diseases seen in the last 20 years in developed countries, as the 'hygiene hypothesis'. Recently, it was proposed that early exposure to lipopolysaccharide (LPS) could be protective against atopic diseases. However, it is well established that exposure to LPS can induce asthma symptoms, both in animals and humans, although it induces a Th1 inflammatory response. In addition, most infections induce asthma exacerbations and Th1 responses. Recently, some studies have showed that some Th1 cells were present in asthmatic patients, which could be related to bronchial hyperreactivity. There is therefore an 'infectious paradox' in asthma, which contributes to show that the Th2 paradigm is insufficient to explain the whole inflammatory reaction of this disease. We propose that the Th2 paradigm is relevant to atopy and inception of asthma albeit a Th1 activation would account at least in part for bronchial hyperreactivity and asthma symptoms.

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Key words: allergy; asthma; atopy; environment; Th1; Th2.

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Accepted for publication 30 April 2003

Atopy is an individual predisposition to develop IgE-mediated allergies against environmental allergens such as house dust mites, pets, and pollen (1). It is a genetically and environmentally determined condition, predisposing to asthma, allergic rhinitis and conjunctivitis, and atopic dermatitis. Most atopic subjects develop a rhinitis, but in a number of subjects, atopy remains clinically silent, being only detected by the presence of specific sensitizations to aeroallergens, e.g. positive skin tests and/or presence in serum of specific IgE. Bronchial hyperresponsiveness (BHR) and asthma occur only in a subgroup of atopic patients (2). We had previously found that about 50% of rhinitic patients display asthma (3).

A considerable body of data has accumulated in the past 15 years to show that atopic disorders are driven by the Th2 subset of CD4⁺ T cells. Mosmann et al. first described (4) two types of T helper (Th) clones in mice, namely Th1 and Th2 cells, which could be distinguished from one another, based on their profile of cytokine secretion. Th1 cells were then shown to be involved in delayed-type hypersensitivity, through their production of IFN- γ , and to be the main effectors of the phagocyte-mediated host defense (5, 6). Th2 cells produce IL-4, IL-5,

IL-13, IL-9 and IL-10. IL-4 and IL-13 induce IgE and IgG1 synthesis in mice and IgE and IgG4 in humans; IL-5 enhances eosinophil differentiation and is a major eosinophil-activating cytokine.

This distinction led to a series of studies which, by showing an increase of Th2 cytokine expression in atopic diseases, established the so-called Th2 paradigm, upon which Th2 cells would completely account for rhinoconjunctivitis, asthma and atopic dermatitis. However, recent data reviewed herein challenge this paradigm by showing to some extent that (a) Th2 activation is necessary but not sufficient to explain these diseases and (b) that IFN- γ producing cells are found in atopic diseases.

The Th2 paradigm in atopic diseases

Animal models

Several animal models, mainly in guinea pigs, rats and mice sensitized to ovalbumin have been used to establish the pivotal role of Th2 activation in atopic diseases. Notably, IL-4 knockout mice are resistant to ovalbumin

sensitization and therefore to subsequent eosinophilic lung infiltration upon allergen challenges (7). In IL-5 knockout mice, the allergen-induced eosinophilic lung infiltration is impaired, and the development of nonspecific airway hyperresponsiveness (AHR) is abolished (8). Reciprocally, mucosal IFN- γ gene transfer inhibits pulmonary allergic responses in mice (9). In a murine model of tropical pulmonary eosinophilia (TPE), a severe asthma-like syndrome due to an allergic response to microfilariae, IL-12 suppresses pulmonary eosinophilia and AHR by switching the *in situ* T cells from a Th2 to a Th1 profile of cytokine secretion (10). In addition to these experiments, passive transfer of Th2 cells induces AHR in mice (11–15).

The role of IL-10, produced by Th2 cells, must be considered separately. Indeed, this cytokine, produced by Th2 cells, is an immunoregulatory molecule, which inhibits rather than stimulates the allergic reaction, both at the stage of sensitization and at that of allergen challenge (16). A rising number of studies suggest that IL-10 could be produced in bronchi by non-Th2 cells, namely regulatory T cells (Tr) (17, 18). These cells are reminiscent of the well-known Th3 cells in the bowel, which by secreting IL-10 and TGF- β , down-regulate in normal conditions any deleterious inflammatory reaction against intestinal antigens. It is well established that in models of inflammatory bowel diseases, this population of T cells is impaired (19). In asthma, the impairment of Tr cells is still to be demonstrated (17). Recently, CD4+ T cells engineered to produce IL-10 prevented allergen-induced airway hyperactivity and inflammation (20).

Human studies

Whereas in mice a clear distinction between Th1 and Th2 cells can be made, in humans T cell activation varies between a Th1 pole in which Th1 cytokines are predominant to a Th2 reciprocal pole in which Th2 cells predominate (21). Every stage between both poles is possible, with a Th0 stage in which both kinds of cytokines can be produced (22). This continuum gave the substratum for the concept of Th1/Th2 balance.

Atopy. It has been conclusively demonstrated that the immune system from newborn infants is skewed to a spontaneous Th2 activation. This skewing is thought to be consecutive to the necessary tolerance established during pregnancy by the maternal immune system to avoid early abortion (23). Indeed, pregnancy must be considered as a particular case of allogenic situation in which paternal antigens must be tolerated. In this view, any immune activation leading to cytotoxicity, the main mechanism of allograft rejection, a Th1 process, must be avoided during pregnancy. Indeed, IFN- γ administration triggers early abortion in pregnant mice (23). The IL-4 and IL-10 production by maternal T cells during pregnancy avoids the deleterious IFN- γ secretion and

thus induces a natural predisposition of newborn infants to produce Th2 cytokines. Accordingly, Sudo et al. (24) had shown that mice bred in a germ-free environment spontaneously produce IgE. Prescott et al. (25) showed that in response to allergens, cord blood T cells produce a Th2 profile of cytokines, whatever the atopic status of infants.

However, infants predisposed to atopy display an impaired capacity of T cells to produce IFN- γ in response to nonspecific stimuli than nonatopic controls (26). Martinez et al. (27) demonstrated an inverse relationship between the production of IFN- γ by peripheral blood mononuclear cells (PBMC) in nine-month-old babies and the number of positive skin tests in the parents. We confirmed, in adults, that the number of T cells producing IFN- γ was lower in atopics as compared to controls (28). We showed that the number of T cells producing IFN- γ in response to a nonspecific stimulus was inversely correlated to the number of positive skin tests of the patients. Prescott et al. (25) showed that although a Th2 bias was found in both populations of infants, those predisposed to atopy responded to allergen stimulation *in vitro* by producing a smaller amount of cytokines. Furthermore, they showed that in infants who will be atopic at the age of 18 months the cytokine profile remained stable, although in the nonatopic counterparts an increase in IFN- γ production and a decrease in IL-4 production occurred at six months of life.

Asthma

Descriptive studies. Activation of T cells in asthma was first described among peripheral blood mononuclear cells (PBMC) before the Th1/Th2 balance was discovered. PBMC activation was found in asthmatics challenged with allergen (29), in acute severe asthma (30), and in mild atopic asthmatics (31, 32). Walker et al. (33) identified an association between broncho-alveolar lavage (BAL) T-cell activation, eosinophilia and asthma severity, and suggested that lymphocyte deactivation might lead to clinical improvement. Such deactivation was shown to occur after steroid treatment (34). Robinson and co-workers demonstrated an increased expression of the IL-3, IL-4, IL-5 and GM-CSF genes, but not of the IFN- γ gene in BAL from asthmatics compared to controls (35). Corrigan and colleagues found elevated levels of IL-5 in serum from patients requiring oral prednisolone therapy during asthma exacerbations (36). In response to allergens, Leonard et al. showed that in atopic asthmatics the PBMC IFN- γ /IL-4 ratio was lower in the more severe patients (37). In allergic rhinitis (38) and atopic dermatitis, a similar pattern of results was found in several studies, showing that Th2 cells predominated *in situ* during these conditions (39).

Specific challenges. Repeated low-dose allergen exposure model provides a gradual worsening of the asthmatic

response with airway eosinophilia and increased IL-5 in induced sputum, associated with BHR, but without development of marked acute bronchoconstriction (40, 41). Following seven week days of repeated low-dose allergen exposure, a significant increase in the expression of IL-13 mRNA, and a trend towards a reduced expression of the pro-inflammatory cytokines IL-6 and IL-8 and of the Th1 cytokines IFN- γ and TNF- α was found in mild asthmatics (42). One additional study showed that the cumulative dose provocation regimen can induce a more pronounced Th2-like immune response in asthmatic patients than the low dose provocation model (43). The effect on T cell activation of natural pollen exposure during the relevant season was studied in allergic patients, and most of these studies showed a seasonal increase of Th2 cytokines with no variation or a decrease of IFN- γ (44–48).

Taken together, these studies show that a Th2 skewing (a) is present in atopic subjects soon after birth, (b) is observed in blood or *in situ* in atopic subjects and (c) is increased upon low dose specific stimulation (Table 1). Therefore, one could consider that the Th2 paradigm gives a complete view of the immunopathology of atopic diseases. Once in the organism, the allergen would induce the Th2 recruitment and/or differentiation of specific T cells. These cells would induce IgE production by plasmocytes, under the action of IL-4 and IL-13. IgE

Table 1. Respective relevance of the Th2 paradigm and the Th1 paradox in atopic diseases. References in parentheses (LPS, lipopolysaccharide; BHR, bronchial hyperresponsiveness)

<i>Th2 paradigm</i>
Th2 cytokines necessary in animal models of IgE mediated inflammation
Th2 skewing of T cells at birth in atopic new-born infants
Th2 genes over-expressed in blood and <i>in situ</i> in atopic diseases compared to controls
Hygiene hypothesis
Protective effect of Th1 stimulation in animals against allergic inflammation
<i>Th1 paradox</i>
Early microbial exposure not protective against asthma
Th1 cells present in allergic diseases
Infections and LPS, Th1 inducers, trigger asthma symptoms in patients
Increase in Th1 cells during symptoms compared to baseline
Targeting of Th2 cells ineffective on BHR

would in turn be the substratum for immediate signs of allergy via the degranulation of effector cells and the release of vaso- and broncho-active mediators (e.g. histamine, leucotrienes, etc.). In the same way, IL-5 production by Th2 cells would attract and activate eosinophils, responsible for the delayed phase of allergic reaction, and in case of continued exposure, of the chronic disease (Fig. 1).

However, these conclusions, derived from these studies might be misleading for several reasons. Indeed, descrip-

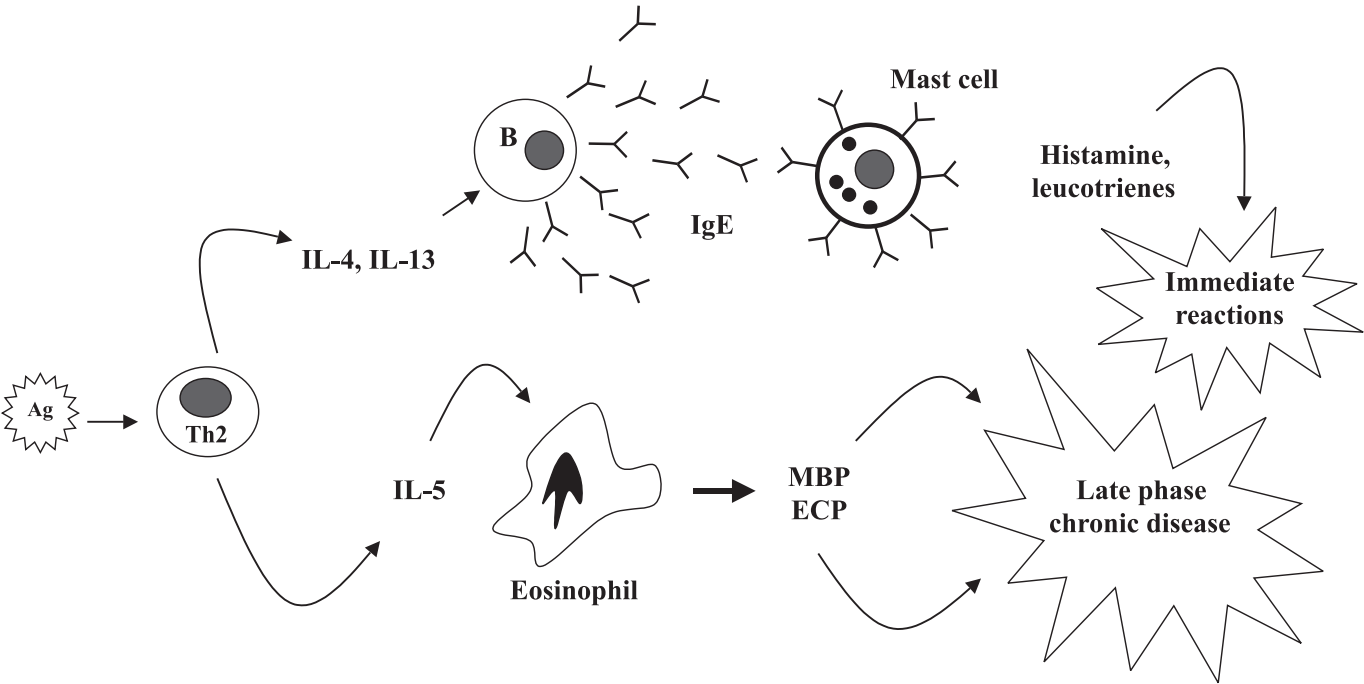


Figure 1. Th2 paradigm in atopic diseases (hypothesis). Once into the organism, the allergen triggers a Th2 activation. This activation leads to IgE production by B cells and to the early phase of allergic response via the release of vaso- and broncho-active mediators by mastocytes and other effector cells. In parallel, IL-5 activates eosinophils, leading to the late phase reaction and if allergen exposure is prolonged, to the chronic disease, via the production of basic proteins. MBP, major basic protein; ECP, eosinophil cationic protein; Ag, allergen.

tive studies indicate the state of activation of T cells at one date of the disease only by comparison with normal nonatopic controls. Therefore, the differences seen can be relevant to the patients' background (atopy) but not to the disease itself or to the cause of symptoms. Furthermore, they do not indicate the variation of T-cell activation in patients according to symptoms. In this view the effect of allergen stimulation is more relevant, but studies of low dose allergen exposure do not trigger symptoms, and studies performed during pollen seasons only assessed the T-cell activation either before and after or during and after the season but not according to symptoms.

The Th1 paradox in allergic diseases

Th1 activation protects from atopy: the hygiene hypothesis

Recent epidemiological studies have found a lower prevalence of allergic rhinitis, asthma, and allergen sensitization in subjects exposed to infectious agents in the first months of life. This was first suggested by sibling studies (49), which showed that children with more than three siblings were protected from atopy, in as much as they were the youngest. The inverse relationship between atopy and early microbial exposure was also suggested by studies comparing the prevalence of atopy and allergic diseases between populations with a distinct way of life but similar environmental exposure and genetic background. For instance, atopy was found to be twice as frequent in former western Germany as compared to eastern Germany at the time of reunification (50); the anthroposophic way of life (51), in which consumption of fermented food containing lactobacilli is frequent and vaccinations are avoided is protective against atopy. This inverse relationship was confirmed later. Indeed, Matricardi (52) showed that the presence of serum antibodies directed against various microbial antigens was inversely correlated to the presence of atopy. Recently, this author elegantly showed by analyzing the large American database of the Third National Health and Nutrition examination Survey that the increase of the prevalence of rhinitis and asthma from 1920 to 1970 was evident in HAV seronegative people but absent in HAV seropositive subjects (53). These results are in keeping with experiments performed in mice, in which microbial stimulation prevents or inhibits allergic processes. Indeed, although in mice bred in germ free conditions a spontaneous IgE production occurs, in mice normally exposed to microbes, this IgE production is suppressed, suggesting that the protection conferred by microbial exposure against allergy is more related to early gut colonization than to infection (24). Furthermore, stimulation by BCG (54, 55), lactobacillus (56) or bacterial deoxynucleotides CpG (57, 58) of ovalbumin-sensitized mice led to an inhibition of specific IgE production, pulmonary eosinophilia or AHR

during subsequent OVA challenges. In some cases, pretreatment by such microbial substances prevented the IgE dependent sensitization (55).

These epidemiological or experimental studies suggest that a lack of early Th1 stimulation creates the conditions for the development of the spontaneous Th2 deviation, according to the universal skewing to allergy previously proposed (23). This hypothesis is now widely known as the 'hygiene hypothesis' (59).

Recently, it was proposed that the substratum of the protective effect of early microbial exposure against atopy was related to the early exposure to lipopolysaccharide (LPS) (60). This role for LPS was brought by a series of studies showing that birth and early life in traditional farms (61–69), were protective against atopy. Indeed, in such an environment, exposure to LPS is high (70), due to the presence of farm animals. Indeed, in 61 infants at high risk of developing asthma, allergen sensitized individuals displayed significantly lower LPS levels at home than nonsensitized infants (71). In a subset of them, LPS levels correlated with IFN- γ producing T cells (Th1) but not with IL-4, IL-5, or IL-13-producing cells (Th2).

Th1 activation induces asthma symptoms

Infection, a Th1 activator, triggers asthma symptoms

According to the hygiene hypothesis, one would expect that infections would prevent and not induce asthma. Indeed, most infections induce a protective cytotoxic Th1 response. In the case of respiratory syncytial virus (RSV), known to be associated to asthma in infancy, Th2 activation was reported, with IgE production (72). However, it was recently shown in mice that although RSV induces a Th2 response when administered in newborn animals it induces Th1 activation when the infection occurs later (73).

It is well known, nevertheless, that asthma symptoms are frequently triggered by infections, as shown in various studies. Respiratory tract infections caused by viruses (74), chlamydia (75, 76) and mycoplasma (77) have long been involved in the triggering of asthma. Moreover, previous studies have shown that LPS, far from protecting against asthma, can correlate with and trigger the symptoms (78). Indeed, an essential role for LPS exposure in some patients with occupational asthma was proposed (79). Furthermore, Michel et al. (80) previously showed a positive association between indoor LPS levels and the severity of asthma.

In the same view, according to the hygiene hypothesis, attendance of day care centers in early life, by promoting a high microbial exposure of children is expected to protect from asthma. Kramer et al. (81) showed indeed in children from former East Germany that the entry in day care before 1 year of age protected from atopy the children with less than three siblings. However, other studies showed that day care attendance was associated to infectious respiratory diseases and asthma (82, 83).

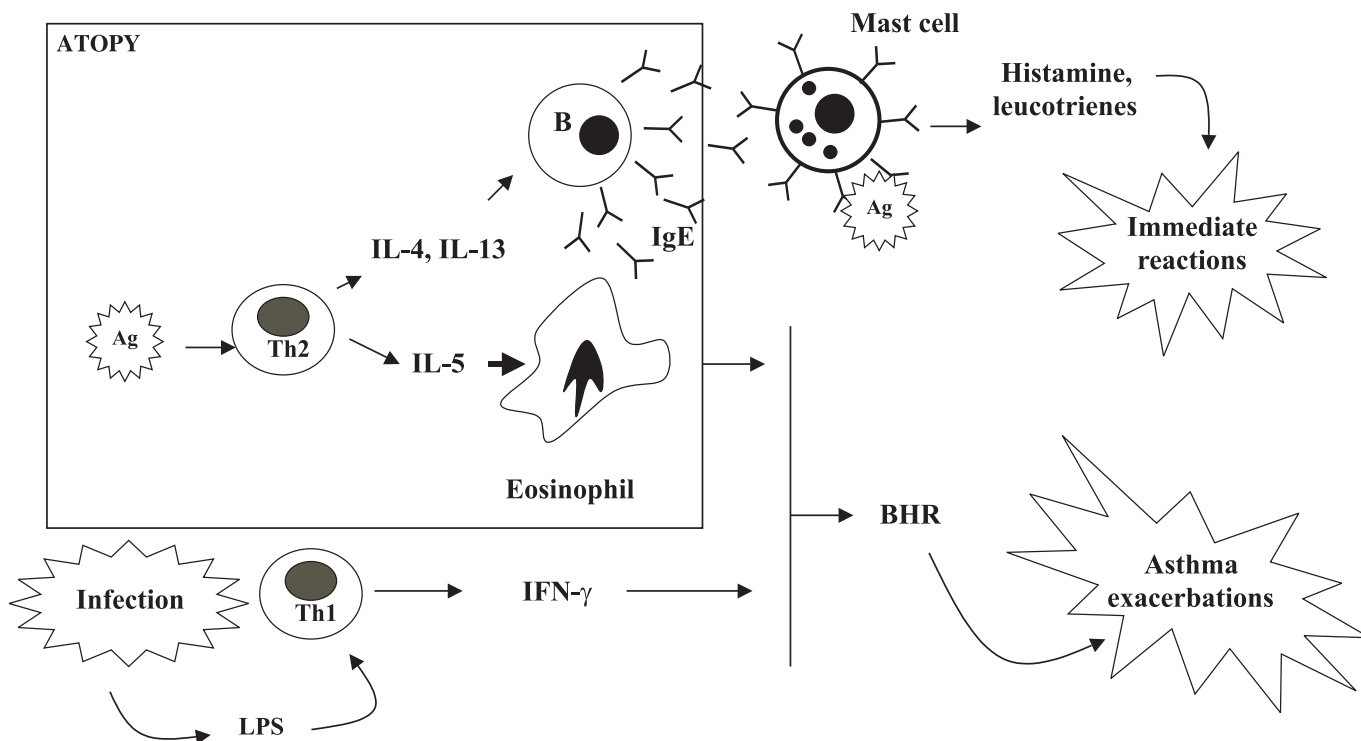


Figure 2. Th1 paradox in atopic diseases (hypothesis). The Th2 activation is relevant for the inception of atopy. Another kind of inflammation, in which Th1 cells are present, is necessary to induce the development of the disease and in patients, the triggering of symptoms. BHR, bronchial hyperreactivity; Ag, allergen; LPS, lipopolysaccharide.

Th1 activation in asthma. Krug et al. had previously showed at the single cell level an increase of IFN- γ producing T cells in BAL from asthmatics (84). Others (including ourselves) have later clearly established that Th1 activation occurs in asthma (28, 85). Indeed, we studied the IL-4 and IFN- γ production by blood T cells in 69 asthmatic patients at the single cell level by flow cytometry (28). We confirmed the intrinsic Th2 skewing in atopy, with a higher level of IL-4 in cell culture supernatants and a lower number of T cells producing IFN- γ in nonasthmatic atopics. In asthma, the number of T cells producing IFN- γ was not different from controls, and this was explained by the presence of an increase of IFN- γ producing CD8 $^{+}$ T cells (Tc1 cells). This finding was confirmed by Cho et al., who showed an increased frequency of IFN- γ producing CD4 $^{+}$ and CD8 $^{+}$ T cells in asthmatics compared with normal subjects (85).

Furthermore, IFN- γ -producing cells, present in asthma at baseline, increase during symptoms. In Chinese children, percentages of CD4 $^{+}$, CD8 $^{+}$, and IL-2R $^{+}$ lymphocytes in peripheral blood were elevated during asthma attacks and returned to baseline after resolution (86). Kuo et al. (87) found that among CD4 $^{+}$ cells, both IL-4 $^{+}$ and IFN- γ $^{+}$ cells decreased after asthma attacks. The Th1/Th2 ratio did not vary during symptoms, and was lower than controls. Corrigan et al. (88) found that

IFN- γ was increased in acute severe asthma, and returned to baseline with treatment. Recently, we showed an increase in IFN- γ producing T cells in induced sputum from symptomatic asthmatics compared to control patients (89). During cypress allergy, we developed a model of study of inflammation in which patients are sampled before, twice during, and after the season. We found a Th2 deviation in patients at baseline compared to controls, but during the pollen season the proportion of blood IL-13 producing T cells decreased whereas those of IFN- γ $^{+}$ T cells increased (90).

The role of IFN- γ producing cells found in these studies is questionable. It could reflect a negative feed back on Th2 activation. However, several works suggest that endogenous IFN- γ could induce rather than suppress BHR. Hessel et al. have elegantly shown that the eosinophilic infiltration of ovalbumin-sensitized mice can be dissociated from AHR by a prior treatment of animals with anti-IL-5 or anti-IFN- γ antibodies (91). In anti-IFN- γ treated mice, the response to metacholine was abolished, but not the eosinophilic infiltration. The source of the IFN- γ -induced BHR could be CD8 $^{+}$ T cells, as depletion of this subset in sensitized mice led to the incapacity to develop AHR, the IgE response being conserved (10, 92, 93).

The limitations of ovalbumin models reside in the fact that the effect of specific stimulation is transient, and

disappears in the hours following the ovalbumin challenge. Therefore, Mathur et al. have developed a model of IgE response against *Schistosoma mansoni*, in which the nonspecific AHR induced by a specific challenge is sustained and still present 10 days after challenge (94). The level of IFN- γ in alveolar lavage and airways sensitivity to metacholine increased at 10 days in parallel. In this model, a treatment with anti-IL-5 inhibited the eosinophilic influx but had no effect on airways hyperreactivity nor on IFN- γ , although dexamethasone abolished eosinophilia, airways hyperreactivity and IFN- γ production. This experiment is a further instance of the dissociation between bronchial hyperreactivity and eosinophilia. The former could be dependent on Th1 activation, the latter being Th2-dependent. Accordingly, it was shown that although LPS was able to prevent allergic sensitization, its effect, once mice are sensitized to ovalbumin, is not always protective. Indeed, in one study (95), LPS exposure 1 day after challenge reduces the allergen-induced lung inflammation, but increases this inflammation if administered 6–10 days after challenge. Recently, it was shown that LPS reduces the allergen-induced pulmonary eosinophil accumulation but with no effect on AHR (96). Accordingly, trials conducted in asthma with drugs specifically targeting the Th2 activation by anti-IL-5 (97) or recombinant IL-12

(98) induced a decrease in allergen-induced eosinophilia but had no effect on allergen-specific bronchial hyperreactivity.

Thus, whereas Th1 activation has a protective effect against sensitization and inception of asthma, it can increase bronchial hyperreactivity and trigger symptoms once the allergic disease is present. There is, therefore, an 'infectious paradox' in asthma, illustrated by the fact that Th2 activation and early microbial avoidance induce asthma, although Th1 activation and late microbial exposure trigger asthma symptoms (99) (Table 1).

Accordingly, one could now consider that the Th2 cell activation is necessary for the expression of atopy, required for the inception of atopic disease but not sufficient. A subsequent Th1-like activation would be necessary for the development of BHR and the triggering of symptoms (Fig. 2).

Concluding remarks

The Th2 dogma in allergic diseases does not account for the whole allergic inflammation. Relevant for the inception of atopy, it only partially explains the inflammation present in asthma (Fig. 3). In asthma, a Th1 component of inflammation is present, which is related

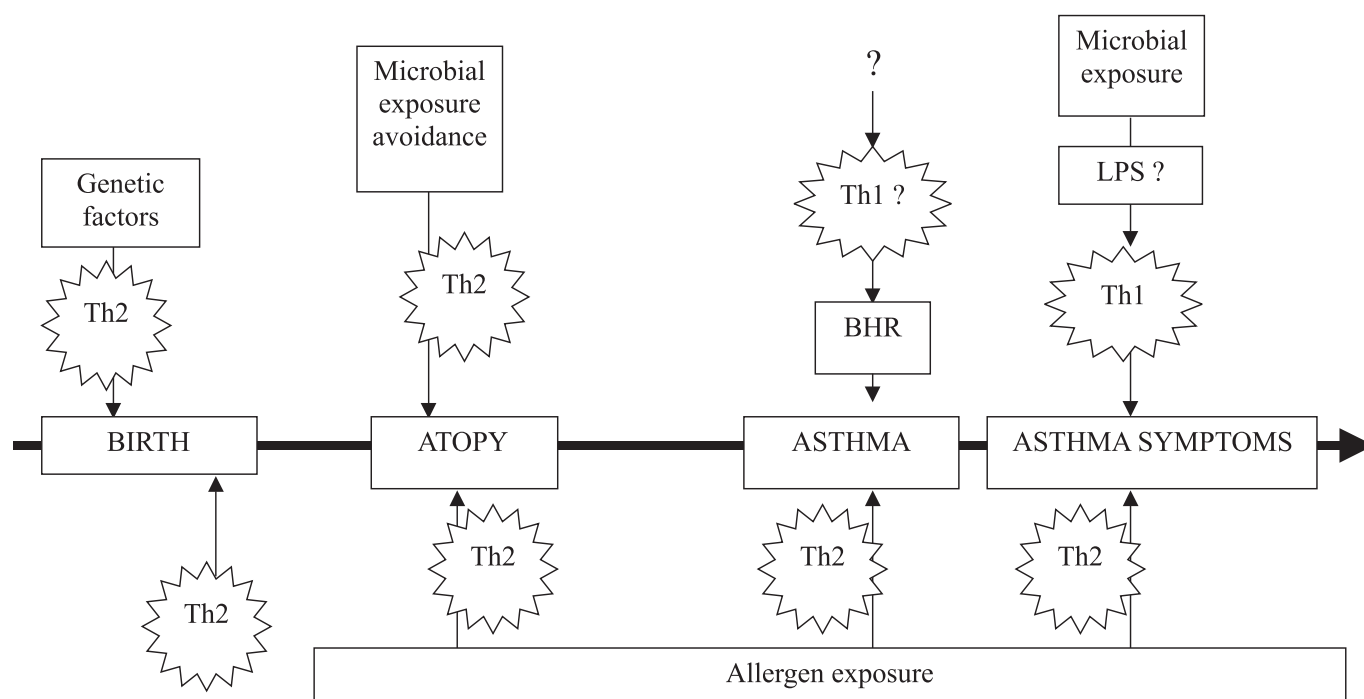


Figure 3. Natural history from atopy to asthma (hypothesis). The immune system is skewed at birth to a Th2 activation, in as much as genetic factors predisposing to atopy are present. Atopy develops in response to allergens in case of early microbial avoidance, according to hygiene hypothesis. Later, allergen exposure always stimulates the Th2 activation, but bronchial hyperreactivity and asthma are associated to Th1 activation. Once asthma is present, symptoms are triggered during microbial exposure via an additional Th1 activation. BHR, bronchial hyperreactivity; LPS, lipopolysaccharide.

to BHR and increases during symptoms. Only longitudinal cohort studies, assessing inflammation prospectively from the inception of atopy to the development of asthma, and from asymptomatic periods to exacerbations, will establish the relative extent of both kinds of inflammation.

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Acknowledgments

We thank Assistance Publique-Hôpitaux de Marseille, Collège des Professeurs de Pneumologie, and Pneumologie-Développement for the funds. Virginie Kosher is supported by a grant from Stallergènes and the Agence Nationale pour la Recherche in the frame of a 'Convention industrielle de formation par la recherche'.

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