

# Primary Prevention of Allergic Diseases: Current Concepts and Mechanisms

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**Atopic diseases, the new “epidemic of the twenty-first century” and a central health problem of industrial nations, call for the development of innovative primary prevention strategies. The present review provides an overview of current experimental and immunomodulatory procedures and their underlying mechanisms.**

**Key words:** *asthma, Th1/Th2-cytokines, immunomodulation, mouse model*

As a new “epidemic of the 21st century”<sup>1</sup> causing growing health problems, particularly in industrialized countries, atopic diseases such as hay fever, bronchial asthma, and atopic dermatitis call for the development of innovative primary prevention concepts (Figure 1, Table 1).

Pathophysiology of allergic diseases is based on extreme T helper (Th)2 immune responses to commonly harmless environmental antigens. The key cytokines interleukin (IL)-4 and IL-13 induce immunoglobulin (Ig) class switch in B cells, leading to excessive IgE production with subsequent mast cell activation and mediator release, and IL-5 contributes to development of eosinophilic inflammation and enhances mucus production of the airway epithelia (recently reviewed by Coffmann<sup>2</sup>). The reasons for dysregulation and the resulting imbalance in cellular immune responses on allergens are still not certainly identified. Genetic predisposition, especially gene–gene interactions,<sup>3</sup> seems to be a fundamental factor but does not explain the extensive increase in the incidence and prevalence of atopic diseases within the last 40 years. Numerous environmental triggers might account for this increase, such as altered climate conditions with increasing global warming, resulting in lengthened pollen seasons and thus increased exposure to environmental allergens, or lifestyle factors, such as improved hygiene.<sup>4</sup> Simple

allergen avoidance for primary prevention of allergy appeared not to be practical or sufficient,<sup>5</sup> and present antiphlogistic therapies with antihistamines or steroids just diminish symptoms for a short time but potentially cause side effects and are not curative.<sup>6</sup>

New immunomodulatory strategies aim to support naturally occurring regulatory mechanisms that may protect against predominant Th2 immune responses and maintain the immunologic balance, thus preventing the development of allergen sensitization as the first step of the atopic march in high-risk children.<sup>7</sup> Most of these new methods are currently under experimental investigation, and only a few have already been employed in humans. The present review provides an overview of these various immunomodulatory strategies and their principal mechanisms.

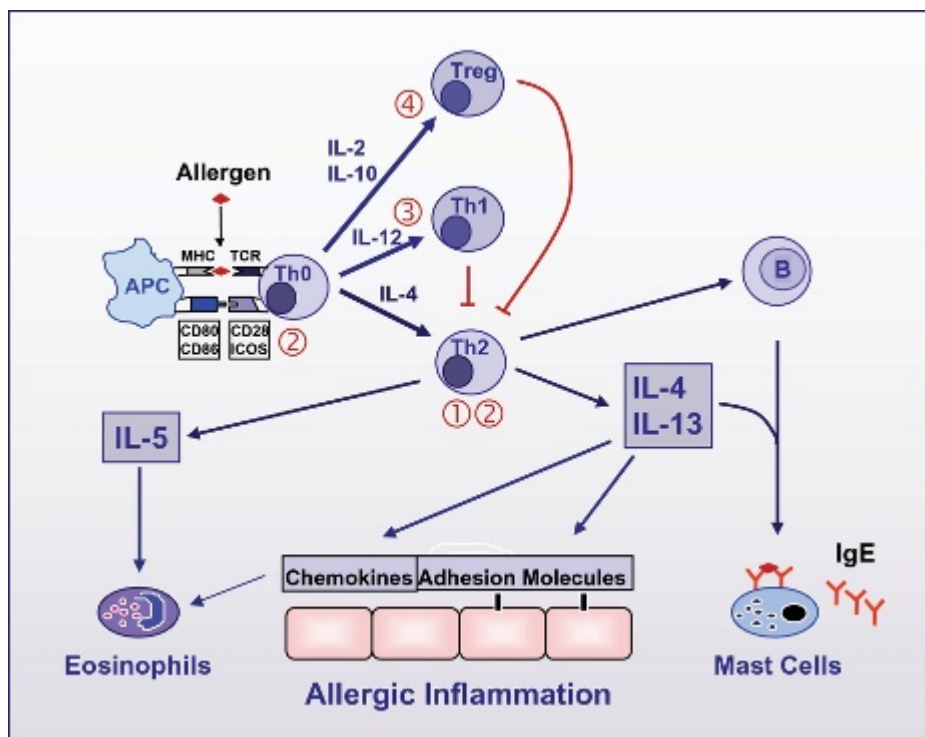
## Th1/Th2 Concept: Center of Immunomodulatory Prevention Strategies

Polarization of the adaptive cellular immune response is based on antigen presentation by dendritic cells (DCs) or other antigen-presenting cells (APCs) that leads to differentiation of naive CD4<sup>+</sup> T cells into Th1 or Th2 effector cells. Immature skin or mucosa-associated DCs phagocytize a foreign antigen on its entry site and migrate via blood and lymph to secondary lymphatic organs while they are differentiating to mature APCs. In secondary lymphatic organs, DCs create an immunologic synapse with naive CD4<sup>+</sup> T cells: they present the phagocytized and processed antigen in a complex with major histocompatibility complex molecules to the respective T-cell receptor, secrete cytokines, and express costimulatory molecules that interact with specific coreceptors on the T cell.

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**Figure 1.** Allergen-induced immune response and new concepts for primary prevention. Allergen-mediated activation of antigen-presenting cells (APCs) induces allergen-specific T helper (Th)2 cells. Th2 cells produce Th2 cytokines, resulting in increased production of immunoglobulin E by B cells, activation of chemokines and adhesion molecules, and, finally, allergic inflammation. In round brackets, targets for primary prevention concepts, as shown in Table 1.

In the presence of regulatory factors such as thymic stromal lymphopoietin (TSLP),<sup>8</sup> which is produced by epithelial cells, of the costimulatory proinflammatory molecule OX40 ligand,<sup>9</sup> and of IL-4, allergen-induced activation of mature CD8a<sup>-</sup> myeloid DCs of the lungs initiates differentiation of naive CD4<sup>+</sup> T cells to Th2 cells. IL-4 activates cytoplasmic janus kinases (JAKs) 1, 2, and 3 through its two T-cell receptor subsets that phosphorylate tyrosine rests and subsequently activate transcription factor signal transducer and activator of transcription (STAT)6. STAT6 mediates induction of transcription factor GATA-3. Both of them initiate transcription of the Th2 cytokines IL-4, IL-5, and IL-13, most likely through activation of the respective promoter genes.<sup>10,11</sup>

Intracellular pathogens promote mature CD8a<sup>+</sup> plasmacytoid DCs to produce IL-12, IL-23, and interferon (IFN)- $\gamma$ . Binding of IL-12 to the  $\beta_2$ -subset of the IL-12R on CD4<sup>+</sup> T cells activates JAK2 and subsequently STAT4. STAT4 activates the IFN- $\gamma$  promoter gene, which probably directly induces production of IFN- $\gamma$ . Further, IL-12 is able to intensify Th1 immune responses through activation of mitogen-activated protein kinase (MAPK) p38, resulting again in STAT4 activation. IFN- $\gamma$ , which is secreted by mature plasmacytoid DCs and by T cells in an autocrine pathway, activates the transcription factors STAT1 and subsequently T box expressed in T cells (T-bet). As a so-called “master controller,” T-bet promotes

the Th1 immune response indirectly via suppression of GATA-3.<sup>12</sup>

In terms of the dichotomy of the adaptive cellular immune response first described by Mosmann and colleagues,<sup>13</sup> the Th1 immune response acts as a natural antagonist of the Th2 immune response. Thus, various prevention concepts aim at generation of Th1 effector cells to suppress Th2 immune responses. At the same time, predominance of Th1 immune responses is believed to trigger development of autoimmune diseases such as type 1 diabetes, autoimmune thyroiditis, or rheumatic diseases. But as recently shown, the rise of autoimmune inflammation depends on IL-17-producing Th17 cells. In contrast to former assumptions, Th17 cells do not develop from precursor Th1 cells but represent a third Th cell population, which is directly induced by DCs producing IL-23 and inhibited by both cytokines, IL-4 and IFN- $\gamma$ . Therefore, IL-4 and IFN- $\gamma$  prevent development of autoimmune diseases, which has also been increasing within the last 40 years.<sup>14,15</sup> Use of Th1 cytokines (IFN- $\gamma$ , IL-12) in clinical surveys was ineffective or showed high rates of side effects.<sup>16</sup>

### Modulation of the Signal Transduction Cascade by Inhibition of Transcription Factors

Specific blockade of Th2 effector cytokines by monoclonal antibodies is used to treat already existing allergic diseases.

**Table 1.** Immunomodulatory Concepts for Prevention of Allergen-Mediated T Helper 2 Immune Response

No. in Figure 1	Principle	Target	Mechanism	Examples
1	Inhibition of Th2 cytokine synthesis	Transcription factors of Th2 cytokines	Inhibition of synthesis of transcription factors on the level of transcription Inhibition of synthesis of transcription factors on the level of translation	Imiquimod/resiquimod ODN decoys Antisense ODN siRNA
2		Protein kinases of signal transduction cascade	Inhibition of signal transduction cascade following activation of TCR and/or costimulatory receptor molecules Inhibition of signal transduction cascade on the way to synthesis of costimulatory receptor molecules	Inhibitors of ERK, MEK 1/2 Inhibitors of ICOS-inducing protein kinases
3	Induction of Th1 immune response	Pattern recognition receptors on APCs	Activation of TLR-2 Activation of TLR-9 Activation of TLR-4 ?	Mycobacterial antigens CpG motifs Lipopolysaccharides Probiotics
4	Induction of tolerance-inducing Tregs	Transcription factors of regulatory cytokines?	Induction of Foxp3, TGF- $\beta$ and IL-10	SIT Parasites

APC = antigen-presenting cell; CpG = cytosine guanine dinucleotide; ERK = extracellular signal-regulated protein kinase; Foxp3 = forkhead box protein 3; ICOS = inducible costimulator; IL = interleukin; ODN = oligonucleotide; siRNA = small interfering ribonucleic acid; SIT = allergen-specific immune therapy; TCR = T-cell receptor; TGF = transforming growth factor; Th = T helper; TLR = Toll-like receptor; Treg = regulatory T cell.

On the contrary, molecular concepts aim at inhibition of the distinct transcription factors STAT6 and GATA-3 for primary prevention of allergen-induced sensitization and Th2 immune responses. Antiviral activity of imidazoquinolines such as imiquimod is based on inducing Th1 immune responses in macrophages and DCs that was exploited to antagonize Th2 immune responses. In our mouse model of allergen-induced airway inflammation, local application of the imiquimod derivative resiquimod via the airways after allergen sensitization but prior to airway allergen challenges inhibited development of eosinophilic airway inflammation and airway hyperreactivity that was associated with a shift from a predominant Th2 immune response toward a predominant Th1 immune response.<sup>17</sup> Induction of T-bet and suppression of GATA-3 were recently described to be the fundamental

and protective mechanisms of imidazoquinolines.<sup>18</sup> Inhibition of Th2-inducing transcription factors can also be performed by so-called “gene silencing,” the inhibition of distinct gene transcription. Oligonucleotide (ODN) decoys competitively inhibit binding of transcription factors at the deoxyribonucleic acid (DNA) of specific promoter genes and therefore inhibit transcription of respective genes. Indeed, inhibition of STAT6 by means of ODN decoys did diminish proliferation of murine and human Th2 cells in vitro<sup>19</sup> and did suppress IgE synthesis and development of the late-phase inflammatory response in vivo in a mouse model of atopic dermatitis.<sup>20</sup>

Although STAT1 directs Th1 immune responses, it also supports development of allergen-induced airway inflammation by enhancing expression of the costimulatory molecule CD40 on APCs and B cells. CD40 interacts with

CD40L on T cells and activates them to produce Th2 cytokines. In accordance, intranasal application of STAT1-inhibiting ODN decoys did diminish Th2 cytokine production and expression of IL-4-dependent vascular cell adhesion molecule (VCAM)-1 on endothelial cells, which is known to promote leukocyte infiltration of the airways and therefore did prevent development of allergen-induced airway disease in sensitized mice.<sup>21</sup> Further experimental studies are required to analyze the effects of STAT1 on allergen sensitization.

Competitive inhibition of production of transcription factors and cytokines at the ribonucleic acid (RNA) level might also result in diminished Th2 cytokine production (recently reviewed by Popescu<sup>22</sup>). Specific antisense ODNs containing 15 to 20 ODNs activate ribonuclease H, which splits the RNA rest out of DNA-RNA double strands and therefore degrades target messenger RNA, or antisense ODNs inhibit translation via steric blockade of ribosomes. In fact, in a mouse model, local application of specific antisense ODNs did diminish expression of GATA-3, which resulted in dramatically suppressed Th2 cytokine production and allergen-mediated airway inflammation.<sup>23</sup> In contrast, suppression of STAT6 by antisense ODN decoys showed divergent therapeutic effects *in vitro* and *in vivo*.<sup>24,25</sup>

Compared to antisense ODN decoys, the small interfering ribonucleic acid (siRNA) technique promises to be more efficient. Specific endonucleases, so-called “dicer enzymes,” split long double-strand RNA into siRNA containing 21 to 23 nucleotides. Alternatively, synthesized siRNAs are commercially available. siRNAs are integrated into the RNA-induced silencing complex (RISC), which contains helicases, endonucleases, and exonucleases. RISC degrades specifically target RNA molecules by means of the antisense strand of siRNA to interrupt protein biosynthesis.<sup>26</sup> Trian and colleagues recently showed that siRNA inhibited expression of mast cell protease-activated receptor (PAR)-2 in human airway smooth muscle cells *in vitro*.<sup>27</sup> PAR-2 is probably involved in activating airway smooth muscle cells; therefore, it might provoke airway obstruction and hyperreagibility in bronchial asthma.<sup>27</sup> At present, we are analyzing in our mouse model of allergen-induced airway inflammation whether local application of siRNA suppresses expression of STAT6 and GATA-3 and subsequently inhibits allergen-induced airway inflammation.

### Modulation of the Signal Transduction Cascade by Inhibition of Protein Kinases

Receptor-dependent cytoplasmatic protein kinases are responsible for phosphorylation and activation of tran-

scription factors; thus, they fundamentally control differentiation of naive CD4<sup>+</sup> T cells in Th1/Th2 effector cells and synthesis of mediators, inducing development of allergen-induced inflammation. Inhibition of JAKs, which take part in differentiation of both Th1 and Th2 effector cells, might result in unspecific effects. In contrast, the extracellular signal-regulated protein kinase (ERK), which belongs to the MAPK, mediates activation of the eosinophilic IL-5R and eotaxin-R, initiating accumulation and degranulation of eosinophils in the airways.<sup>28,29</sup> Systemic application of a specific inhibitor (UO126) inhibited ERK through competitive inhibition of upstream MAPK/ERK-kinase (MEK)1/2 and suppressed allergen-induced IgE production, VCAM-1 expression in lungs, mucus production in the airway, and airway hyperreactivity in mice.<sup>30</sup>

Th2-cell differentiation requires further costimulatory signals, particularly interactions between CD28 and inducible costimulator (ICOS) on T cells on the one hand and their ligands CD80/86 and ICOS-L on DCs, B cells, and other APCs on the other hand.<sup>31</sup> ICOS acts through activation of MAPK, ERK, and Jun NH2-terminal kinase (JNK). Systemic application of U0126 or SP600125 selectively inhibited ERK or JNK, which, respectively, prevented local allergen-mediated Th2 immune responses and eosinophilic airway inflammation in allergen-sensitized mice following airway allergen challenges.<sup>32</sup> ICOS transcription is regulated by two independent pathways, the Fyn-calcineurin-NFATc2 pathway and the MEK2-ERK1/2 pathway.<sup>33</sup> Thus, expression of the proinflammatory costimulatory molecule ICOS might be diminished by inhibiting members of these pathways, such as the protein kinase Fyn, the transcription factor nuclear factor of activated T cell (NFAT)c2 or MEK2/ERK1/2. Methods might include direct kinase inhibitors or “gene silencing” techniques.

### Modulation of Immune Responses through Stimulation of Innate Immunity

DC activation by foreign antigens represents the first step on the way toward T-cell activation and maturation and therefore the first step on the way toward allergen sensitization. Most allergens are immunologic inert proteins that typically do not induce inflammatory responses but allergen-specific tolerance. However, the presence of so-called “danger signals” such as proteolytic enzyme activity of allergens themselves or microbial antigens leads to DC activation. Particularly, DCs express pattern recognition receptors (PPRs) such as Toll-like receptors (TLRs) for microorganism-associated molecular

patterns (MAMPs) that are invariant and consistent molecular structures of bacteria and other microorganisms. PPR activation induces MAMP-dependent signal transduction and activation of transcription factor nuclear factor (NF)- $\kappa$ B and of MAPK, followed by transcription of proinflammatory cytokines such as tumour necrosis factor (TNF)- $\alpha$ , IL-6, and IL-12 and expression of costimulatory molecules such as CD40 and CD80/CD86 (recently reviewed by Kaisho and Akira<sup>34</sup>). Regular development of the immune system and the balance of adaptive Th1/Th2 immune responses is probably based mainly on natural exposition to microbial antigens as TLR ligands via the gastrointestinal tract, skin, and airways or on several infectious diseases during early infancy and childhood. A variety of immunomodulatory prevention concepts attempt to reconstitute the natural balance of the adaptive immune response by specific activation of PPRs by means of microbial antigens.

### Mycobacterial Antigens

Mycobacterial antigens such as lipoproteins activate TLR-2 in complex with TLR-1 and TLR-6 or TLR-4; induce production of IL-12, TNF- $\alpha$ , IL-10, and IL-15; and initiate development of Th1 effector cells.<sup>35</sup> In numerous mouse models, vaccination with live or inactivated pathogenic or apathogenic *Mycobacteria* prevented development of allergen-mediated sensitization and airway inflammation.<sup>36–39</sup> Recent clinical trials showed a therapeutic effect such as subcutaneous injection of heat-inactivated *Mycobacteria bovis* bacille Calmette Guérin on pre-existing asthma in adults<sup>40</sup> or intradermal application of *Mycobacterium vaccae* on moderate or severe atopic eczema in children.<sup>41</sup> Nevertheless, primary preventive effects of *Mycobacteria* on atopic diseases in humans need to be further investigated.

### CpG motifs

Unmethylated cytosine guanine dinucleotides (CpGs) are common components of prokaryotic bacterial or viral DNA; they are also synthetically produced (CpG motifs). CpGs are incorporated by DCs via endocytosis; they bind and activate cytosolic TLR-9 and induce activation of NF- $\kappa$ B, followed by secretion of type I interferons, IL-12, IFN-inducing protein 10, and other cytokines and chemokines. The resulting innate Th1 immune response is short and limited to proliferating T cells; it is not able to modulate memory Th2 cells.<sup>34</sup> Further, CpG motifs activate the tryptophan-degrading enzyme indolamine-2,3-deoxygenase (IDO) via the STAT1 pathway in CD19<sup>+</sup> DCs.

Intracellular lack of tryptophan and its metabolites causes toxic and other unknown effects, causing diminished T-cell proliferation and immune suppression. Thus, CpG motifs support development of regulatory T cells (Tregs).<sup>42</sup> Accordingly, they induced Th1 cells and/or Tregs that inhibited Th2 immune responses and prevented allergen-induced sensitization and airway inflammation in many animal models and clinical trials (lately reviewed by Racila and Kline<sup>43</sup>). At present, CpG motifs are more and more used as adjuvants for allergen-specific immune therapy (SIT), even in humans. CpG motifs are conjugated with allergens; local or systemic administration of these conjugates generates allergen-specific long-lasting adaptive Th1 immune responses, induces Tregs, and probably also stimulates memory Th2 cells to shift into Th1 effector cells after further allergen contacts.<sup>44</sup>

### Lipopolysaccharides

The so-called “farming effect” belongs to the best-described environmental factors that are associated with a diminished risk of atopic diseases.<sup>45</sup> It is based on intensive exposure to organic dust and thus to a variety of microbial antigens in stables on farms from early infancy on. Peters and colleagues recently confirmed protective properties of organic dust from stables with regard to allergen-mediated sensitization and airway inflammation in a mouse model.<sup>46</sup> Several experimental studies in mice and humans have analyzed, in particular, the immunomodulatory allergy-preventing effects of lipopolysaccharides (LPSs), the cell wall component of gram-negative bacteria and an important ingredient of organic dust. In serum, LPSs bind their soluble receptors lipopolysaccharide-binding protein (LBP) and CD14 and activate TLR-4; LBP and CD14 catalyze TLR-4 activation.

TLR-4 activation activates through the intracellular adaptor molecule MyD88-associated cytoplasmatic protein kinases such as IL-1 receptor-associated kinase (IRAK)4 and others (TRAF6, TAK1, IKK $\beta$ ), which leads to I $\kappa$ B phosphorylation and finally to NF- $\kappa$ B activation.<sup>34</sup> Epidemiologic studies suggested that polymorphisms for CD14 and TLR-4 resulting in reduced responsiveness of DCs on LPSs are associated with an increased risk of developing atopic diseases.<sup>47</sup> In our own work in adult mice, local and systemic application of LPSs later suppressed allergen-mediated sensitization and airway inflammation in an IL-12-dependent way.<sup>48</sup> In neonatal mice, repetitive exposure to simple aerosolized LPSs did not prevent subsequent allergen sensitization, but in combination with allergen-induced mucosal tolerance,



LPSs elicited an unspecific Th1 immune response, which might diminish the susceptibility of organisms to a variety of environmental allergens.<sup>49</sup>

Further, Wang and McCusker showed in a similar model that repetitive exposure of neonatal mice to LPS and ovalbumin led to development of tolerance-inducing Tregs in later sensitized mice.<sup>50</sup> Prenatal initiated and postnatal continued exposition to aerosolized LPS inhibited development of allergen-induced sensitization and airway inflammation in the offspring that was associated with a shift from a predominant Th2 immune response toward a predominant Th1 immune response and was most likely mediated by upregulation of the LPS receptors LBP, CD14, TLR-2, and TLR-4, as well as of the Th1 regulatory transcription factor T-bet.<sup>51</sup>

At present, we are investigating in a prospective, double-blind, placebo-controlled, interventional trial in high-risk infants the potentially preventive effect of orally given apathogenic *Escherichia coli* strains on the development of atopic dermatitis within the first 7 months of life.

### Probiotics

Colonization of the gut by commensal microbes within the first months of life represents the first and probably most important stimulus for the development of the gut-associated immune system, the largest organ-associated immune system. Composition of the gut flora might influence allergen sensitization decisively since epidemiologic observations demonstrated that countries with a high or low prevalence of allergic diseases and atopic and non-atopic individuals showed different microbial strains in the gut,<sup>52</sup> and oligosaccharides (prebiotics) might prevent allergies by supporting the growth of distinct microbes.<sup>53</sup> Thus, at present, animal models and clinical trials are used to elucidate the potentially preventive effects of probiotics, living apathogenic bacteria with health-supporting effects. Indeed, in a prospective clinical trial, *Lactobacillus rhamnosus*, which was given orally during pregnancy and further on during the first months of life, inhibited manifestation of atopic dermatitis in high-risk infants.<sup>54</sup> The probiotics employed are lactobacilli and bifidobacteria in particular, which are acid resistant and adherent to gut mucosa and further colonize the gut. The mechanisms are unclear. In neonatal mice, probiotics induced development of transforming growth factor (TGF)- $\beta$  producing T cells, resulting in diminished IgE and Th2 cytokine production<sup>55</sup>; another clinical trial showed enhanced Th2-antagonizing IFN- $\gamma$  production.<sup>56</sup> Increased permeability of gut epithelia for allergens, which was shown for children

with atopic dermatitis, is also suggested to cause allergen sensitization.

Distinct gut bacteria produce toxic metabolites such as D-lactic acid or acetaldehyde, which inhibit adenosine triphosphate-dependent synthesis of the epithelial cytoskeleton, resulting in defective barrier functions. In young infants, these metabolites accumulate even more as a consequence of immature degrading enzymes. Probiotics, which do not induce toxic metabolites, might provide a balance of the gut flora and compensate for toxic effects, such as breast milk.<sup>57</sup>

### Modulation of Immune Responses by Tolerance Induction

The immune system physiologically does not respond to self-molecules or harmless environmental antigens. Tregs are thought to mediate this phenomenon of antigen-specific tolerance. Natural Tregs develop in the thymus, express constitutively CD25 (IL-2R $\alpha$  chain) and the transcription factor forkhead box protein 3 (Foxp3), and act in an antigen-independent manner immunosuppressively. In the periphery, a xenogeneic group of adaptive antigen-specific Tregs (aTregs) develop from still unknown (CD25<sup>-</sup>) precursor cells in response to foreign antigens. ATregs become CD25<sup>+</sup> during their development; only some of them express Foxp3, especially following activation through CD3, CD28, and TGF- $\beta$ .<sup>58</sup> IL-2 is a decisive growth factor for Tregs; CD28 acts as a costimulatory factor<sup>58</sup>; Foxp3 forms a complex with histone acetyltransferases, histone deacetylases, and chromatin remodeling factors, and inhibits acetylation of histones that results in stopping of DNA transcription as the first step in T-cell proliferation and differentiation.<sup>58</sup> Akdis and colleagues first described diminished numbers of Tregs in atopic patients.<sup>59</sup> Thus, an imbalance between Th2 (and Th1) cells on the one hand and Tregs on the other hand might be responsible for the development of atopic diseases, and immunomodulatory prevention concepts focus on induction of Tregs. The Foxp3 complex itself might be a target; inhibitory factors of histone deacetylases mediate stopping of the cell cycle, diminish cytokine expression, and increase apoptosis, but low target specificity causes serious side effects. At present, more specific Foxp3-associated molecular targets are being extensively investigated to modulate the effects of the Foxp3 complex.<sup>58</sup>

Myeloid and plasmacytoid, immature and mature DCs induce aTregs by producing anti-inflammatory cytokines, particularly IL-10. In a positive feedback mechanism, IL-10 from DCs and IL-10 and TGF- $\beta$  produced by Tregs initiate the development of tolerogenic DCs.<sup>60</sup> Further, Tregs

suppress expression of costimulatory molecules such as CD80/CD86 on maturing DCs. Thus, antigen-activated Tregs are able to inhibit sufficient presentation of further antigens by the same DC.<sup>61</sup> Allergens in higher doses than required only for allergen sensitization activate CD8<sup>+</sup> myeloid DCs that initiate differentiation of aTregs through their costimulatory molecule ICOS-L and transient production of IL-10.

At present, allergen-specific immunotherapy (SIT) represents the only established curative but merely secondary preventive and antigen-specific therapy for allergic diseases. Subcutaneous applications of increasing doses of allergen over 3 to 5 years induce allergen-specific Foxp3<sup>+</sup> Tregs, which express surface molecules such as cytotoxic T-lymphocyte antigen (CTLA)-4 and programmed death (PD)-1 and secrete IL-10 and TGF- $\beta$ . Therefore, these cells induce a lifelong allergen-specific tolerance through intensive immunosuppressive and anti-inflammatory properties.<sup>62</sup> CTLA-4 of these Tregs also activates mature DCs via CD80/CD86, which consequently express IDO and may suppress T-cell functions the other way round.<sup>61</sup>

Following mucosal allergen exposition via the airways, plasmacytoid DCs are activated, which generate Tregs and cause allergen-specific mucosal tolerance in mice.<sup>49,62</sup> Our own preliminary data showed that repetitive exposures of pregnant mice to aerosolized allergen consistently prevented later allergen sensitization and airway inflammation in the offspring associated with diminished allergen-specific T-cell responses in vitro and development of IFN- $\gamma$ -producing T cells (unpublished data).

Heat-inactivated *Listeria monocytogenes*, which was given as an adjuvant together with an allergen, activated mature CD8<sup>+</sup> plasmacytoid DCs to produce IL-10 and IL-12, resulting in development of IL-10- and IFN- $\gamma$ -producing allergen-specific Tregs. These Th1-like Tregs expressed Foxp3 and later prevented allergen-mediated airway hyperreactivity in mice.<sup>63</sup>

### Modulation of Immune Responses by Parasites

During their acute infectious state, helminthes secrete proteases that act as virulent factors and induce a strong Th2 immune response and a massive unspecific IgE production in the host. Further, proteases act as “danger signals” and activate DCs that might promote allergen sensitization.<sup>64</sup> Additionally, parasite antigens such as tropomyosins might show cross-reactivity with allergens, resulting in enhanced allergen sensitization.<sup>65</sup> In contrast, the anti-inflammatory effects of helminthes in the chronic state might be responsible for inverse correlations between

parasitic and allergic diseases.<sup>66</sup> The anti-inflammatory property of helminthes is more and more used for immunomodulatory therapeutic and prevention concepts, although the underlying mechanisms have not been clarified. Both DCs and APCs, as well as CD4<sup>+</sup> T cells, might play a key role. According to experimental data, helminthes induce Foxp3<sup>+</sup> IL-10- and TGF- $\beta$ -producing Tregs that inhibit development of allergen-mediated sensitization and airway inflammation in mice.<sup>67,68</sup> Helminthes also induce CD1<sup>+</sup> natural killer T cells, a subgroup of T cells that express natural killer cell markers and produce immunoregulatory cytokines.<sup>69</sup>

Filarias produce the anti-inflammatory molecule ES62, which suppresses B-cell activation and proliferation by interaction with the signal transduction cascade of the B-cell antigen receptor and inhibits production of proinflammatory cytokines by interaction with the TLR signal transduction cascade.<sup>70</sup> Further, oligosaccharides with immunomodulatory capacities such as lacto-N-neotetraose, which helminthes express on their surface, induce a subgroup of natural Gr1<sup>+</sup>CD11b<sup>+</sup>F4/80<sup>+</sup> suppressor cells, immature myeloid cells that produce IL-10 and TGF- $\beta$  and inhibit proliferation of naive CD4<sup>+</sup> T cells via IFN- $\gamma$ -dependent cell-cell contact.<sup>71</sup> Development of derivatives of these natural immunomodulatory molecules might be of use for primary prevention against allergen-mediated diseases.

### Conclusion

Enormous progress in clarifying the genetic and molecular mechanisms of allergic sensitization allows the development of novel immunomodulatory strategies aimed at primary prevention of allergen-mediated diseases. These are based either on the inhibition of their most relevant pathogenetic elements or in the induction of natural immunoregulatory mechanisms. The achievement of balance in adaptive immune responses against allergens represents the common goal of novel preventive concepts. Ultimately, these specific and curative treatment procedures shall remove symptomatic and often unspecific therapies with potentially severe side effects. The first promising experimental data are giving hope but need to be carefully validated in clinical trials for practicability, safety, and efficiency.

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