Essentials for starting a pediatric clinical study (2):
Role of environment and immunity in the development of childhood allergic and immunologic disorders

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ABSTRACT — Regulatory T- (T_{REG}) cells are considered to inhibit the development of both type 1 and type 2 helper T (T_{H1} and T_{H2}) cells. However, the number of T_{REG} cells in patients with allergic diseases who have high levels of serum IgE and blood eosinophils is reduced as compared to individuals who have similarly high levels of IgE and eosinophils but are asymptomatic. Therefore, T_{REG} cells may suppress the onset of allergic disease by downregulating other types of immune cells besides T_{H1} and T_{H2} cells. The newly discovered interleukin (IL)-17-producing helper T- (T_{H17}) cells responsible for autoimmune inflammatory diseases may counteract with T_{REG} cells even in allergic diseases. T_{REG} cells capable of producing of high levels of tumor necrosis factor (TNF)-α may also be involved in the inflammation in allergic diseases. In this review, the role of T_{H1}, T_{H2}, T_{H17} and T_{REG} cells in allergic diseases is further discussed by using the balancing square model and the factors differentiating between patients with clinical manifestations of allergic symptomatic versus atopic individuals who are sensitized but asymptomatic.

Key words: Helper T-cells, Regulatory T-cells, Interleukin 17, Mast cells, Thymic stromal lymphopoietin

INTRODUCTION

There is an inverse correlation between the levels of endotoxin in house dust and the incidence of atopic sensitization and hay fever (Braun-Fahrlander et al., 2002). The major role of endotoxin is considered to be the stimulation of macrophages or antigen-presenting cells to produce interleukin (IL)-12, which triggers the development of antigen-specific type 1 helper T- (T_{H1}) cells and inhibits the development of type 2 helper T- (T_{H2}) cells. As such, the hygiene hypothesis associated with an increasing prevalence of allergic diseases has been theorized by the T_{H1}/T_{H2} paradigm (Mosmann et al., 1986) for a long time.

Most epidemiological studies supporting the hygiene hypothesis also indicate that preventive effect on allergy of an “unhygienic” environment surrounded by many microbial components is limited to early childhood (Braun-Fahrlander et al., 2002). According to the classical T_{H1}/T_{H2} paradigm theory, this could be typically speculated as shown in Fig. 1. A T_{H1} dominant immune system develops in an individual when the immune system is exposed to allergens without prior exposure to microbial components such as endotoxin early in life. On the other hand, development of allergen (antigen)-specific T_{H1} cells is triggered by simultaneous exposure to the antigen and microbial components. After childhood, the proportion of T_{H1}/T_{H2} cells is not drastically altered by microbial exposure due to a decrease in the number of naïve helper T-cells that can react with common allergens.

The incidence of T_{H1}-mediated autoimmune diseases is also known to have increased in the last half century in parallel with the increase of T_{H2}-mediated allergic diseases (Bach, 2002). The Classical T_{H1}/T_{H2} paradigm cannot be used to explain this point.

RESULTS AND DISCUSSION

Roles of regulatory T-cell populations

Several subsets of CD4+ cells are able to prevent immune responses against self-antigens or allergens. These cells are called regulatory T- (T_{REG}) cells. Since T_{REG} cells inhibit both T_{H1} and T_{H2} cell development in vitro, increases in the incidence of T_{H1} diseases and T_{H2}-mediated diseases are now thought to be related to an insufficient development of T_{REG} cells. However, there is no evidence yet whether the “hygienic” environment with exposure to less microbial components during early life affects the development of T_{REG} cells.
Among the several subsets, naturally-occurring T_{REG} (nT_{REG}) cells have been well investigated. These cells originate in the thymus, express the repertoire of CD4^+ CD25^+ (mouse) or CD4 CD25^{high} (human) and the transcription factor forkhead box protein P3 (Foxp3), and have a major role in modulating the activity of self-reactive cells by inducing the destruction of autoreactive T-cells mainly via cell-cell contact-dependent mechanisms (Sakaguchi et al., 2008). Therefore, nT_{REG} cells are considered to mainly have a preventive role in autoimmune diseases.

In humans, a population of CD4^+ CD25^{high} T-cells with regulatory function very similar to nT_{REG} cells but derived from peripheral memory CD4^+ CD25^+ T-cells has recently been described (Mindi et al., 2005). They are called as adaptive T_{REG} (aT_{REG}) cells (Bluestone et al., 2003) or inducible T_{REG} (iT_{REG}) cells. One of iT_{REG} cell types is the IL-10 producing type 1 regulatory T- (Tr1) cells, whose suppressive function has been well documented in allergy and autoimmunity (Roncarolo et al., 2006). The term “iT_{REG}” is often used for the IL-10-producing Tr1, whereas the term aT_{REG} is often used as CD4^+ CD25^{high} T-cells derived from peripheral memory CD4^+ CD25^+ T-cells. Therefore, in this review, we have used the term “aT_{REG}” as Foxp3^+ CD4^+ CD25^{high} T-cells as shown in the recent review article written by Bacchetta et al. (2007). Nevertheless, all subsets of T_{REG} cells require a cytokine, transforming growth factor (TGF)-β for their development. IL-10 is also produced not only by Tr1 cells but also by various cell types including regulatory dendritic cells that can induce aT_{REG} (Svensson et al., 2004). As such, these two cytokines play an important role in immune regulation.

Foxp3 mutant mice develop an intense multiorgan inflammatory response including allergic airway inflammation, striking hyperimmunoglobulinemia E, eosinophilia, and dysregulated T_{H1} and T_{H2} cytokine production (Schubert et al., 2001; Hori et al., 2003). In human, genetic defects in Foxp3 cause immune dysregulation, polyendocrinopathy, and enteropathy, X-linked (IPEX) syndrome (Wildin et al., 2002). Most IPEX patients suffer from food allergy and atopic dermatitis-related symptoms immediately after birth. It is thus suggested that Foxp3^+ T_{REG} cells play an important role in regulating common allergic disorders as well as IPEX. The number of Foxp3^+ T_{REG} cells is decreased in skin lesions in patients with atopic dermatitis and in psoriasis patients (Verhagen et al., 2006).

Although Foxp3 is transiently expressed by antigen-activated helper T-cells (Allan et al., 2007), only persistent and high-level Foxp3 expression is related to the immunosuppressive functions (Sakaguchi et al., 2008).
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(IFN)-γ derived from T_{H1} cells often prevents the IL-17-mediated inflammation in mice with experimental autoimmune diseases (Nakae et al., 2002; Steinman, 2007). A T-cell subpopulation that exclusively produces IL-17 (T_{H17}) is now credited for causing and sustaining tissue damage. Human and mouse T_{H17} cells may require different sets of cytokines for their development. Thus, the identification of the T_{H17} cells triggered a shift in the immunologists’ perspectives regarding the basis of tissue damage or autoimmune diseases, where as for over 20 years the role of T_{H1} cells was considered paramount (Steinman, 2007).

However, it has been recently revealed that the expression of IL-17 in human CD4+ T-cells may be completely different from mice (Evans et al., 2007). Unlike mouse, human IL-6 and IL-21 do not induce IL-17 expression in either naïve or effector T-cells. TGF-β inhibits human T_{H17} cell development but promotes mouse T_{H17} cell development when co-stimulated with IL-6 (Evans et al., 2007). It should also be noted in human adult peripheral blood, a large proportion of helper T-cells can produce both IFNγ and IL-4. The proportion of T_{H17} cells in peripheral blood CD4+ cells are consistently less than 1% in the peripheral blood from healthy individuals, and slightly higher among CD4+ T-cells derived from the patients with Crohn’s disease (Annunzio et al., 2007). Autologous T_{REG} cell clones suppress T_{H1} or T_{H2} cells but not T_{H17} cells (Annunzio et al., 2007). Nevertheless, IL-6 is the most important growth factor for this cell type since it inhibits the development of both human and mouse T_{REG} cells (Romagnani et al., 2006).

The key cytokine of T_{H17} cells, IL-17, is known to induce the production of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 as well as proinflammatory chemokines CXCL1, 2, and 8 by acting on various cell types (Schmidt-Weber et al., 2007). In humans, sputum IL-17A mRNA levels are significantly elevated in asthmatics as compared to healthy controls (Bullens et al., 2006). Endogenous IL-17 contributes to the development of allergen-induced airway hyperresponsiveness and there is also evidence that IL-17 stimulates the release of several cytokines with known capacity for airway remodeling, from cells normally residing in the airways (Linden, 2006).

With the discovery of T_{H17} and T_{REG} populations, the balancing square model is now needed to explain the pathogenesis of various immunological diseases. It also enables us to explain the epidemiological data demonstrating an increase in allergic diseases (Fig. 2). According to this model, we speculate that substantial amounts of plant antigens, parasites, molds, viruses and bacteria are required for balancing the total immune system (Fig. 2). Autoimmune diseases are now considered to be initiated by an upregulation of T_{H17} cells and a defect in nT_{REG} cell function, whereas peritumor tissues are strikingly infiltrated with Foxp3+ nT_{REG} cells implying that these cells impinge upon immune-mediated rejection of the tumor (Betts et al., 2007).

![Diagram of immune cells and cytokines](image-url)

**Fig. 2.** The balancing square model.

The four T-cell types (T_{H1}, T_{H2}, T_{H17} and T_{REG}) antagonize each other. T_{H1}-promoting cytokine, IL-12 is inhibitory to T_{H2} cell development, whereas T_{H2}-promoting cytokine, IL-4 blocks T_{H1} development. T_{H17}-derived IFNγ blocks T_{H17} development. T_{REG} inhibits development of both T_{H1} and T_{H2} cells by direct contact.

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Role of aT$_{REG}$ or iT$_{REG}$ cells in the onset of allergic diseases

Allergic diseases are caused by uncontrolled T$_{H2}$-based immune responses to environmental antigens. It has been demonstrated that healthy nonatopic subjects have detectable IL-10-producing allergen-specific Tr1-like T$_{REG}$ (iT$_{REG}$) cells, whereas the proportions of these T$_{REG}$ cells are very low in symptomatic allergic patients (Akdis et al., 2004).

Several studies suggest a possible defect and impaired function of nT$_{REG}$ cells in the pathogenesis of immune responses toward allergens. However, like studies on autoimmune diseases, it is often difficult to distinguish the overlapping phenotypic characteristics between T$_{REG}$ cells and activated helper T-cells (Bacchetta et al., 2007). Moreover, nT$_{REG}$ cells are found to act only to auto-antigens but not to exogenous antigens, which cause allergic diseases. Therefore, aT$_{REG}$ or iT$_{REG}$ cells are surely more important than nT$_{REG}$ cells.

We recently demonstrated that symptomatic atopic patients had a lower Foxp3$^+$CD4$^+$ ratio than asymptomatic controls having similar levels of serum IFN-$\gamma$, total IgE and eosinophils. These results suggest that circulating Foxp3$^+$CD4$^+$ cells regulate unknown factor(s) affecting the onset of allergic diseases which are unrelated to these T$_{H1}$/T$_{H2}$ markers. Measurement of Foxp3$^+$CD4$^+$ cells has the potential to aid in evaluating the presence of active inflammation, which cannot be evaluated by known T$_{H1}$ and T$_{H2}$-related markers in patients with allergic diseases (Orihara et al., 2007).

One of the many reasonable explanations for this observation is considered as follows; i.e., downregulation of Foxp3$^+$ aT$_{REG}$ cells is often related to upregulation of T$_{H1}$ cells. In mice, IL-17 activates mast cells to release a proinflammatory cytokine, tumor-necrosis factor (TNF)$\alpha$ and thus can cause neutrophilic inflammation (Nakae et al., 2007). In humans, T$_{H1}$ cells may enhance allergic inflammation by stimulating the tissue resident cells to release TNF-$\alpha$, and are proven to evoke marked inflammation and airway remodeling. Using the balancing square model, the immunological feature of symptomatic allergic diseases may be illustrated as in Fig. 3A. Among T$_{H1}$ cell subtypes, TNF-$\alpha$-rich inflammatory T$_{H2}$ (iT$_{H2}$) cells may be developed by stimulation of dendritic cells with T$_{H2}$ adjuvants associated with allergens or thymic stromal lymphopoietin (TSLP) often found in inflammatory tissues in allergic diseases (Liu, 2007). Thus, iT$_{H2}$ and T$_{H17}$ cells can be both upregulated in symptomatic allergic patients, where mast cells are also activated. On the other hand, in asymptomatic controls having similar high levels of IgE and eosinophils, both T$_{H2}$ and aT$_{REG}$ cells may be upregulated. Due to T$_{H17}$ cells, the levels of IFN-$\gamma$ may be kept at considerably high levels. However, these patients are often infected with viruses at the site of inflammatory tissues due to downregulation of classical T$_{H1}$ cells (Gern et al., 2000) capable of producing antiviral cytokine IFN-$\alpha$.

Among T$_{H2}$ subsets, IL-10-producing regulatory T-

![Fig. 3](image-url)

Fig. 3. Onset of allergic diseases may be determined by the ratio of proinflammatory T-cell subsets (T$_{H17}$ and iT$_{H2}$) versus T$_{REG}$ subsets.
A. In patients with chronic allergic diseases, proinflammatory T-cell subsets, i.e., T$_{H1}$ cells and T$_{H2}$ cells capable of producing high levels of TNF-$\alpha$ (iT$_{H2}$ cells) are upregulated.
B. In asymptomatic atopic individuals, T$_{H2}$ cells capable of producing IL-10 (iT$_{H2}$ cells) may be upregulated and T$_{H17}$ cells may be inactivated.
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(rT_{H2}) cells may be upregulated in asymptomatic atopy individuals. Mast cells are therefore not activated, in spite of the presence of high levels of IgE antibodies and constant exposure to common allergens as shown in Fig. 3B. Nevertheless, it will be necessary to determine why some people do not have marked mast cell activation even though they are sensitized to multiple allergens.

T_{REG} cells can suppress not only T-cells but also natural killer cells, dendritic cell maturation, and antibody production by B cells (Miyara et al., 2007). Recently, mast cells and T_{REG} -derived IL-9 are found indispensable in T_{REG} -mediated peripheral tolerance to allograft transplantation in a mouse model (Lü et al., 2006). Pollen immunotherapy that is known to induce allergen-specific aT_{REG} or Tr1 cells inhibits seasonal increases in IL-9 protein expression and c-Kit+ mast cell infiltration in the nasal mucosa during the pollen season (Nouri-Aria, et al., 2005). It is of particular interest to investigate further relationship between aT_{REG} or iT_{REG} and mast cells in future studies.

In conclusion, An atopic predisposition is acquired via upregulation of T_{H2} cells compared to IFN-γ-producing T_{H1} cells (T_{H1} cells and T_{H1'} cells) specific for each allergen as shown in Fig. 1. Numerous epidemiological studies indicate that microbial components affect the balance between these T-cell types (Braun-Fahrländer et al., 2002). It should be noted, however, that the majority of people who acquired an atopic predisposition in a “hygienic” environment are still asymptomatic or having very mild symptoms (Orihara et al., 2007). At present, we have no answer as to why some further develop the clinical manifestations of allergic disease while others remain asymptomatic.

We have reported that active atopic patients had a lower Foxp3^CD4^ ratio than asymptomatic controls having similar levels of serum IFN-γ, total IgE and eosinophils (Orihara et al., 2007), suggesting that the development of clinical manifestations of allergic diseases may be determined by the ratio of proinflammatory T-cell subsets (T_{H1'} and iT_{H1'}) versus T_{REG} subsets. Increased ratio of proinflammatory cytokines (IL-17, TSLP and IL6) versus regulatory cytokines (IL-10 and TGF-β) in severe allergic diseases (Bacchetta et al., 2007; Romagnani, 2006, Schmidt-Weber et al., 2007, Liu, 2007) would activate further the balance shift and form a positive feedback loop in chronic inflammation (Fig. 4). Nevertheless, we

**Fig. 4.** A model for development of allergic and autoimmune diseases in the absence of microbial components. Increased ratio of proinflammatory cytokines (IL-17, TSLP and IL6) versus regulatory cytokines (IL-10 and TGF-β) may play a key role in determining inflammatory allergic/autoimmune diseases versus asymptomatic individuals.
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will have to identify the factors influencing the balance shift of proinflammatory and regulatory T-cell population.

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