

Summary



The incidence of allergies in the Western World is rising over the past few decades. There are several kinds of allergies known, such as inhalation allergies and food allergies. These allergies are characterized as a Type I hypersensitivity of the immune system. Typical for these allergies is the production of certain antibodies directed against the allergen. There are several allergens known that underlie such reactions, such as inhalation allergens (e.g. tree pollen and grass pollen) and food allergens (e.g. cow's milk and peanuts). The antibodies that are specifically directed against the allergens are called immunoglobulin E, IgE. When a person becomes sensitized for an allergen, allergen-specific IgE is formed which eventually leads to aberrant reactions upon subsequent contact with the allergen. Clinical symptoms of an allergic reaction can occur in the skin (eczema), airways (hay fever and asthma), and sometimes systemic reactions develop (anaphylactic shock). For inhalation allergies treatments are available in the form of immunotherapy. Immunotherapy is applied by repeated administration of the allergen, which induces a certain form of tolerance for the allergen, thereby reduces the clinical symptoms. However, the mechanism of immunotherapy is not yet fully understood. For food allergies, such a curative therapy has not been developed so far. This thesis describes that T cells may serve as a possible target to develop therapies in the future.

T cells are an important population of cells of the immune system and can be subdivided into several groups. The different T cell subsets exert their function by the production of certain molecules, cytokines (such as interleukins, ILs and interferons, IFNs). A particular subset of T cells (Th2 cells, characterized by the production of IL-4) is responsible for the production of the allergen-specific IgE, produced by B cells, and play therefore an important role in allergy. Next to Th2 cells, other subsets exist, such as Th1 cells (characterized by the production of IFN- γ), and regulatory T cells that are characterized by the production of regulatory cytokines as IL-10 and TGF- β .

Since the frequency of allergen-specific T cells in blood is very low, the technique of T cell cloning was used in chapter 2 and 3. Cow's milk protein (CMP)-specific T cells were isolated from blood and cultured in a CMP-specific culture system. This technique was

used to amplify the amount of allergen-specific T cells from blood of children with cow's milk allergy (CMA), allergic controls and non-allergic controls. The obtained T cell clones (TCCs) were further characterized for their CMP-specificity, their ability to produce certain cytokines and the expression of cell surface markers.

In chapter 2 CMP-specific TCCs were generated and analyzed. The T cell response in three different donor groups (age 4-12 years) was studied: children with a diagnosed CMA, allergic controls with a diagnosed food allergy other than CMA, and non-allergic controls. The cytokine production of the T cells was an important parameter. T cells isolated from children with CMA produced mainly Th2 cytokines (IL-4 and IL-13). Also, from children without CMA it was possible to isolate CMP-specific T cells, but these T cells differed remarkably from the T cells isolated from children with CMA. T cells isolated from allergic controls produced high amounts of IL-10, whereas T cells isolated from non-allergic controls produced little or no cytokines in response to CMPs. The results presented in chapter 2 suggest that two forms of tolerance may exist. In the non-allergic children a form of 'natural tolerance' may exist, characterized by T cells that produce low amounts of IL-4 and IFN- γ . In the allergic controls an important role for IL-10 is shown. A high concentration of this cytokine may be necessary to induce a form of 'acquired tolerance'. The high levels of IL-10 are probably involved in the maintenance of a tolerogenic environment towards cow's milk in the allergic controls.

In chapter 3, the CMP-specific T cell response of infants and children without CMA was characterized, and it was analyzed how this response changed over time. It appeared that the atopic background of an infant without CMA influences the cytokine pattern of the T cells. T cells obtained from atopic infants (without CMA) produced more Th1 cytokines (IFN- γ) compared with T cells isolated from non-atopic infants. This Th1-skewed pattern changed over time in a cytokine profile as observed in the T cells of the healthy controls (a Th0 profile, equal amounts of IL-4 and IFN- γ). These results suggest that the CMP-specific T cells of atopic infants need a Th1-skewed response to prevent the development of CMA.

Another population of T cells (CD4⁺CD25⁺ regulatory T cells, CD4⁺CD25⁺ Tregs) is involved in the regulation of Th1 as well as Th2 cells. Whether the CD4⁺CD25⁺ Tregs exert their suppressive function via the production of IL-10 and/or TGF- β or via cell-cell contact has not been resolved yet. In chapter 4, it is demonstrated that this important population of T cells, with regulatory capacities, is present in food allergic patients and that these cells are also suppressive in *in vitro* assays. In chapter 5, it was analyzed whether the effect of birch pollen specific immunotherapy can be explained by differences in the Treg population. Both the percentage as well as the function of CD4⁺CD25⁺ Tregs was not altered after immunotherapy. Unfortunately, the investigation of the CD4⁺CD25⁺ Treg population was hampered by the fact that CD4⁺CD25⁺ Tregs

could only be isolated from blood, and not from organs, such as the mucosal epithelia. Furthermore, their suppressive function was studied in *in vitro* systems. Therefore, it might be possible that CD4⁺CD25⁺ Tregs do play an important role *in vivo* during immunotherapy in the process of tolerance induction, but that this is not reflected by the CD4⁺CD25⁺ Tregs isolated from blood.

To investigate whether it is possible to modulate T cells of allergic children by regulatory cytokines (such as IL-10 and TGF- β), the response of T cells in the presence and absence of TGF- β was determined in chapter 6. TGF- β strongly inhibited the proliferation of T cells, derived from allergic as well as non-allergic donors. However, TGF- β was not able to modulate the cytokine production of the T cells concomitantly. On the other hand, TGF- β did induce a change in T cells by enhancing the expression of the activation marker, CD69. This marker has been described earlier in animal models to be a possible indicator of the deviation towards a more tolerogenic microenvironment.

Since the TCCs used in chapter 6 were not easily modulated by TGF- β , other T cell populations were investigated for their response towards TGF- β in chapter 7. In this chapter, naïve and memory T cells from adult peripheral blood and naïve T cells from cord blood were used. TGF- β was only able to inhibit their proliferation in the absence of costimulation. Remarkable differences were found between the three T cell populations used, with regard to the effect on the cytokine production. T cells isolated from peripheral blood (both naïve and memory) produced less cytokines in the presence of TGF- β compared with T cells isolated from cord blood, which produced high amounts of IL-10 in the presence of TGF- β . This TGF- β -induced IL-10 induction was not abrogated by culturing under Th1 polarizing conditions (IL-12 and anti-IL-4), but disappeared under Th2 polarizing conditions (IL-4 and anti-IL-12).

In conclusion, these chapters demonstrate the important role for the cytokine IL-10 in the *in vivo* situation in which tolerance is maintained. Moreover, the *in vitro* data show that the cytokine TGF- β may be important in the induction of IL-10 early in T cell development. The question remains whether already differentiated T cells (memory T cells) are prone to modulation by cytokines such as IL-10 and TGF- β and whether the activation of CD4⁺CD25⁺ Tregs is involved in this process.