

## SUMMARY

Allergy is an important disease that affects a substantial proportion of the general population, and which has seen an increasing incidence during the past three decades. Cow's milk allergy (CMA) is one of the major food-allergies during infancy and early childhood. This thesis focuses on patients with CMA and atopic dermatitis (AD), which is the major presentation of CMA in the majority of patients during childhood. The aim of this thesis is to investigate the role of antigen-specific T cells in the etiology of CMA and AD. In addition, the currently used methods to diagnose CMA, *in vivo* and *in vitro*, are evaluated.

In **Chapter 1**, a general introduction to CMA and AD in infancy and childhood is given. CMA plays a pathogenic role in approximately a third of the infants with AD in the first year of life, and is associated with the spontaneous development of clinical tolerance within a relatively short period of two to three years. Similar to inhalant-allergy, in which Th2 cells that secrete high levels of interleukin (IL)-4, IL-5 and IL-13 are important, T cell reactivity towards the proteins in cow's milk has been implicated in the aetiology of allergy to cow's milk. This has however not been thoroughly investigated so far.

**Chapter 2** describes differences in the cow's milk protein (CMP)-specific T cell response between blood of infants with AD with and without CMA. Using an antigen-specific T cell culturing system with autologous B cells as antigen-presenting cells, CMP-specific T cell clones were established from blood. Results show that T cells specific for the various proteins in milk are present in blood of infants with AD, irrespective of their atopic state. Analysis of cytokine release shows that the CMP-specific T cell response in infants with CMA is Th2-skewed, with production of high levels of IL-4, IL-5 and IL-13. In contrast, infants without CMA have a Th1-skewed response, with high levels of interferon (IFN)- $\gamma$  and low levels of the Th2-cytokines.

In **Chapter 3**, infants without an atopic predisposition and without allergic disease are investigated. Results show that in these individuals, a T helper cell response directed against the major proteins in milk is evident. These T cells show a balanced, Th0-like cytokine production with an equal production of IL-4 and IFN- $\gamma$ . Comparison of these T cells with those described in **Chapter 2** emphasizes the Th0-like phenotype of cells, and the low release of Th2 cytokines. This suggests that similarly to individuals with an atopic predisposition, tolerance to milk in normal individuals is not caused by absence of circulating, CMP-specific T cells, but is probably determined by low release of Th2-cytokines by these cells.

**Chapter 4** investigates the changes that occur in the CMP-specific T cell response during the spontaneous development of clinical tolerance for cow's milk in infants with CMA and AD. Results show that CMP-specific T cells are still present in blood of CMA patients that have become clinically tolerant for CMPs. However, production of the Th2 cytokines by these T cells is lower than during the allergic

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state. In contrast, production of IFN- $\gamma$  is increased, which results in a Th1-skewed cytokine pattern. These results emphasize the important role of antigen-specific Th2 cytokine-release in the development of allergic reactions to CMPs.

In **Chapter 5**, the expression of several cell-surface markers by CMP-specific T cells is investigated. Results show that the expression of the activation markers CD25 and CD30 by CMP-specific T cells is significantly higher in infants with CMA, which suggest a higher activation state of these T cells. After the development of clinical tolerance for CMPs, cell-surface expression of activation markers decreases, suggesting a downregulation of CMP-specific T cell reactivity. Comparison of the expression of CD26 and CD30 with the release of IL-4 and IFN- $\gamma$  confirms earlier studies which demonstrated that on the level of the individual T cell, CD26 and CD30 cannot be used as exclusive markers for Th2- and Th1-cells.

In **Chapter 6**, CMP-specific T cells from blood and skin are investigated, and compared with regard to cell-surface expression of the skin-specific homing marker; cutaneous lymphocyte antigen (CLA). Results show that expression of CLA by CMP-specific T cells in patients with CMA and AD is comparable with patients without CMA. This suggests that the skin-homing potential of T cells from allergic and non-allergic individuals is not essentially different. Analysis of CLA-expression by T cells in skin of patients with AD however, shows significant higher expression levels of CLA on skin T cells compared to T cells from blood. This finding suggest that the level of CLA-expression might be important for the retention of allergen-specific T cells in the skin.

**Chapter 7** evaluates the screening for food-specific sensitization using *in vitro* IgE detection and skin prick-testing in infants and children. Results show a substantial mismatch between the outcome of both tests when screening for food-specific sensitization in infants and children with suspected food allergy. This indicates that the screening for food-specific sensitization in the pediatric age group should be performed using both *in vitro* IgE detection as well as skin prick testing, to prevent false-negative results.

**Chapter 8** provides a description of a new protocol for a double-blind, placebo-controlled cow's milk challenge (DBPCCMC) to diagnose immediate-type hypersensitivity reactions in infants and children, in a normal pediatric clinical setting. Results of the use of this protocol in a cohort of 154 children referred to the pediatric outpatient clinic of the University Hospital in Utrecht showed that it is possible to perform blinded-challenges routinely in the diagnostic work-up of children with suspicion of CMA. This indicates that DBPCCMCs should be implemented into routine pediatric practice, which may prevent inaccurate diagnosis and unnecessary elimination diets.

Finally, **Chapter 9** is a general discussion which summarizes and discusses the presented data on CMP-specific T cells in this thesis. In this chapter, a model is presented that describes the postnatal development of the T cell response to cow's

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milk in atopic and non-atopic individuals. Especially during infancy, clear differences exist between the CMP-specific T cell response in atopic and non-atopic individuals with regard to cytokine release. This suggests that the mechanisms that regulate the T cell response to milk are of vital importance in the development of clinical tolerance to CMPs. Future studies should therefore be focused on the elucidation of these mechanisms, to give directions for the development of therapeutic and preventive strategies in allergy to cow's milk.