

REVIEW

The cow's milk protein-specific T cell response in infancy and childhood

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Introduction

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. In the first years of life, the immune system is relatively immature and highly susceptible to the development of sensitization and allergy to environmental allergens such as foods and inhalants. Cow's milk is the most frequently encountered dietary allergen in infancy. Allergy to cow's milk is therefore the most important allergy in infancy, and is usually the first presentation of the atopic state of an individual. Unlike other (food) allergies, cow's milk allergy (CMA) in infancy is associated with rapid and spontaneous development of clinical tolerance. Elucidation of the mechanisms that determine the development of allergy and tolerance to cow's milk is therefore of importance, because it may not only provide insights for therapeutic and preventive strategies for CMA, but also other (food) allergies.

The focus of this review is the relevance of circulating cow's milk protein (CMP)-specific T cells in the aetiology of CMA. Results from literature are summarized and discussed. We present a model which describes the development of the CMP-specific T cell response in blood of atopic and non-atopic individuals during infancy and early childhood.

The relevance of CMP-specific T cell reactivity in the aetiology of CMA

The protein-fraction in cow's milk is the major immunogenic component. Therefore, cow's milk protein (CMP)-specific T cell reactivity has been extensively studied. It is clear that a CMP-specific T cell response is not only present in blood of patients with CMA, but also in individuals without CMA, irrespective of their atopic state [1–4]. This shows that CMA is not determined by a CMP-specific T cell response per se. In addition, CMP-specific T cell reactivity has been demonstrated in blood of children that have outgrown their CMA [5, 6], which further emphasizes that the presence of a CMP-specific T cell response per se does not cause CMA. Both in allergen-stimulated PBMCs [1, 3, 7], and at the clonal level [4] it has been shown, that the CMP-specific T cell response in patients with CMA is Th2-biased compared to milk-tolerant individuals. This is shown by a high production of IL-4 and low production of IFN- γ . Furthermore, these cells produce high levels of IL-5

and IL-13, two cytokines that are closely related to the Th2 response [4]. In combination with this Th2-biased cytokine pattern, the activation state of antigen-specific T cells is probably also of importance in the aetiology of allergic reactions to CMPs. Studies that investigated the nature of proliferative PBMC responses suggested that CMP-specific T cells from allergic patients have an enhanced activated state, with high proliferative capacity [2, 8–10]. Analysis of cell surface expression of CD25 (IL-2R α), showed that the expression of this activation marker by CMP-specific T cells of infants with CMA is significantly higher than in atopic and non-atopic infants without CMA [6]. Similar results were found for the expression of CD30, another marker of T cell activation, which is closely associated with CD25 expression [6]. These findings suggest that the CMP-specific T cell response in patients with CMA is by itself more vigorous than in non-allergic patients. In line with this, it was demonstrated that the development of clinical tolerance for CMA in patients with CMA is associated with a down-regulation of the CMP-specific Th2-response in blood [5], and a lower activation state of the circulating CMP-specific T cells [6].

Taken together, there is convincing evidence that in patients with CMA, highly activated Th2 cells, that are specific for cow's milk proteins, circulate in blood. For inhalation allergens such as house dust mite it has been extensively shown that these Th2 cells can migrate to peripheral tissues such as the lungs or the skin, where they initiate allergic inflammation by stimulation of IgE synthesis and eosinophil recruitment. Evidence for similar mechanisms in CMA is accumulating. In an experimental animal model, it was shown that skin lesions in cow's milk-induced dermatitis are dominated by Th2 cells [11]. Human studies have demonstrated T cells expressing homing-markers specific for the gastro-intestinal tract [12] or skin [13, 14] in the blood of patients with cow's milk-induced allergic symptoms. Furthermore, a recent study by Beyer and co-workers showed that in patients with cow's milk allergic gastro-enteritis, CMP-specific T cells expressing a Th2-profile are present in the gut [15]. Organ-specific Th2 cell-responses in patients with cow's milk-induced skin and/or lung symptoms still need to be demonstrated, and are currently under investigation.

Differences in T cell reactivity towards the various proteins in cow's milk

The protein fraction of cow's milk consists of at least 20 different proteins that may provoke an allergic reaction in humans [16]. The whey proteins, α -lactalbumin and β -lactoglobulin,

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together with the casein proteins, are proposed to be the most important allergenic proteins [17]. Studies that investigated the IgE response in blood have indicated that caseins are more immunogenic than whey proteins, and that patients with CMA mostly have an allergic reaction against caseins [16–18]. In addition, patients who fail to develop tolerance within a few years (persistent CMA) are most often allergic to the caseins [19]. Differences in T cell reactivity towards the different proteins in milk are less well investigated. It is clear that both in individuals with and without CMA T cell reactivity against all protein fractions is present [2, 4], however, the small number of patients that have currently been investigated does not yet make it possible to detect an immunodominant fraction at the T cell level.

Intact milk proteins consist of multiple joined amino acids, sometimes up to 200, while the components of the immune system recognize only small parts of the proteins (epitopes) consisting of several amino acids [20]. Investigations into IgE binding (B cell) epitopes have shown that patients with and without CMA do differ in their epitope recognition of the various CMPs [21, 22]. Interestingly, it has also been shown that patients with persistent CMA have IgE reactivity to different epitopes than patients with 'transient' CMA [23, 24]. It has been speculated that the structure of protein epitopes (e.g. linear vs. conformational), influences the way they are handled by the immune system [25]. Consequently, this difference in handling of various epitopes determines the interval by which tolerance to cow's milk develops [26]. On the T cell level, epitope information is still sketchy. Explorative research into T cell epitopes on α S1-casein [27, 28] and β -lactoglobulin [29, 30] has been performed in patients with CMA. It is currently unclear if there are differences in the epitope recognition between T cells from patients with and without CMA, or patients with transient and persistent CMA. Studies are underway to investigate potential differences. If indeed there are also differences in epitope recognition at the T cell level, then characterization of the 'allergenic' epitopes may provide methods for the development of highly (epitope)-specific therapy for CMA.

Model of the development of the CMP-specific T cell response during infancy and early childhood (Fig. 1)

The CMP-specific T cell response in individuals without an atopic predisposition

Proliferative responses to the proteins in cow's milk have been shown in cord blood of atopic and non-atopic individuals [29, 31–33]. This means that CMP-specific T cells are already present in blood of atopic and non-atopic individuals at birth. Priming of T cells against CMP-proteins thus may occur *in utero*, through mechanisms which are currently unknown [34, 35]. Studies by Prescott and co-workers have shown that the allergen-specific T cell response to environmental allergens at birth is Th2-skewed, both in atopic and non-atopic individuals [32, 36]. This is probably secondary to the fact that T cell responses in all individuals are Th2-skewed *in utero*, to prevent the toxic effects of the Th1 cytokines on the placenta and the 'allogenic' fetus [37]. Postnatally however, the development of T cell responses to environmental allergens in individuals with or without an atopic predisposition is essentially different [35, 36]. It is thought that in non-atopic individuals these Th2 responses are gradually suppressed to prevent sensitization to environmental allergens [36]. From investigations in PBMCs it was suggested that T cell responses in individuals without an atopic predisposition generally deviate to a Th1-biased pattern [35, 36]. However, in these studies cytokines were measured in bulk cultures of T cells, making it difficult to extrapolate these results to the individual T cell. Recently it was shown that, when investigated at the single cell level, the T cell response to milk in non-atopic infants displays a balanced, Th0-like cytokine profile, with equal production of IL-4 and IFN- γ [38]. Usually, cow's milk is encountered in large quantities during the first year of life. As the strong inflammatory capacities of Th1-cytokines, such as IFN- γ , can be harmful, it seems therefore logical that a balanced (i.e. Th0-like) cytokine response to dietary antigens such as cow's milk is better than a full-blown Th1 response that is dominated by IFN- γ release.

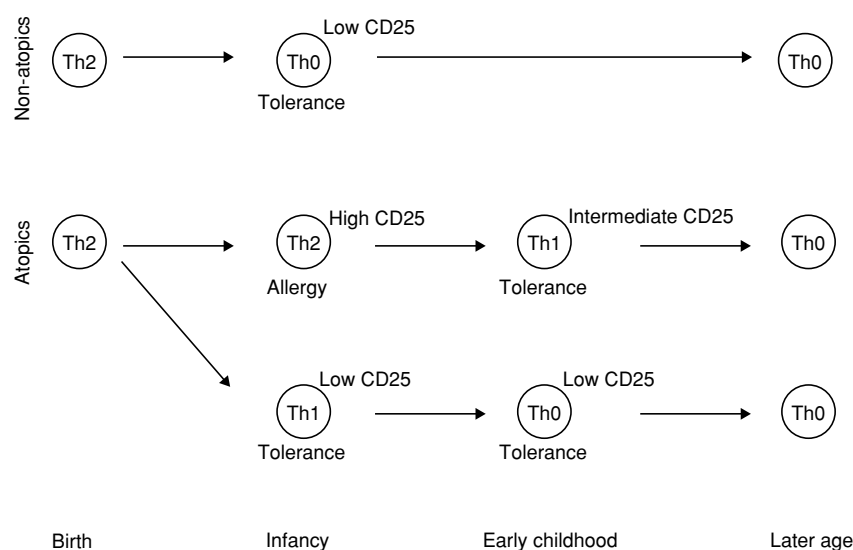


Fig. 1. Model that describes the development of the CMP-specific T cell response in non-atopic and atopic individuals. Results from birth are described in [32, 36]. Results from infancy and early childhood are described in [4–6, 38]. Results from late childhood are extrapolations of results described in [4–6, 38].

For this reason, it is likely that the CMP-specific T cell response remains Th0-like throughout life. This still needs to be confirmed however. Studies have shown proliferative responses to cow's milk in PBMCs of non-atopic adults [39–41] but cytokine release by these CMP-specific T cells has not yet been evaluated.

The CMP-specific T cell response in atopic individuals who develop CMA

Several studies have suggested that, in contrast to individuals without atopy, fetal (Th2-biased) T cell responses aberrantly persist in individuals with an atopic predisposition [33, 36]. This causes T cell responses to common allergens to remain Th2-skewed during infancy and childhood. It is clear that atopic infants with allergy to CMPs have a Th2-biased, CMP-specific T cell cytokine response in blood. This suggests that CMA is the result of an (aberrant) persistence of Th2 response. Remarkably, CMA in infancy is very transient, i.e., most patients become spontaneously tolerant to milk within 2 years. It has been shown that the development of spontaneous clinical tolerance to cow's milk in patients with CMA is associated with a down-regulation of the Th2 response, while at the same time the production of IFN- γ is up-regulated [5]. This is similar to the redirection of the allergic Th2 response towards a Th1 profile that is seen in patients in whom tolerance is induced by immunotherapy [42, 43]. It might be that in infants with persisting Th2 responses to milk, who have subsequently developed CMA, an 'overshoot' deviation of the (Th2-biased) CMP-specific T cell response to a Th1-response is needed to induce a CMP-tolerant state. This suggests that CMA is the result of a temporary delay in the (normal) redirection of the fetal Th2 response, which then is spontaneously 'corrected' by a deviation of the CMP-specific T cell response.

It seems likely that in due time however, this CMP-specific Th1-biased T cell response will be down-regulated to a balanced, Th0-like response. As argued before, a full-blown Th1 response with high levels of IFN- γ may have harmful effects. It is therefore plausible that in the long term, patients that have become clinically tolerant to milk after 'transient' CMA will obtain a T cell response that is comparable with atopic and non-atopic individuals who never had CMA. Long-term follow-up of cytokine responses in patients who have become tolerant are needed to confirm this hypothesis.

The CMP-specific T cell response in atopic individuals who do not develop CMA

As stated before, it has been suggested that atopic individuals have a prolonged period of fetal Th2-skewed allergen-specific T cell responses [33, 36]. However, not all atopic individuals develop CMA, and previous studies have not discriminated between T cell responses in atopic patients with, and without allergic reactions to the studied allergen. In a recent study we investigated, at the clonal level, the CMP-specific T cell response in individuals with an atopic predisposition and atopic dermatitis, but who did not have allergy to cow's milk as proven by formal challenge [4]. In accordance with the hypothesis that production of high levels of Th2 cytokines by allergen-specific T cells is important in the aetiology of allergy, it was shown that CMP-specific T cells from these infants do not have high Th2 cytokine levels. Interestingly however, it

was found that the majority of these infants have a CMP-specific Th1-biased response, with high levels of IFN- γ . This finding raises important questions. The hallmark characteristic of individuals with an atopic predisposition is their tendency to develop sensitization and allergy to common allergens. The fact that these patients have not developed CMA means that, despite their atopic state, allergy to cow's milk has somehow been successfully prevented. It is tempting to speculate that the Th1-biased nature of the cytokine response is of importance in this respect. Atopic individuals have an increased risk of developing allergic sensitization during the first year of life, the so-called 'window of sensitization' [36, 44]. Usually CMPs are encountered in large quantities during infancy. It can be argued that during this period, with its lingering fetal T cell responses which cause a Th2-biased milieu in blood, the T cell response to milk is firmly Th1-skewed in these patients to prevent sensitization. Follow-up of these patients showed that the CMP-specific T cell response in these infants deviates to a Th0-like cytokine pattern after infancy [5]. It is tempting to speculate that after infancy, when the 'window of sensitization' has passed and allergy to milk has been successfully prevented, there is no longer need for a 'compensatory', Th1-biased T cell response. For this reason, to prevent the potential harmful effects of a full blown Th1-response upon exposure to cow's milk, the response is down-regulated to a balanced, Th0-like response. It seems logical that this Th0 response will persist throughout life, but this has to await formal proof.

Regulation of the CMP-specific T cell response

Suppression of allergen-specific T cell reactivity

From the investigations of the past decade, it has become clear that the nature of the T cell response against the proteins in cow's milk is important in the aetiology of tolerance and allergy to cow's milk. This suggests that the understanding of the regulation of T cell responses to cow's milk proteins is of utmost importance for the development of therapeutic and preventive strategies for CMA. Several recent reports have provided insights in these regulatory mechanisms. It was demonstrated that in patients with allergy to birch pollen and insect venom, artificial induction of tolerance via allergen-specific immunotherapy induces an increase in IL-10 production by PBMCs [42, 43, 45]. This is attributed to an increased production of IL-10 by a subset of regulatory T cells, designated Tr1 [46–48]. These regulatory Tr1 cells are induced by IL-10, but also produce high levels of IL-10, low levels of IL-2, and no IL-4 [48, 49]. It is thought that (Tr1-produced) IL-10 is important for the suppression of the allergen-specific T cell response through inhibition of proliferative responses and cytokine production of allergen-specific T cells [45, 48, 50]. Another important cytokine that can suppress the allergen-specific T cell response is TGF- β , and T cells that predominantly produce this cytokine have been designated Th3 [51]. TGF- β modulates the T cell response by inhibiting proliferation [52]. It has been shown that the induction of tolerance in auto-immune diseases is dependent on the production of TGF- β [53, 54]. Recent *in vitro* experiments have shown the suppressive effect of TGF- β on the proliferative capacity of CMP-specific T cells [55]. This suggests that the production of TGF- β is also important for the existence of

tolerance to cow's milk proteins. In support of this hypothesis, it was shown that T cells in the gastro-intestinal mucosa of patients with CMA and gastro-intestinal symptoms produce virtually no TGF- β [15]. Future studies should therefore set out to investigate whether Tr1 and/or Th3 cells play a role in tolerance and allergy to cow's milk, and whether the development of (spontaneous) tolerance in CMA is mediated by these cells.

Recent studies have identified a regulatory subset of (CD4⁺) T cells that express high levels of CD25 (IL-2R α) [56–58]. It has been shown that these CD4⁺ CD25⁺ T cells are able to induce anergy of antigen-specific T cells, causing a suppression of proliferation and cytokine release. The exact mechanism behind it is currently unclarified, but it requires cell–cell contact and is not mediated by IL-10, TGF- β or IL-4 [58]. The effect of these CD4⁺ CD25⁺ T cells is essentially a suppression of the antigen-specific T cell response. It has been suggested that this is the crucial mechanism by which peripheral tolerance to dietary antigens such as cow's milk is maintained [59, 60]. *In vitro* experiments have shown that this subset of regulatory T cells can suppress T cell reactivity specific for cow's milk proteins [58]. In patients with CMA, the CD25⁺ suppressor population is present and it displays, *in vitro*, a strong suppressive effect on CMP-specific T cell reactivity [41]. This is interesting, because it suggests that functionally intact regulatory T cells are present in patients in whom, by a currently unclarified mechanism, a breakdown of peripheral tolerance to cow's milk has occurred. Future studies that are aimed at the specific stimulation of these naturally occurring regulatory T cells in patients with CMA, might contribute to the (re-)establishment of clinical tolerance in patients with cow's milk allergy.

Modulation of cytokine patterns

Another important mechanism by which the allergen-specific T cell response is regulated is by direct modulation of the cytokine profiles that are secreted by allergen-specific T cells. Recent studies have shown a crucial role for antigen-presenting cells (APCs) in this respect. It has been shown that the signals that APCs in the central lymphoid organs provide during the priming of T cell responses in the perinatal period, can determine the outcome of the Th1- or Th2-biased nature of the T cell response. There are distinct subsets of dendritic cells (DCs) that are responsible for primary polarization of naïve T cells in the lymph nodes [61, 62]. These DC subsets provide stimulatory signals during antigen-presentation that determine if the T cell cytokine profile becomes Th1- or Th2-biased [63, 64]. Although the exact signals remain to be elucidated, both soluble factors such as IL-12, as well as cell–surface interactions are probably of importance in this process [62, 63]. During the postnatal development of allergen-specific T cell responses, it has been proposed that the peripheral APC network plays a crucial role [36, 44, 65]. It has been demonstrated that the DC network in the lung can orchestrate the postnatal T cell response towards inhalation allergens [66, 67]. The mechanisms that regulate the postnatal immune response to foods are less clarified, and are under investigation [68]. The APCs in the gut-associated lymphoid tissue are proposed to be of vital importance in this respect [59, 69, 70], but the nature of the relevant APCs, and the exact processing events still remain to be elucidated [59, 70].

Conclusion

In summary, it has become clear that there are distinct differences between CMP-specific T cell reactivity in blood of infants with and without CMA. These differences are especially expressed by the (allergen-specific) release of Th2 cytokines. In blood of patients with CMA, circulating cow's milk-specific Th2 cells are present, that can migrate to peripheral tissues such as the skin or the lungs. There they can initiate allergic inflammation by stimulation of IgE synthesis and eosinophil recruitment. It is thought that the presence of these cells is the determining factor for the aetiology of cow's milk allergy.

Furthermore, recent investigations have indicated that during infancy differences exist between the development of the CMP-specific T cell response in atopic and non-atopic individuals (Fig. 1). While in individuals with atopy the (pre-natal) Th2 responses aberrantly persist, leading to clinical CMA, these Th2 responses are effectively down-regulated in individuals without an atopic predisposition. There is increasing evidence that particular T cell subsets (regulatory T cells) play a crucial role in this process. Future studies should therefore be focused on the role of regulatory T cells and other mechanisms that can modulate the CMP-specific T cell response, to give directions for the development of therapeutic and preventive strategies in CMA.

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