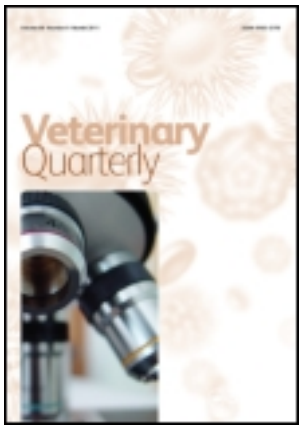

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E.Z. Veenhof [a], E.F. Knol [b], T. Willemse [a c] & V.P.M.G. Rutten [c d]

[a] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

[b] Department of Dematology/Allergology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

[c] Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

[d] Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

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REVIEW ARTICLE

Immune responses in dogs with cutaneous adverse food reactions

E.Z. Veenhof[a], E.F. Knol[b], T. Willemse[ac] and V.P.M.G. Rutten[cd]*

[a]*Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands;* [b]*Department of Dematology/Allergology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands;* [c]*Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands;* [d]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa*

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Adverse food reactions (AFR) in dogs are reactions due to apparently harmless food antigens, with an unknown aetiology, i.e. immunopathogenesis. Despite the entry of food allergens via the intestinal tract, in the majority of dogs with AFR, clinical symptoms are only associated with the skin (CAFR). In the present review, factors are presented of relevance in triggering the differentiation of naive T cells into effector T cell types and the role of these T cell types in allergy. More specifically, the allergic immune responses in intestine and skin are discussed in this article as well as the potential pathways, e.g. homing of antigen presenting cells or allergen-induced T cells to the skin, of induction of cutaneous symptoms.

Keywords: dog; allergy; adverse food reaction; skin; intestine; IgE

1. Adverse food reactions in dogs

Allergy is considered an inappropriate immune response to harmless environmental substances, named allergens. The term allergy is mainly used to describe type I hypersensitivity reactions that are due to activation of mast cells via cross-linking of membrane-bound allergen-specific immunoglobulin E (IgE) by allergens. It is unclear whether the adverse reactions to food antigens in dogs are the result of undesired immune responsiveness, such as type I hypersensitivity, or intolerance to food. Therefore, the generic term adverse food reactions (AFR) is used, rather than food allergy. Although the clinical signs of canine AFR may occur at several locations, including the gastrointestinal tract and the respiratory system, they are usually restricted to the skin, hence referred to as cutaneous adverse food reactions (CAFR). The distribution and clinical manifestations are often indistinguishable from those in atopic dermatitis (AD). The prevalence of AFR in the dog population is up to 8% (Wills and Harvey 1994; Chesney 2002) and approximately 7–25% of all allergic skin disorders are CAFR (Chesney 2002; Picco et al. 2008). Only 10–15% of dogs with skin symptoms show concurrent intestinal symptoms, such as vomiting and diarrhoea (Carlotti et al. 1990). There is no breed or sex predisposition for CAFR. In 33–48% of cases the age of onset of CAFR is below 1 year, in 51–85% 1–3 years and in 16% 4–11 years of age (Rosser 1993; Picco et al. 2008).

Food consists of proteins, carbohydrates, lipids and water-soluble glycoproteins of which the latter are the most important allergens. Most allergens are resistant to heat, acid and proteases, and some even become allergenic after cooking or digestion (Untersmayr and Jensen-Jarolim 2008). Constituents that are not resistant to digestion may, when eaten in large quantities, act as allergens after intact passage through the stomach (Untersmayr and Jensen-Jarolim 2008). Beef, lamb, chicken, wheat, soybean, milk, eggs and corn have been described as causative for AFR (White 1986; Carlotti et al. 1990; Jeffers et al. 1996; Vaden et al. 2000). However, the exact allergenic molecules of these foods, except for cow's milk and beef, have not been identified to date. It has been shown that in dogs with cow's milk and beef-related AFR, bovine IgG and also phosphoglucomutase in beef are major allergens (Martin et al. 2004). However, this analysis was based on allergen-specific IgE levels, of which the role in (C)AFR is not yet proven. Currently, CAFR is diagnosed by dietary elimination and provocation tests, using the complete original food for provocation after initial feeding of an elimination diet.

2. T cell introduction

Food is digested in the gastrointestinal tract and degraded to oligopeptides, which are predominantly adsorbed via M cells in the small intestine and thereafter internalised by antigen presenting cells (APC). The APC, such as dendritic cells (DC), macrophages and B cells, present antigen via major histocompatibility complex class II (MHC-II) molecules on their surfaces, in conjunction with B7.1 or B7.2 as costimulatory molecules (Murphy et al. 2008).

*Corresponding author. Email: v.rutten@uu.nl

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The T cell receptor (TCR) is responsible for the specific recognition of the antigen–MHC-complex and CD28 molecules expressed on the T cell bind to the B7 molecule. Interactions between TCR, MHC-antigen complex, CD4 or CD8 and that between B7 and CD28, result in activation of T cells and differentiation of effector T cells (Murphy et al. 2008). These effector T cells circulate throughout the body, and the receptors on their surface enable them to migrate to specific locations directed by chemokines and integrines. CCL17 (also named Thymus and Activation-Regulated Chemokine: TARC) is a chemokine expressed in the skin by dermal cells, and its expression is increased in human AD (Zheng et al. 2003). T cells expressing the CCL17 receptor CCR4 (chemokine [C-C motif] receptor 4) home to the skin disorders (Imai et al. 1999; Zheng et al. 2003). Both CCR4 and CCL17 also increased in dogs with AD (Maeda et al. 2002; Maeda et al. 2004). In addition, the intestinal-derived dendritic cells and mesenteric lymph node stromal cells can induce $\alpha4\beta7$ integrin expression on T cells. This integrin binds to MAdCAM-1, an adhesion molecule expressed specifically on intestinal endothelial cells, causing migration of activated T cells into intestinal tissue (Berlin et al. 1993; Molenaar et al. 2009). In this way, the intestine-derived dendritic cells, which internalised the antigen, are responsible for return of the antigen-specific T cells to the intestine.

Classically, there are two main types of T lymphocytes: $CD8^+$ T cells (cytotoxic T cells) and $CD4^+$ T cells (T helper cells). $CD8^+$ T cells are mainly involved in defence against intracellular pathogens, such as viruses and function by killing the infected cell. The $CD4^+$ T cell population, existing of several subtypes, serves to help other immune cells, hence they are called T helper (Th) cells. Th2 as well as Th1 cells are involved in the activation of B-cells and Th1 cells may additionally activate phagocytes to eradicate intracellular pathogens, such as bacteria and fungi. Regulatory T cells (a CD4 subtype named Treg) have an immunomodulatory or suppressive function, whereas Th17 cells are involved in the elimination of extracellular and fungal infections and exert (pro-)inflammatory functions (Liang et al. 2007; Ouyang et al. 2008). Important effector molecules of T cells are cytokines, small soluble proteins that influence the functional properties of many cells, including T cells. Typical Th1 cytokines are IFN-γ, IL-18 and TNF-α; Th2 cytokines are IL-4, IL-5 and IL-13; Th17 cytokines are IL-17A, IL-17F and IL-22; and Treg cytokines are TGF-β and IL-10 (Harrington et al. 2005; Zhu et al. 2010). Regulatory T cells can be further categorised into three types: $CD4^+CD25^{high}$ Tregs (appearing to suppress T cells in a cytokine-independent manner by cell-cell contact), Th3 cells (immunomodulate and suppress through TGF-β) and Tr1 cells (immunomodulate and suppress through IL-10)(Zhu et al. 2010). The selection of the immunoglobulin (Ig) (sub)isotype produced by B cells depends on the T helper subtype and the cytokines they produce. Thus, IL-4 stimulates the production of IgG1, IgG3, IgG4 and IgE (Gascan et al. 1991; Fujieda et al. 1995; Geha et al. 2003; Avery et al. 2008) and IL-13 stimulates the production of IgG4 and IgE (Punnonen et al. 1993; Geha et al. 2003; Kanari et al. 2010). IL-6 stimulates the production of all IgG types (Kawano et al. 1995), whereas IL-10 stimulates the production of IgG1, IgG2, IgG3, IgG4 and IgA and inhibits the production of IgE (Defrance et al. 1992; Briere et al. 1994; Jeannin et al. 1998; Akdis et al. 1998). In addition, TGF-β induces IgA (Zan et al. 1998) production and inhibits that of IgG4 and IgE (Armitage et al. 1993), IL-21 stimulates the production of IgG1 and IgG3 in human B cells (Avery et al. 2008; Pene et al. 2004) and IFN-γ can inhibit the overall production of immunoglobulins (Reynolds et al. 1987; King and Nutman 1993; Hussain et al. 1999).

2.1. *Involvement of various types of $CD4^+$ T cells*

The differentiation of a naive $CD4^+$ T cell to either the Th1, Th2, Th17 or Treg subtype is not fully understood. It is believed to depend on the cytokines present in the environment of the cell during activation, the co-stimulator molecules used to drive the response, antigen dose (Hosken et al. 1995), type and differentiation status of APC (Moser and Murphy 2000), and the nature of the antigen-MHC-II molecule and binding strength to TCR (Constant and Bottomly 1997; Sloan-Lancaster et al. 1997). Interactions with T cells lead to the activation of transcription factors within the cell that will bind to promotor sites for cytokine genes specific for a Th subtype. Figure 1 is a simplified schematic overview of the key transcription factors involved in $CD4^+$ T cell differentiation.

2.1.1. *T helper 1 cell*

T helper 1 differentiation can be induced by the cytokines IFN-γ, IL-12, IL-18, IL-23 and IL-27. IL-12, especially in combination with IL-18, acts on T cells to trigger IFN-γ expression. It activates the transcription factor STAT4, which induces and stimulates the IFN-γ promoter (Nakahira et al. 2002). Next, STAT4 and IFN-γ can regulate the expression of T-bet in T cells, which regulates the IFN-γ and IL-12Rβ2-chain gene expression (Figure 1). However, the exact pathway of IFN-γ regulation is not yet known (Lighvani et al. 2001; Szabo et al. 2002; Mullen at al. 2002; Hwang et al. 2005; Usui et al. 2006; Zhu et al. 2010). The T cell differentiation into Th2 cells is inhibited by the Th1-type cytokine expression (IFN-γ and IL-12) and T-bet inhibits the Th2 promoting GATA3 expression (Ouyang et al. 1998; Elser et al. 2002; Zhu et al. 2010). After differentiation into Th1, the cell starts to produce IFN-γ and IL-12 (Figure 1).

2.1.2. *T helper 2 cell*

T helper 2 cell differentiation can be induced by the Th2-specific cytokines IL-4 and IL-13 (Figure 1).

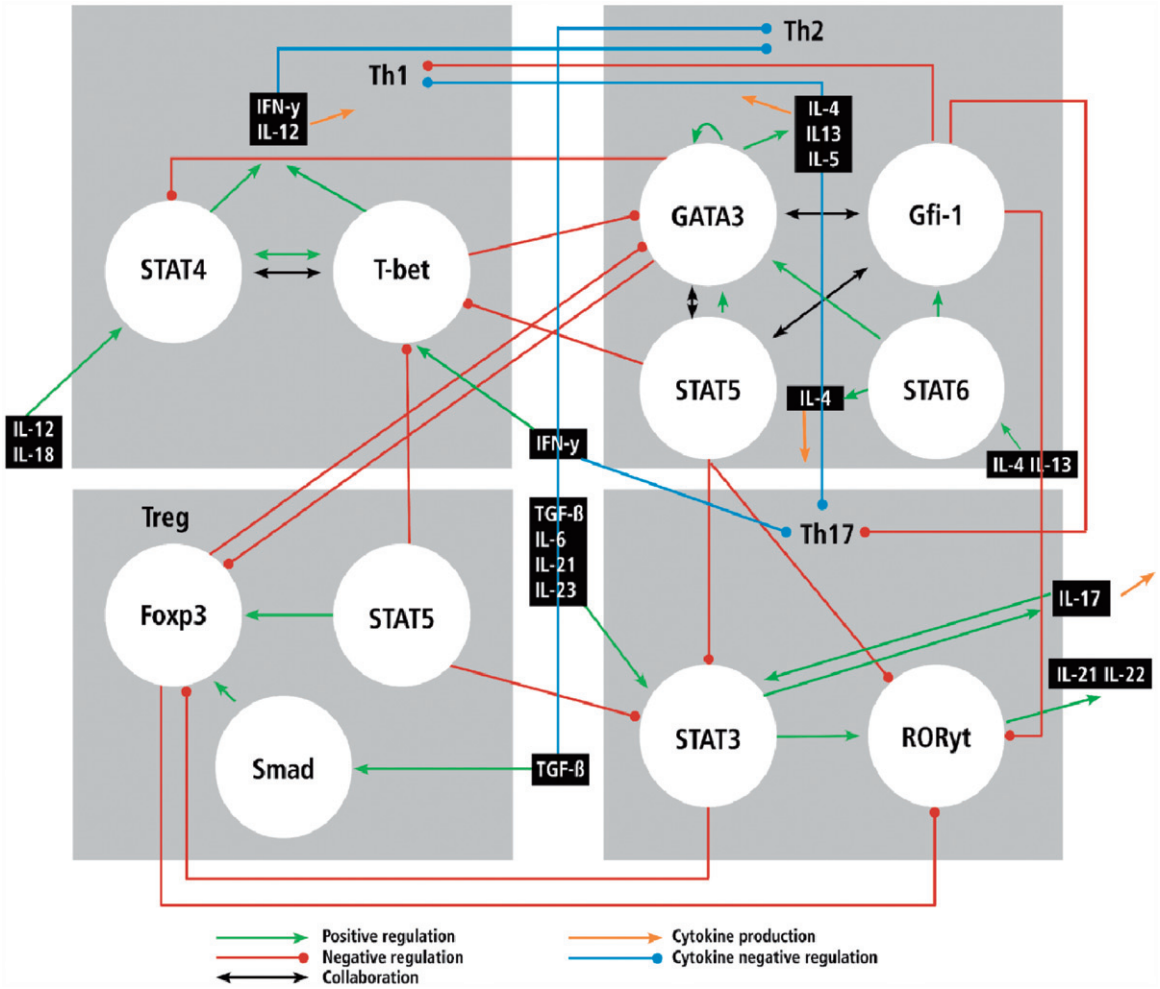

Figure 1. Simplified schematic overview of key transcription factors and their influences on T helper cell differentiation and function.

These cytokines trigger the phosphorylation of the STAT6 transcription factor, which triggers the expression of IL-4 and GATA3 genes (Kaplan et al. 1996; Kim et al. 1999; Zhu et al. 2010). GATA3 activates the IL-4 promotor, regulates IL-13 and IL-5 expression and can autoregulate its own expression (independent of STAT6)(Rosser 1993; Ouyang et al. 2000; Das et al. 2001; Zhu et al. 2004; Zhu et al. 2010). Moreover, the T cell differentiation into Th1 cells is inhibited by the Th2-type cytokine expression and GATA3 inhibits the expression of STAT4, a transcription factor involved in Th1 cell differentiation (Ouyang et al. 1998; Usui et al. 2003). STAT5 is a transcription factor involved in differentiation into all T helper cell types. Its enhanced expression is needed for differentiation into Th2 or Treg cells, in contrast this enhanced expression inhibits the differentiation into Th1 or Th17 cells (Zhu et al. 2010). Gfi-1 is a transcription factor involved in T cell development of most $CD4^+$ T cell subtypes. It stimulates $GATA3^{hi}$ cells to cell growth and differentiation, suggesting it is selective for Th2 cell differentiation. Moreover, it seems to suppress the Th17 and Th1 development (Zhu et al. 2010). As a consequence of GATA3 activation, the cell develops into a Th2 cell (Figure 1).

2.1.3. *T helper 17 cell*

The exact activation pathway of the recently described Th17 cells is not yet known. In mice, TGF-β, IL-6, IL-21 and IL-23 can induce naive T cells to become Th17 via activation of the STAT3 transcription factor, which triggers RORγt (Harrington et al. 2005; Veldhoen et al. 2006; Bettelli et al. 2006; Zhu et al. 2010), a transcription factor in mice of which the function is not completely known. In addition, the activation of STAT3 via IL-6 suppresses Foxp3 expression, suggesting that IL-6 may inhibit differentiation into regulatory T cells and stimulates the differentiation into a Th17 cell (Figure 1) (Yang et al. 2007; Zhu et al. 2010). Moreover, both IL-4 and IFN-γ can inhibit the activation of Th17 cells, suggesting that both Th1 and Th2 cells can inhibit Th17 differentiation (Zhu et al. 2010). In humans, Th17 memory cells have mainly been investigated. The cytokines IL-1β, IL-23 and IL-6 promote production of IL-17. In contrast to

mice, in humans TGF-β inhibited IL-17 when added alone or in combination with IL-1β, IL-23 or IL-6 (not shown in Figure 1) (de Jong et al. 2009). Upon differentiation Th17 cells produce IL-17, IL-21 and IL-22 (Figure 1).

2.1.4. *Regulatory T cell*

The $CD4^+CD25^{high}$ Treg cells are a natural occurring population that expresses the specific transcription factor Foxp3, the key transcription factor for these cells. It was long believed that Treg cells could not be induced, however in mice it was shown that induction did occur by TGF-β through activation of Foxp3 via Smad (Chen et al. 2003). STAT5 activation is also critical for Treg development (Figure 1). Although not proven, it is suggested that STAT5 binds the Foxp3 promoter, thereby influencing Treg cell development (Zhu et al. 2010). Moreover, Foxp3 can interact with RORγt (transcription factor involved in Th17 cells) and herewith suppresses the differentiation into Th17 cells (Figure 1). TGF-β induces both Th17 and Treg in mice, but Treg need high concentrations of TGF-β to be induced (Zhu et al. 2010). Suppression of T cells by Tregs is cell–cell contact dependent, but the exact pathway is not known. It possibly depends on IL-2 inhibition, expression of the inhibitory co-stimulatory molecule CTLA-4 or suppression via membrane-bound TGF-β (Thornton et al. 2000; Nakamura et al. 2001).

Tr1 and Th3 cells have also been described, however it is unknown whether these cells are specific CD4 subtypes lineages or belong to other Th cell subsets (Th1, Th2, Th17, Treg). Tr1 cells are characterised by secretion of high levels of IL-10, but IL-10 can also be produced by other T cells such as Th1, Th2 and Th17 cells (Saraiva and O'Garra 2010). In addition, oral tolerance induces TGF-β-producing cells, which have been characterised as Th3 cells (Weiner 2001). However, recently it was observed that approximately 40% of these cells also expresses Foxp3, thus possibly part of the so-named Th3 cells may be induced by Tregs (Oida et al. 2003; Chen et al. 2008).

2.2. *Plasticity of T cell differentiation*

For years it was believed that the differentiation of the $CD4^+$ T cell into a Th1, Th2, Th17 or Treg cell was an irreversible step. Recently, however, it has been shown that differentiated cells can change into other types of T helper cells depending on the cytokine micro-environment (Zhu et al. 2010). For example, Tregs can produce IL-17 when they are exposed to IL-6, and show an upregulation of RORγt (Xu et al. 2007; Akimzhanov et al. 2007). Another example is that Th2 cells can be induced by IL-12 to produce IFN-γ (Zhu et al. 2010). This indicates that differentiated $CD4^+$ T cells can still be converted into other T helper types through the influence of their micro-environment.

3. Allergic immune reactions

$CD4^+$ T cells and their cytokines play an important role in the initiation of allergic reactions. A prominent characteristic of allergy is the production of allergen-specific IgE. IL-4 or IL-13 are produced by Th2 cells and induce class switching in B cells, giving rise to IgE production. The secreted IgE binds to specialised high affinity receptors (FcεRI) on mast cells, basophils, Langerhans cells (LC) and dendritic cells from which it dissociates only very slowly (Hunt et al. 2005). When an allergen crosslinks the IgE on the mast cells and basophils, these cells respond by an immediate release of their prestored mediators, which are responsible for the allergic symptoms, that occur rapidly. This process is named type I hypersensitivity. The substances released from mast cells ensure that inflammatory cells, including activated T cells, in the course of time enter the tissue and respond to the allergen or the pro-inflammatory environment, hence the so-called early and late-phase reactions may be observed.

In humans, food allergy has been associated with Th2 responses, possibly caused by a Th2-like micro-environment, resulting in an impaired Th1 response and/or impaired Treg response after allergen challenge. Stimulation of PBMC with cow's milk results in a Tr1 or Th1 response in healthy and non-cow's milk allergic persons and a Th2 response in cow's milk allergic persons (Schade et al. 2000; Akdis et al. 2004; Rautava and Isolauri 2004; Tsuge et al. 2006). Moreover, when PBMC of peanut allergic children are compared with those of children who became tolerant to peanut over time, the tolerant children show a Th1 profile after allergen stimulation rather than a Th2 reaction (Turcanu et al. 2003). In addition, cow's milk allergic children who became tolerant, showed a high level of IL-10 (Tiemessen et al. 2004), which may have been produced by Tr1 cells or macrophages. In humans, a diagnosis of atopy is supported by the presence of increased levels of total IgE and allergen-specific IgE in blood. In human food allergy, IgE serology needs to be combined with food challenges to confirm allergy and to find the causative allergen, as allergen-specific IgE levels alone are poor predictors for clinical reactions. AD in dogs is commonly associated with allergen-specific IgE. In contrast, in canine CAFR there is no evidence that it is associated with allergen-specific IgE levels, thus canine CAFR is diagnosed via dietary tests only (Jackson et al. 2003; Pucheu-Haston et al. 2008). Although high serum concentrations of allergen-specific IgE were found in some experimental canine models of orally-induced gastrointestinal hypersensitivity (Puigdemont et al. 2006), there is currently no evidence that spontaneous CAFR is caused by type 1 hypersensitivity reactions. Local and systemic immune

responsiveness in CAFR are largely unexplored, hence its causative mechanisms remain to be elucidated.

4. Intolerance reactions

Food allergy is described as an immunological reaction to food proteins and food intolerance is described as an adverse reaction to the chemicals in foods, resulting in, e.g. gastrointestinal and skin symptoms. In children, food intolerance reactions are more common than true allergic reactions and the signs and symptoms are similar to allergic reactions (Clarke and McQueen 1996). One of the differences is that for food allergy often small amounts of allergen are needed to trigger a fast allergic reaction (within hours), while intolerance reactions are dose-related and cause a slower reaction (within 48 h). However, there are also exceptions and allergic reactions can occur slower and intolerance reactions faster than 48 h. Food intolerance reactions may be caused by the absence of specific enzymes needed to digest a food substance, or an abnormality in the absorption of specific nutrients, or by naturally occurring chemicals in food (Ozdemir et al. 2009). Food intolerance is diagnosed, in general, by excluding immunological reactions to the food and performing elimination diets and dietary challenges (Kitts et al. 1997). The presence of immunological reactions to food proteins is not yet proven in dogs with CAFR, thus part of these dogs may actually suffer from intolerance reactions instead of allergic reactions.

4.1. *Intestine*

The mucosal immune system encounters large quantities of antigens daily and generally suppresses immune reactivity to harmless foreign antigens, such as food proteins and commensal bacteria. On the other hand, it may adequately react to pathogens. The induced non-reactivity of immune cells to foreign antigens is called oral tolerance. Antigens may pass the mucosal barrier in four different ways (Figure 2). First, mainly particulate and some soluble antigens are actively transported by microfold cells (M-cells) into the dome region of Peyer's patches. This region contains DC that internalise, process and present the antigen to lymphocytes in the underlying lymphoid structure that contains B cell follicles surrounded by T cells. Second, DC in the lamina propria may sample antigens from the gut lumen by dendrites extruding between the epithelial cells (Rescigno et al. 2001). Third, epithelial cells may internalise soluble antigens by fluid-phased endocytosis, antigens are transported in small vesicles and are digested when they combine with lysosomes (Chehade and Mayer 2005). Fourth, CD23 (low affinity IgE receptor) is expressed on small intestinal epithelial cells in normal and food allergic humans. CD23 can transport allergen into the intestinal tissue with the help of IgE and CD23/IgE-transported allergens seem to be protected from lysosomal degradation. Thus, allergens keep their original allergenic properties (Yu et al. 2001; Bevilacqua et al. 2004; Li et al. 2006). Recently, also the high affinity IgE receptor FcεRI has been found on human intestinal cells. However, the clinical implication of this finding needs to be investigated (Untersmayr et al. 2010).

The cellular constitution of the healthy canine intestine is similar to that of human (James 1993; Lundqvist et al. 1995; German, Hall, and Day 1999; German, Hall, Moore, et al. 1999; Sonea et al. 1999, 2000). The epithelium predominantly contains $CD8^+$ T cells and the intraepithelial lymphocytes mainly express the $\alpha\beta$ T-cell receptors (TCR) and for one-third $\gamma\delta$ TCR.

The lamina propria contains more $CD4^+$ T cells than $CD8^+$ T cells and expresses mostly $\alpha\beta$ TCR (James 1993; Lundqvist et al. 1995; German, Hall, and Day 1999; German, Hall, Moore, et al. 1999; Sonea et al. 2000). The cytokine environment of human healthy intestine differs from the canine intestine. The human healthy intestine has an immunosuppressive profile with a predominance of TGF-β, IL-10 and IL-4 (Jump and Levine 2004; Sanchez-Munoz et al. 2008), in which IL-4 seems to play an immunosuppressive role and not a Th2-inducer role (Rogler and Andus 1998). The intestinal cytokine profile of healthy dogs has a mixed Th1 (IFN-γ, IL-18) and tolerant profile (TGF-β, IL-10)(Peters et al. 2005).

The intestinal homeostasis is largely maintained by the APC and epithelial cells, although the underlying mechanisms are not yet elucidated. Dendritic cells in the lamina propria of the intestine may secrete IL-10 and IL-4, thus contribute to suppression (Chehade and Mayer 2005; Frossard et al. 2004). Moreover, intestinal epithelial cells may present luminal antigens in the context of MHC-II molecules on their surface, but they lack the costimulatory molecules B7.1 and B7.2 rendering them tolerogenic rather than activating (Lin et al. 2005; Westendorf et al 2006). Food allergy can result from a breach in oral tolerance and by the exposure of food allergens via other tissues and organs. This has been shown for the respiratory system in humans (Leser et al. 2001; Borghesan et al. 2008) and for the skin in murine models (Hsieh et al. 2003). Moreover, in AD it has been shown that allergens may pass the skin due to skin barrier dysfunction (Pucheu-Haston et al. 2008; Proksch et al. 2006), a possibility that may occur in AFR as well. If so, this may indicate that the intestine may not be the initial exposure site for food allergens. The intestine of CAFR dogs has not been investigated yet, whereas the human food allergic intestine is only sparsely examined. In cow's milk allergic humans with intestinal signs, the intestinal lymphocytes showed a Th2 cytokine profile (Beyer et al. 2002). In addition, decreased TGF-β1 expression was seen in duodenal epithelial and laminia propria lymphocytes in children with multiple food allergies (Pérez-Machado et al. 2003), suggesting a switch from a Th1 to a Th2 environment and/or a failure in oral

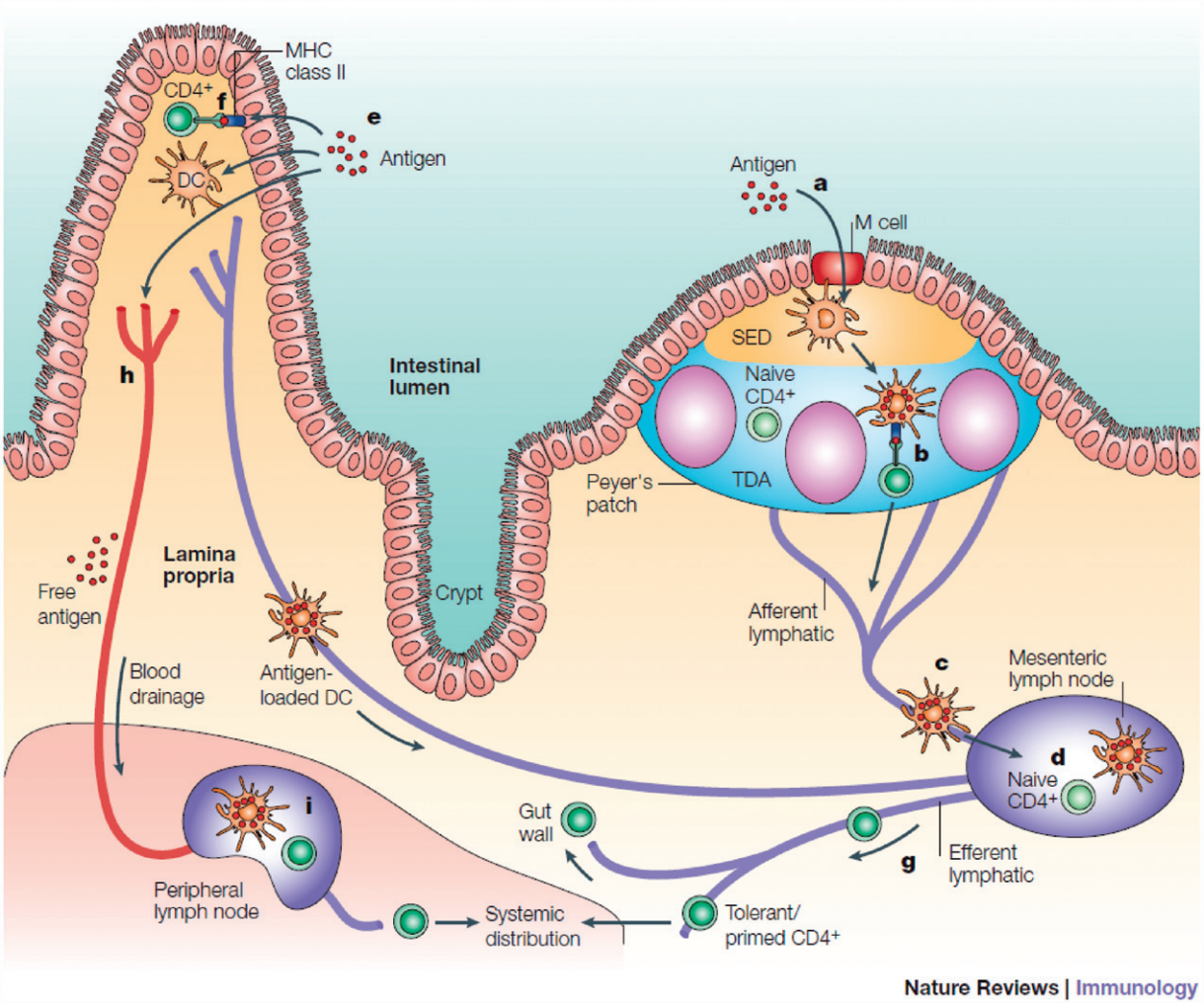

Figure 2. Overview of the intestinal villus and Peyer's patch and possible antigen pathways. Antigen may enter through the microfold (M) cells in the follicle-associated epithelium (FAE) (a), and after transfer to local dendritic cells (DC), may then be presented directly to T cells in the Peyer's patch (b). Alternatively, antigen or antigen-loaded DC from the Peyer's patch may gain access to draining lymph (c), with subsequent T-cell recognition in the mesenteric lymph nodes (MLN) (d). A similar process of antigen or antigen-presenting cell (APC) dissemination to MLN may occur if antigen enters through the epithelium covering the villus lamina propria (e), but in this case, there is the further possibility that MHC class II^+ enterocytes may act as local APC (f). In all cases, the antigen-responsive $CD4^+$ T cells acquire expression of the $\alpha_4\beta_7$ integrin and the chemokine receptor CCR9, leave the MLN in the efferent lymph (g) and after entering the bloodstream through the thoracic duct, exit into the mucosa through vessels in the lamina propria. T cells which have recognised antigen first in the MLN might also disseminate from the bloodstream throughout the peripheral immune system. Antigen may also gain direct access to the bloodstream from the gut (h) and interact with T cells in peripheral lymphoid tissues (i). Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology (Mowat, 2003), copyright 2003.

tolerance with allergy as the outcome. In contrast, no change in T-cell phenotypes or a distinct Th1, Th2, or Treg profile was detected in the duodenum of dogs with only cutaneous clinical signs of food hypersensitivity, suggesting that the intestinal mucosa is not the primary site of T-cell activation that eventually leads to cutaneous food hypersensitivity (Veenhof et al. 2010).

4.2. *Skin*

Currently, it is unknown how exposure to food can lead to adverse reactions in the skin. There are three hypothetical pathways, one is that free allergen is transported from the intestine into skin or peripheral lymph nodes. The second hypothetical pathway is that allergens within APC or exosomes are transported from the intestine to other tissues (van Niel et al. 2001; Admyre et al. 2007). The third option is allergen exposure via the skin (Cork et al. 2006; Marsella et al. 2011). The skin (Figure 3), existing of epidermis, dermis and subcutis, protects against pathogens, toxin penetration and other damage. Although the skin is protective against pathogen penetration and is water-resistant, the epidermal permeability in human and canine AD is increased (Proksch et al. 2006; Pucheu-Haston et al. 2008), enabling allergens to enter the skin. This increased permeability process is due to the skin barrier dysfunction. Filaggrins are part of the keratin cytoskeleton and together with intracellular

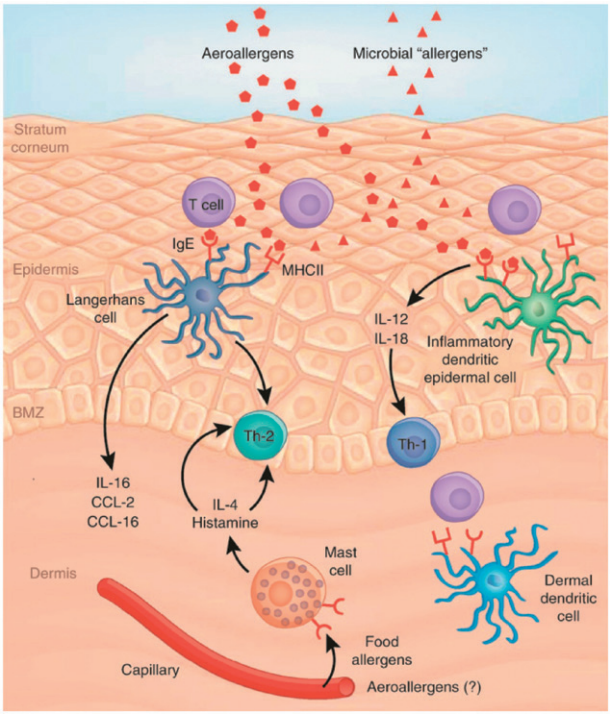

Figure 3. Schematic figure showing the effects of allergens on the cutaneous inflammation in AD. This schematic figure contains more T cells and LC then normally are present in skin for explanatory reasons. Allergens can enter the skin both through the bloodstream (for example, food allergens) or through the stratum corneum (e.g. environmental allergens). In the skin, IgE bound on Fc receptors on cutaneous mast cells and on dendritic cells contributes, together with specific T cells, to the inflammatory response. Reprinted by permission from Macmillan Publishers Ltd: Journal of Investigative Dermatology (Werfel, 2009), copyright 2009.

lipids and epidermal proteases, influence the skin barrier. Mutations in genes encoding filaggrin have been found in a part of the AD patients, potentially leading to the increased permeability of the skin (Palmer et al. 2006; Sandilands et al. 2007; Leung 2009). In addition, the cytokines IL-4 and IL-13 are able to inhibit filaggrin expression (Howell et al. 2007), thus Th2 responses in the skin may also alter the filaggrin-mediated skin barrier and further increase exposure to allergens. Abnormal filaggrin protein expression and decreased ceramide levels are also observed in canine AD and loss-of-function mutations in filaggrin are suggested (Chervet et al 2010). CAFR dogs show cutaneous signs in appearance and distribution often similar as AD. If food allergens can penetrate the skin, as a result of a decreased skin barrier function, this could explain the presence of cutaneous signs and the lack of intestinal symptoms in the majority of dogs with AFR.

When allergens penetrate the skin, they can be internalised by APC present in the skin. LC and inflammatory dendritic epidermal cells are two distinct APC in the skin present in lesional and in a lesser extent non-lesional skin. In allergic humans and dogs, both types of APC have the high affinity IgE receptor (FcεRI) on their surface. Allergens penetrating the skin can be efficiently taken up by the FcεRI-bound IgE molecules on the LC (Olivry et al. 1996; Novak et al. 2003), facilitating allergen processing in the skin. APC containing allergen from the skin or intestine can travel via blood and lymph to peripheral lymph nodes. In the lymph nodes naive T and B cells can recognise the allergen and upon recognition interact with the APC; subsequently, the B and T cells become activated. It is not completely elucidated yet how T cells are triggered to express specific tissue homing receptors, but the origin of the APC or the location of lymph nodes may play a role (Johansson-Lindbom and Agace 2007).

CCR4 and CCR10 are chemokine receptors expressed on the human activated skin homing T cells (Wang et al. 2010). Their ligands CCL17 (TARC) and CCL21 (MDC) are chemokines expressed by cutaneous dendritic cells and bind to CCR4 (Hammad et al. 2003), while CCL27 (CTACK) and CCL28 are expressed by keratinocytes and bind CCR10 (Reiss et al. 2001; Homey et al. 2002). With the help of CCR4, T cells can enter the skin at the site of the microvasculature of the dermis and migrate further in the epidermis with the support of CCR10 (Fuhlbrigge et al. 1997; Reiss et al. 2001; Homey et al. 2002; Hammad et al. 2003; Wang et al. 2010). Memory T cells may express a skin-specific homing molecule (Cutaneous Lymphocyte Antigen: CLA), leading the cells to the skin (Fuhlbrigge et al. 1997; Reiss et al. 2001). In canine skin, CCL28 and CCL17 (TARC) are expressed, and CCR4 is found on canine Th2 cells. The expression of these chemokines and ligands are increased in lesional skin of canine AD (Maeda et al. 2002, 2004, 2005, 2008). The presence and the role of CCL17, CCL28 and CCR4 in canine CAFR is not investigated yet. The acute lesional skin in AD in humans shows initially a more Th2-skewed reactivity and the skin of chronic AD patients shows a more Th1-skewed reactivity (Hamid et al. 1994; Thepen et al. 1996; Leung et al. 2004). In addition, human lesional skin contains more eosinophils and macrophages than non-lesional skin and their products stimulate or prolong the inflammatory reaction (Ou and Huang 2007). The inflammatory cell profile in skin of canine AD is similar to that of human AD. Skin inflammation is characterised by an influx of $CD4^+$ and $CD8^+$ T cells in the lesional skin (Olivry et al. 1997; Sinke et al. 1997) and a mixed cytokine profile with predominant expression of IL-6, IL-4 and IL-13 in the early stage followed by IFN-γ, IL-12 and IL-18 later on (Olivry et al. 1999; Nuttall et al. 2002; Marsella et al. 2006). The T cell subsets and cytokine profiles in the skin of canine CAFR have not been extensively investigated. In the skin of canine CAFRs, a predominant presence of $CD8^+$ T cells and increased expression of the IL-4, IL-13, Foxp3 and SOCS-3 genes were observed. IFN-γ gene expression was increased in lesional compared to non-lesional skin. The predominance of $CD8^+$ T cells indicates that the immunopathogenesis of CAFRs is different from that of canine AD. The elimination diet relieved clinical signs, but did not influence T cell

phenotypes or expression of the cytokine and transcription factor genes in the skin of dogs with CAFRs, indicating a continuously pre-activated immune status in dogs sensitised to food constituents (Veenhof et al. 2011).

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