Chapter 6

Histamine in Atopic Disorders: Atopic Dermatitis and Pruritus

W. Bäumer1,2, F. Glatzer3, K. Roßbach1, H. Ohtsu4, M. Seike5, S. Mommert3, T. Werfel3, R. Gutzmer3

1Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine Hannover, 30559 Hannover, Germany

2Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, USA, e-mail: wbaeumer@ncsu.edu

3Division of Immunodermatology and Allergy Research, Hannover Medical School, Germany

4Applied Quantum Medical Engineering, Department of Engineering, Tohoku University, Japan

5Department of Food and Nutrition Science, Sagami Women's Junior College, Japan

6.1. Role of Histamine and Histamine H4 Receptor in Inflammatory Skin Diseases in Man

6.1.1. Introduction

The skin is the primary interface between the environment and the inside of an organism and protects the body against harmful agents, pathogens, allergens, physical trauma, UV radiation and excessive electrolyte and water loss. Different diseases interfere with this function, including the extremely common chronic inflammatory skin diseases. Chronic inflammatory skin diseases are characterized by chronic inflammation of the skin, often with severe pruritus. The most diseases include psoriasis and eczema, including atopic dermatitis (AD) and allergic contact dermatitis (ACD). Numerous different inflammatory cell populations are involved in the pathogenesis of inflammatory skin diseases, including T cells, antigen presenting cells (APC), granulocytes and keratinocytes. The inflamed skin in these diseases becomes dry, red, itchy and scaly, resulting
in decreased quality of life. At present, the responsible mechanisms that are involved in inflammation and pruritus in these disorders and how they lead to pathogenesis are not completely understood.

A major mediator of inflammation and allergic reactions is histamine, which plays an important role in acute and chronic inflammation as well as hypersensitivity reactions. High amounts of histamine are released during allergic and inflammatory disorders, where increased levels of histamine have been reported in lesions of psoriasis and eczematous skin diseases (Stander & Steinhoff, 2002) with possible peak values of $10^{-5}$ to $10^{-3}$ molar during immediate hypersensitivity reactions following mast cell degranulation (Simons & Simons, 1994). Histamine may hold many therapeutic capabilities, but therapeutic blockade of the $H_1$ and $H_2$ histamine receptors are not efficient to relieve pruritus in AD or psoriasis (Akdis et al., 2006). A possible explanation for this observation is that histamine might mediate effects via other receptors, such as the $H_4$ receptor (Thurmond et al., 2008). The $H_4$ receptor is the most recently discovered histamine receptor and there is evidence that it is preferred histamine target in the immune system (Zampeli & Tiligada, 2009). This makes it an attractive target for developing novel therapeutics of inflammatory skin diseases (Tiligada et al., 2009).

This article summarizes the findings regarding the differential expression and function of $H_4$ receptor on cell types relevant for human chronic inflammatory skin diseases and pruritus. The role of histamine in animal models of atopic dermatitis is also discussed.

6.1.2. Histamine $H_4$ Receptor Expression and Function on Cell Types Involved in Inflammatory Skin Diseases

The expression of $H_4$ receptor is described for various cells types relevant for inflammatory skin diseases and for some cell populations, functional studies of the $H_4$ receptor also exist.

6.1.2.1. T Cells

T cells play a key role as immune effector cells in AD (Novak et al., 2003), and various studies have been conducted to characterize their role in this disease. Gene expression studies have shown the expression of the $H_4$ receptor on mRNA and protein level in T cells, where $H_4$ receptor expression on CD4+ T cells can be upregulated by an IL-4-dominated micromilieu. Furthermore, it was demonstrated that $H_4$ receptor expression on Th2 cells is higher compared with Th1 cells and stimulation of $H_4$ receptor with specific agonists results in an induction of transcription factor AP-1 in Th2 but not in Th1 cells (Gutzmer
Stimulation of the H₄ receptor results in upregulation of IL-31 on the mRNA level in Th2 cells (Gutzmer et al., 2009). Moreover, H₄ receptor might be involved in the induction of proinflammatory cytokines in activated monocytes and macrophages by enhanced IL-31 release (Kasraie et al., 2010). Stimulation of the H₄ receptor also inhibits the antigen-specific human T cell responses by decreased IFN-γ and IL-5 expression (Sugata et al., 2007). Th17 cells, another important subtype of T cells, also express the H₄ receptor. Th17 cells are increased in peripheral blood of AD patients and play a potential role in AD, and the number of this cell type is associated with the severity of AD (Koga et al., 2008). The H₄ receptor was detected on human memory T cells, polarized into Th17 cells by IL-1β and IL-23, and on IL-17-positive cells in lesions of inflamed skin. Furthermore, it was demonstrated that a stimulation of H₄ receptor results in an upregulation of IL-17 mRNA and secreted protein and an induction of the transcription factor AP-1 (Mommert et al., 2012).

Ou et al. (2004) observed that the blood of patients with AD showed high numbers of CD4+CD25+ regulatory T cells (Tregs) in combination with increased induction of the transcription factor forkhead box P3 (FOX P3). Another study by Schnopp et al. (2007) demonstrated the presence of Tregs in the skin of AD patients (Ou et al., 2004, Schnopp et al., 2007).

CD8+ T cells also express H₄ receptor at the mRNA and protein levels and stimulation of the H₄ receptor in CD8+ T cells results in a release of the T cell chemoattractant IL-16 (Gantner et al., 2002). Invariant natural killer T cells (iNKT cells) have been found in lesional skin of patients suffering ACD, AD and other types of inflammation (Simon et al., 2009). Activation of these cells causes the expression of IL-4 and IFN-γ, but it is unclear under which conditions they trigger a Th1 or Th2 response.

6.1.2.2. Antigen Presenting Cells

Antigen presenting cells (APCs), which include monocytes and dendritic cells (DCs), are one of the major components in the initiation of allergic inflammation. A major role of these cells in allergic inflammation is antigen uptake and presentation followed by the secretion of cytokines and chemokines to generate a cytokine network. In early investigations, it was demonstrated that human monocytes and monocyte-derived dendritic cells (MoDCs) express the H₄ receptor on the mRNA and protein level and that the receptor is involved in control of cytokine and chemokine production. H₄ receptor stimulation in monocytes and MoDCs result in a suppression of IL-12, IL-27 and CCL2, leading to calcium influx and chemotaxis (Gutzmer et al., 2005, Damaj et al., 2007, Dijkstra et al., 2007, Gschwandtner et al., 2012).

Further studies focused on several subtypes of DC which play an important role in inflammatory skin disorders, such as inflammatory dendritic epidermal
cells (IDECs), plasmacytoid DCs (pDCs), myeloid DCs (mDCs) and Langerhans cells (LCs). The H₄ receptor on murine and human LCs is expressed on the mRNA and protein levels and stimulation of the H₄ receptor results in decreased expression of CCL2 in human LCs as well as enhanced the migration of LCs from the epidermis in ex vivo migration assays using human epidermis (Gschwandtner et al., 2010). Recent findings showed the expression of H₄ receptor on different subtypes of DCs demonstrated different functional activities. IDEC represents a DC subtype, which was found in lesions of AD. Stimulation of the H₄ receptor expressed on IDECs result in a decreased expression of IL-12 and CCL2 (Dijkstra et al., 2008). Inflammatory DCs characterized by 6-Sulfo LacNAc groups on the cell surface (SLAN-DCs), a major population of DCs in human blood, expresses the H₄ receptor on mRNA and protein level and shows high proinflammatory capacity through T cell response induction and the release of proinflammatory cytokines. SLAN-DCs are recruited in inflamed skin, where subsequent stimulation of the H₄ receptor results in a strong decrease of lipopolysaccharide-induced TNF-α and IL-12 production. Through this mechanism, histamine reduces the proinflammatory capacity of these cells (Gschwandtner et al., 2011b). Expression of the H₄ receptor has also been detected on pDCs and mDCs. Interestingly, pDCs from patients with psoriasis express more H₄ receptor compared with healthy or AD donors. Preliminary functional studies on the role of H₄ receptor in pDC revealed decreased expression of the pro-inflammatory cytokines TNF-α, Interferon-α and the chemokine CXCL8. Moreover, an active migration of pDCs in response to stimulation with a H₄ receptor agonist was observed (Gschwandtner et al., 2011a). For mDCs, the stimulation of H₂ and H₄ receptors lead to reduced expression of the Th1-associated CXCL10. This suggests that the H₄ receptor has major anti-inflammatory effects in APCs by downregulating proinflammatory cytokines such as IL-12, TNF-α and IL-27 and the chemokines CCL2, CXCL8 and CXCL-10.

6.1.2.3. Granulocytes

An increased number of circulating eosinophils in AD patients and the detection of activated eosinophils in lesional skin possibly provide evidence for a role of granulocytes in the pathology of inflammatory skin diseases (Simon et al., 2004, Werfel, 2009). Human eosinophils and neutrophils express the H₄ receptor and histamine acts as chemoattractant for eosinophils. (Clark et al., 1975, Clark et al., 1977, Oda et al., 2000, Morse et al., 2001). Subsequent studies identified the H₄ receptor as the inducer of chemotaxis and showed that histamine stimulation resulted in calcium mobilization, the induction of actin polymerization, the increase in adhesion molecules CD11b/CD18 and CD54 and the alteration in cell shape (O’Reilly et al., 2002, Buckland et al., 2003, Ling et al., 2004). H₄ receptor activation also leads to a migration of
eosinophils from blood into inflamed tissues (Barnard et al., 2008). In contrast, for human basophils and neutrophils no chemoattractant effect via H₄ receptor was observed.

6.1.2.4. Natural Killer Cells

Natural killer cells (NK cells) are a subpopulation of lymphocytes in peripheral blood (15%) that act as early effector cells in the innate immune system and are characterized by CD3- and CD56+ expression. In atopic eczema, NK cells are located near DCs in lesional skin and it is suspected that NK cells modulate the function of DCs in AD (Buentke et al., 2002). Expression studies revealed H₁ and H₄ receptor expression on the protein level for human NK cells with induction of chemotaxis by histamine. This effect is inhibited by the H₃/H₄ receptor antagonist thioperamide. NK cells are lacking the H₃ receptor, so this effect has been attributed to thioperamide binding to the H₄ receptor (Damaj et al., 2007).

6.1.2.5. Skin cells (Keratinocytes, Fibroblasts, Mast Cells)

Keratinocytes form the outermost surface of the body and are directly in contact with the environment. These cells are responsible for the recruitment of other cell types to the site of inflammation and regulate the first step in the production of antimicrobial peptides as well as proinflammatory cytokines and chemokines. They play an important role in inflammatory skin diseases, such as AD (Wittmann and Werfel, 2006). Early studies showed keratinocytes express the H₁ receptor and described the various immunomodulatory effects of histamine on these cells (Kohda et al., 2002, Kanda & Watanabe, 2003, Giustizieri et al., 2004, Kanda & Watanabe, 2004). Histamine modulates the differentiation from epidermal keratinocytes and impairs the skin barrier function via H₁ receptor (Gschwandtner et al., 2013).

Yamaura et al. (2009) initially reported the expression of H₄ receptor in keratinocytes through immunohistochemical studies on human epidermal tissue. The H₄ receptor appeared to be upregulated during differentiation of keratinocytes in the upper layer of epidermis versus keratinocytes in basal layer (Yamaura et al., 2009a). The expression of H₄ receptor on human primary keratinocytes has also been demonstrated. Furthermore, outer root sheath keratinocytes from patients with AD show significantly increased expression of the H₄ receptor compared with keratinocytes of healthy donors. The stimulation of the H₄ receptor results in an enhanced proliferation of foreskin and outer root sheath keratinocytes in different in vitro cell proliferation assays and scratch assays. This effect was blocked by pre-incubation with the H₄ receptor specific antagonist JNJ7777120 (Glatzer et al., 2013).
Dermal fibroblasts also express the H₄ receptor on mRNA and protein levels. The expression of this receptor can be upregulated by stimulation of fibroblasts with lipopolysaccharide, indomethacin or dexamethasone (Ikawa et al., 2008). The involvement of fibroblasts in skin diseases like AD has not been well understood until recently. Leung et al. (1982) showed morphology changes and cell mediated cytotoxicity against skin fibroblasts in AD skin (Leung et al., 1982). Another study showed a possible gene expression profile associated with AD in fibroblasts based on proteomic analysis. These findings suggest a possible role for fibroblasts in the pathogenesis of AD (Park et al., 2006).

Human mast cells express the H₄ receptor on the mRNA and protein levels and stimulation of this receptor in mast cells results in enhanced CXCL12-mediated recruitment of precursor mast cells into the dermis (Hofstra et al., 2003, Lippert et al., 2004, Godot et al., 2007). A recent study demonstrated enhanced production of IL-6 via ERK and phosphoinositide 3-kinase (PI3K) activation following stimulation of the H₄ receptor in murine mast cells (Desai & Thurmond, 2011). Based on these investigations, it can be hypothesized that the H₄ receptor on mast cells plays an important role for the accumulation of these cells in allergic tissue. Otherwise, H₄ receptor stimulation has no relevant influence on the mast cell degranulation and subsequent mediator release from these cells (Hofstra et al., 2003).

6.1.3. Molecular Mechanisms in Regulation of H₄ Receptor Expression

Recent studies have reported that alterations in the H₄ receptor gene are associated with numerous diseases, including chronic inflammatory skin disorders. The H₄ receptor is significantly upregulated in the course of different skin diseases (Gschwandtner et al., 2011a, Gschwandtner et al., 2011b), but the possible genetic or epigenetic reasons behind these observations are unknown. Yu et al. (2010) detected three different single nucleotide polymorphisms (SNPs) within the H₄ receptor gene significantly associated with AD, indicating that the H₄ receptor plays a role in the development of this disease. (Yu et al., 2010b). The same group demonstrated a correlation between the presence of increased copies (Copy number variation) of the H₄ receptor gene and the risk of developing systemic lupus erythematosus (Yu et al., 2010a). An association between genetic variations of H₄ receptor and infectious asthma has also been described (Simon et al., 2012).

6.1.4. Conclusions

Together, all of the above studies provide evidence for a pathogenetic and immunomodulatory role of the H₄ receptor in chronic inflammatory skin diseases and pruritus. The H₄ receptor modulates the function of relevant
Table 6.1
Expression and function of the human H4 receptor in relevant cell types for inflammatory skin diseases (AP-1 activating protein 1, DC dendritic cell, IDEC inflammatory dendritic epidermal cell, IFN-γ Interferon gamma, IL Interleukin, SLAN-DC 6-Sulfo LacNac-expressing dendritic cell, Th T helper cell, TNF-α tumor necrosis factor alpha, Treg regulatory T cell).

<table>
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<tr>
<th>Cell subpopulation</th>
<th>Expression H4 receptor</th>
<th>Function H4 receptor</th>
<th>Reference</th>
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<tr>
<td><strong>Antigen presenting cells</strong></td>
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<td>Monocytes</td>
<td>mRNA and protein level</td>
<td>↑ Calcium influx</td>
<td>(Damaj et al., 2007), (Dijkstra et al., 2007), (Gschwandtner et al., 2012a)</td>
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<td></td>
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<td>↓ CCL2</td>
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<td>Monocyte-derived DC</td>
<td>mRNA and Protein level</td>
<td>↑ Chemotaxis</td>
<td>(Gutzmer et al., 2005)</td>
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<td>↓ IL-12</td>
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<td>IDEC</td>
<td>protein level</td>
<td>upregulation by IFN-γ</td>
<td>(Dijkstra et al., 2008)</td>
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<tr>
<td>SLAN-DC</td>
<td>mRNA and protein level</td>
<td>↓ IL-12</td>
<td>(Gschwandtner et al., 2011b)</td>
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<td>Plasmacytoid DC</td>
<td>mRNA and protein level</td>
<td>↑ Chemotaxis</td>
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<td>↓ TNF-α</td>
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<tr>
<td>Myeloid DC</td>
<td>mRNA level</td>
<td>↓ IP10</td>
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<td>Langerhans cells</td>
<td>protein level</td>
<td>↑ Chemotaxis</td>
<td>(Gschwandtner et al., 2010)</td>
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<td>↓ CCL2</td>
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<td>T cells</td>
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<td>CD4+ Th 1/2</td>
<td>mRNA and protein level</td>
<td>↑ IL-31</td>
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<td></td>
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<td>↓ IFN-γ</td>
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<td>CD4+ Th17</td>
<td>mRNA and protein level</td>
<td>↑ IL-17</td>
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<td>Induction of AP-1</td>
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<td>CD4+ Treg</td>
<td>Not described</td>
<td>4-methylhistamine recruited Treg</td>
<td>(Morgan et al., 2007)</td>
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<td>CD8+</td>
<td>mRNA level</td>
<td>↑ IL-16</td>
<td>(Gantner et al., 2002)</td>
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<td>Invariant natural killer T cells</td>
<td>mRNA and protein level</td>
<td>Not described</td>
<td>Unpublished data</td>
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<td>Other</td>
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<tr>
<td>Natural killer cells</td>
<td>mRNA and protein level</td>
<td>↑ Chemotaxis</td>
<td>(Damaj et al., 2007)</td>
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<td>Keratinocytes</td>
<td>mRNA and protein level</td>
<td>upregulation in differentiated cells</td>
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<td>Fibroblasts</td>
<td>mRNA and protein level</td>
<td>↑ Proliferation</td>
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<td>Mast cells</td>
<td>mRNA and protein level</td>
<td>↑ Recruitment of CKCL12</td>
<td>(Thurmond et al., 2008), (Hofstra et al., 2003), (Lippert et al., 2004), (Godot et al., 2007)</td>
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<td>expressing precursors,</td>
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cell populations by influencing chemotaxis and cytokine production, among other mechanisms. By influencing chemotaxis and cytokine production, the H₄ receptor may influence Th cell polarization linking innate and adaptive immune pathways. This makes the H₄ receptor a desirable therapeutic target in chronic inflammatory skin diseases. Table 1 summarizes the different cell populations expressing the H₄ receptor and the possible described functions of this receptor on these cells.

### 6.2. Role of Histamine in Animal Models of Atopic Dermatitis

#### 6.2.1. Introduction

Histamine is a ubiquitous chemical messenger that displays numerous functions mediated through four known pharmacologically distinct receptors. The H₁ receptor is expressed in peripheral nerves, keratinocytes and endothelial cells (Baumer and Rossbach, 2010). The characteristic features of H₁ receptor activation in the skin are exemplified by itching transactions (Hagermark et al., 1979) and increased vascular permeability (Sercombe et al., 1986). As many of these functions contribute to allergic responses, H₁ receptor antagonists have been successfully used as drugs for treating certain forms of allergies (Simons & Simons, 2011). The H₂ receptor is expressed in keratinocytes, melanocytes, macrophages and lymphocytes (Akdis & Simons, 2006), but its exact function in the skin remains unclear. The H₃ receptor is predominantly expressed in the central nervous system (Sander et al., 2008, Baumer & Rossbach, 2010) but the exact physiological roles it plays in the skin remain to be explored. The H₄ receptor is also expressed in mast cells and sympathetic and parasympathetic nerves and may regulate histamine, serotonin, acetylcholine and other neurotransmitters (Sander et al., 2008). Recently, a fourth histamine receptor was identified as H₄ (Nakamura et al., 2000), where H₄ receptor expression has been observed in eosinophils, T cells, dendritic cells, mast cells and primary sensory neurons (de Esch et al., 2005). The H₄ receptor mediates chemotactic activity of histamine for mast cells and eosinophils (Zampeli & Tiligada, 2009).

Assessing the effect of histamine in the development of eczematous lesions in vivo has been difficult, as most observations involve the use of histamine receptor antagonists. When using such agents, problems such as adverse effects, short half-life and selectivity are unavoidable. However, histamine-deficient HDC(-/-) mice, produced by disrupting the histidine decarboxylase (HDC) gene, were studied to circumvent these problems (Ohtsu et al., 2001).
6.2.2. Histamine Facilitates Development of Eczematous Lesions in Contact Dermatitis: Role of Histamine H₁ Receptor

The role of histamine in scratching behaviour (see chapter 6.3) and the extent of chronic eczema was investigated by using HDC(-/-) mice of the 129/Sv inbred strain (Seike et al., 2005a). Chronic contact dermatitis was induced with daily application of diphenylcyclopropenone (DCP) on a hind paw of HDC(+/+) and HDC(-/-) mice for 2 months. Histological examination of the skin sample revealed that the mice displayed inflammatory cell infiltration, hyperplastic epidermis and newly spreading neuronal processes, but the magnitude of these changes were more significant in HDC(+/+) mice.

Further studies using the C57B6 strain were conducted to support the previous evidence, to observe the effects in our new experimental strain and to provide further information about the role of each histamine receptor (Seike et al., 2005b). Development of eczematous lesions in contact dermatitis was reduced in HDC(-/-) mice versus HDC(+/+) mice, as observed in the previous experiment with the 129/Sv strain. The H₁ agonist histamine trifluoromethyl toluidide (HTMT) promoted development of eczematous lesion in HDC(-/-) mice and correspondingly, an H₁ receptor antagonist (loratadine) reduced development of eczematous lesions in HDC(+/+) mice. However, an H₂ agonist (dimaprit) and H₂ antagonist (cimetidine) were ineffective in HDC(-/-) and HDC(+/+) mice, respectively. These results suggest that histamine facilitates the development of eczematous lesions in a murine model of contact dermatitis mainly via H₁ receptors, which was confirmed with another H₁ receptor antagonist, olopatadine hydrochloride. Mice administered with olopatadine develop mild erythema with slight hair loss, whereas mice without olopatadine develop scaly erythema with marked hair loss and erosions. Skin repeatedly challenged with hapten displays marked epidermal hyperplasia and infiltration of inflammatory cells, including mast cells in the dermis, and olopatadine suppresses these histological changes (Hamada et al., 2006).

6.2.3. Histamine H₄ Receptor: a New Player in the Game

Soon after discovery of the fourth histamine receptor, it became apparent that this receptor might mediate central immune and inflammation-mediated processes like immune cell migration and modulation of cytokine secretion (Zampeli & Tiligada, 2009a, Gutzmer et al., 2011). These actions might also have an impact on the development of allergic skin diseases (Gutzmer et al., 2011).

Effects of H₄ receptor antagonists have been tested in several murine models of allergic dermatitis, in the skin of laboratory beagles and in a canine model of atopic dermatitis. In some studies acute models of allergic contact dermatitis
were used and in one additional study acute lesions were compared to chronic lesions induced by repetitive topical administration of the relevant hapten.

The selective H₄ receptor antagonist JNJ7777120 did not reduce the ear swelling induced by the hapten dinitrochlorobenzene (DNCB) and toluenediisocyanate (TDI), which differ in their induction of a Th1- and Th2-dominated inflammatory response (Baumer et al., 2004, Rossbach et al., 2009b). Seike et al. used trinitrochlorobenzene as hapten to elicit ACD in mice, a hapten similar to dinitrochlorobenzene. Even suprapharmacological doses of the H₄ receptor antagonist JNJ7777120 did not reduce the acute allergic response. By chronification of the lesions, however, a reduction of inflammatory response accompanied by a diminished mast cell and eosinophilic infiltration was observed in this study published 2010 (Seike et al., 2010).

More recently, reduction of the inflammatory response induced by topical administration of the hapten fluorescein isothiocyanate (FITC) was also observed in the acute phase of allergic dermatitis by treatment with JNJ7777120 (Cowden et al., 2010). One characteristic of the FITC model is a distinct eosinophilia, which is less pronounced in other models of hapten-induced contact dermatitis (such as by DNCB or TDI). In this respect, it is interesting that the dual H₃/H₄ receptor antagonist thioperamide was effective in reducing inflammation in a modified model of picryl chloride-induced allergic dermatitis, in which blood eosinophilia was induced by cyclophosphamide. A combination of thioperamide with the H₁ receptor antagonist pyrilamine even enhanced the anti-inflammatory effect (Hirasawa et al., 2009).

Taken together, these results indicate that H₄ receptor blockade reduces inflammation in chronic ACD and acute ACD with predominant Th2 milieu, but there appears to be only a minor role for H₄ receptor antagonism in lesions of acute ACD with predominant Th1 milieu.

A very recent study revealed that the H₁ receptor antagonist olopatadine and H₄ receptor antagonist JNJ7777120 improved scratching behavior and skin inflammation in a model of chronic allergic dermatitis established in a NC/Nga mouse model (Ohsawa & Hirasawa, 2012), where mice have a genetic defect in barrier function that provides a model of AD closer to the human disease than the aforementioned contact dermatitis models (Jin et al., 2009). However, atopic-like lesions were triggered by frequent topical administration of the hapten picryl chloride. Thus, this model might be viewed as an intermediate between an allergic contact dermatitis model and an atopic dermatitis model.

Interestingly, in a recent study, mice were sensitized epicutaneously against ovalbumine (OVA), which resembles another widely accepted atopy mouse model (Jin et al., 2009). Wildtype (BALB/c) and H₄ receptor knockout mice were epicutaneously sensitized with OVA according to a standard protocol with slight modifications (Spergel et al., 1998). The clinical development of dermatitis was markedly attenuated in H₄ receptor knockout mice and was accompanied by
a significantly diminished influx of inflammatory cells and reduced epidermal hyperproliferation, as revealed by histological examination. Interestingly, the serum of knockout mice showed a significantly reduced amount of OVA-IgE. In spleen and skin draining lymph nodes, a significantly decreased number of CD4+ T cells and F4/80+ macrophages were observed. The INF-γ production of ex vivo-stimulated splenocytes was also significantly reduced in knockout mice (Rossbach et al., 2012).

In less severe skin lesions, the reduced cell influx into the skin as well as the reduced number of splenocytes and lymph node cells clearly indicate an anti-inflammatory role of the H₄ receptor in this chronic model of atopic dermatitis.

6.2.4. Histamine in Dogs

Dogs suffering from atopic dermatitis share several similarities (albeit with some differences) compared to human AD. This has caused debate as to whether atopic dogs may be a suitable model for human AD (Marsella & Girolomoni, 2009, Marsella et al., 2011). A role of histamine has been disputed for decades and enhanced concentration of histamine has been reported in lesional skin of dogs (Marsella & Olivry, 2001). There also seems to be higher “reagibility” of mast cells in atopic dogs (Marsella & Olivry, 2001). However, H₁ receptor as well as H₂ receptor antagonists show only moderate effects on lesions and pruritus in atopic dogs (DeBoer & Griffin, 2001, Olivry et al., 2010). Nevertheless, the role of histamine was re-evaluated using the first findings, where the H₄ receptor might have immunomodulatory functions. In 2008, Jiang et al reported the first pharmacological characterization of the canine H₄ receptor (Jiang et al., 2008) and robust expression of the H₄ receptor was demonstrated on the mRNA level in canine skin (Eisenschenk et al., 2011). Our group explored the role of H₄ receptor in canine skin by intradermal injection of histamine and putative H₄ receptor agonists to laboratory beagles with the intention to study a classical weal and flare reaction and pruritus. Histamine as well as the H₄ receptor agonists clobenpropit and VUF4830 induced a weal and flare reaction after intradermal injection. Unexpectedly, no pruritus was induced by either substance (Rossbach et al., 2009a). However, when the beagles were pre-treated topically with the H₄ receptor antagonist JNJ7777120 before histamine application, the weal and flare reaction was reduced by approximately 30%, further indicating a role of the H₄ receptor in this reaction (Rossbach et al., 2009a).

The prevention of skin lesions by H₁ and H₄ receptor antagonists was also tested in an alternative canine model of atopic dermatitis (Pucheu-Haston et al., 2008). Six atopic Maltese-beagle crossbred dogs experimentally sensitized to Dermatophagoides farinae (DF) were enrolled into blinded placebo and active controlled experiments. H₄ receptor antagonists (JNJ7777120 or JNJ28307474) were applied topically before allergen challenge and JNJ28307474 was also
given orally after allergen challenge. A triamcinolone acetonide solution applied topically was used as a positive control, and skin lesions that developed after the application of the Df allergen were graded at the site of allergen application. Twenty four hours after the challenge, placebo treated animals and animals treated with topical and oral JNJ28307474 or topical JNJ7777120 showed a comparable lesion score, whereas the triamcinolone solution prevented all dogs from having any lesions (Baumer et al., 2011). The systemic administration of cetirizine and hydroxyzine was also tested in the same model. A prophylactic administration of hydroxyzine and cetirizine in doses, which significantly reduce the histamine or anti-canine, IgE-induced weal and flare reaction, also did not reduce the median inflammatory score of the placebo treatment (Baumer et al., 2011). These data provide evidence that the preventive administration of H<sub>1</sub> receptor as well as H<sub>4</sub> receptor antagonists has no impact on the development of acute inflammatory skin lesions induced by the topical administration of a relevant allergen. It is thus intended to study effects of H<sub>4</sub> receptor antagonists in chronic skin lesions.

6.3. Role of Histamine Receptors in Pruritus Transmission

6.3.1. Introduction

Pruritus has been defined as an unpleasant sensation that triggers a desire to scratch (Ikoma et al., 2011). A wide range of substances have been implicated for the induction of itch (Steinhoff et al., 2006), but histamine remains as the best-known endogenous agent and serves as a classical inducer of itch under experimental settings (Magerl et al., 1990, Schmelz et al., 1997). In a mouse model of chronic contact dermatitis induced by daily application of diphenylcyclopropenone (DCP) for 2 months, HDC(+/+) mice showed significantly increased inflammatory cell infiltration, hyperplastic epidermis and new spreading of neuronal processes than the HDC(-/-) mice (Seike et al., 2005a). Scratching behaviour was induced in HDC(+/+) mice but was barely observed in HDC(-/-) mice, suggesting that histamine production is important for the itch sensation. Aside from the H<sub>2</sub> receptor, all histamine receptors are involved in the transmission of histamine-induced itch (Bell et al., 2004, Rossbach et al., 2011). The first indications that the H<sub>4</sub> receptor is involved in pruritus were made by Bell et al (2004), where it was demonstrated that intracutaneously administered clobenprobit (H<sub>3</sub> receptor antagonist/H<sub>4</sub> receptor agonist) as well as imetit (H<sub>3</sub>/H<sub>4</sub> receptor agonist) induced scratching behaviour in mice. Furthermore, the clobenprobit-induced itch was antagonised by systemic administration of
the H₃/H₄ receptor-antagonist thioperamide. Moreover, intradermal injection of 4-methylhistamine, a H₄ receptor agonist that displays no affinity at the H₃ receptor, induced itch in mice, confirming the findings by Bell et al. (2004) and clearly pointing out the role of the H₄ receptor in histamine-induced itch transmission (Dunford et al., 2007). Pruritic properties of intradermal administered thioperamide and clobenpropit indicated that the H₄ receptor is also involved in histamine-induced itch response (Hossen et al., 2003, Sugimoto et al., 2004). However, since systemically administered thioperamide dose dependently reduced histamine- or clobenpropit-induced pruritus (Hossen et al., 2003, Bell et al., 2004, Sugimoto et al., 2004), the precise role of the H₃ receptor remained unclear. It has recently been shown that the H₃ receptor is also involved in histamine-induced itch. Intradermal injection of the selective H₃ receptor inverse agonist pitolisant dose-dependently induced scratching behaviour in mice. Interestingly, this itch response could be blocked by pre-treatment with H₁ or H₄ receptor antagonists (Rossbach et al., 2011).

Histamine, 4-methylhistamine and thioperamide induce scratching behaviour in mice independently of mast cells or other haematopoietic cells (Hossen et al., 2003, Dunford et al., 2007), indicating a direct effect on sensory nerves. Additionally, stimulation of the H₄ receptor on Th2 cells leads to an increased release of IL-31 (Sonkoly et al., 2006, Gutzmer et al., 2009). This newly discovered cytokine is an important itch inducer and is mainly produced by activated T cells. The IL-31 receptor complex (IL-31 receptor A and the oncostatin-M receptor) is found in the skin on sensory C-fibers and keratinocytes as well as in the dorsal root ganglia (DRG), where it probably contributes to the transmission of itch signal (Raap et al., 2008). Thus, IL-31 might represent a mediator contributing to pruritus induced by H₄ receptor stimulation (Gutzmer et al., 2009).

In humans, itch induced by histamine is transmitted via specific mechanoinsensitive C fibers. These “itch” fibers are preferentially activated by pruritogens like histamine and respond to histamine application with a time course of excitation that reflects the sensation of itch (Schmelz et al., 1997). Besides the histaminergic pathway, electrophysiological studies suggest the existence of a second peripheral pathway for the transmission of itch (Schmelz, 2010). Cell bodies of sensory nerves are located in the DRG and expression of the H₁, H₂ and H₄ receptors have been observed on a subpopulation (about 15%) of DRG neurons (Kashiba et al., 1999, Cannon et al., 2007, Strakhova et al., 2009, Rossbach et al., 2011). Stimulation with H₂ or H₄ receptor agonists as well as inhibition of the H₃ receptor increases intracellular [Ca²⁺]free levels in these neurons (Kim et al., 2004, Han et al., 2006, Shim et al., 2007, Rossbach et al., 2011) and histamine requires the activation of TRPV1 to excite sensory neurons (Kim et al., 2004, Shim et al., 2007, Kajihara et al., 2010, Rossbach et al., 2011). Moreover, mice pretreated with a TRPV1 blocker (or mice lacking TRPV1) showed significantly reduced scratching behaviour in response to histamine application.
Shim et al. (2007) further demonstrated that histamine excites sensory neurons by activating TRPV1 via phospholipase A\(_2\) and lipoxygenase stimulation. In addition, Han et al. (2006) showed that phospholipase C\(_\beta3\) mediates the scratching response induced by activation of the H\(_1\) receptor on C fiber neurons (Han et al., 2006). These results strongly suggest that histamine requires the activation of TRPV1 to excite sensory neurons via H\(_1\) and H\(_4\) receptors and cause itching. The precise mechanisms underlying the mediation of itch via the H\(_3\) receptor are still unclear. The H\(_3\) receptor modifies the release of histamine and other neurotransmitters not only in the CNS but also in peripheral tissues (Arrang et al., 1983, Ohkubo et al., 1995, Nemmar et al., 1999, Blandizzi et al., 2000). The H\(_3\) receptor may modulate the release of histamine directly from DRG neurons or possibly regulate the release of other neurotransmitters such as substance P, which in turn could activate surrounding cells to release histamine. Substance P has found to be involved in the mediation of histamine-induced itch, where H\(_4\) receptor antagonism inhibits substance P-induced pruritus and intradermal injection of a tachykinin NK1 antagonist decreases the pruritus induced by the H\(_1\) receptor antagonist/H\(_3\) receptor agonist clobenpropit (Hossen et al., 2006, Yamaura et al., 2009). A decreased threshold or even an enhanced neurotransmitter release in response to H\(_3\) receptor inverse agonism might activate H\(_1\) receptor and H\(_4\) receptor on a subset of sensory neurons, which in turn could result in the excitation of itch-mediating histamine-sensitive sensory nerves, triggering the itch response (Rossbach et al. 2011). However, it can not be excluded that skin cells other than mast cells, such as keratinocytes, are required for a possible enhanced release of histamine via blockade of the H\(_3\) receptor.

Sensory nerves in the skin transmit the itch signal to the DRG and from there it reaches the spinal cord. From the lamina I, a superficial layer within the dorsal horn of the spinal cord, the signal is projected to the thalamus. The expression of c-Fos was specifically upregulated in lamina I of the spinal dorsal horn following repeated DCP application in mice (Seike et al., 2005a). Increased expression of c-Fos and substance P in this region were downregulated by olopatadine (Hamada et al., 2006), suggesting that scratching behaviour in chronic contact dermatitis in mice are mainly mediated via histamine and the afferent pathways of sensation to the central nervous system are mediated through lamina I of the spinal dorsal horn. Histamine-induced itch is transmitted via a distinct neuronal pathway consisting of specialized mechano-insensitive primary afferent fibers and mechano-insensitive dorsal horn spinthalamic projection neurons (Schmelz et al., 1997, Andrew and Craig, 2001, Schmelz, 2010). These histamine-sensitive spinthalamic tract neurons project mainly to the ventral, posterior and inferior nucleus as well as the ventral periphery of the ventral, posterior and lateral nucleus of the lateral thalamus, whereas nociceptive spinthalamic tract neurons project mainly to the nucleus submedius of the medial thalamus (Ikoma et al., 2011). However, the histamine-responsive fibres
are also excited by at least one algogen, namely capsaicin (ligand of TRPV1) and are thus not itch-specific (Schmelz, 2010). The impact of the different histamine receptor subtypes on the spinal itch transmission to our knowledge has not been elucidated yet. Intrathecal injection of histamine induces a behavioral response consisting of biting and licking with occasional hindlimb scratching in mice, which seems to be dependent on the injected histamine doses mediated by \( H_2 \), NK1 and NMDA receptors (Sakurada et al., 2002, Watanabe et al., 2008, Mizoguchi et al., 2011). Recently, the expression of the \( H_4 \) receptor in the spinal cord has been shown but its functional role has not yet been clarified (Strakhova et al., 2009). While histamine has been a well-known mediator of pruritus for over 100 years, the understanding of itch signalling via histamine receptors is far from being completely understood.

### 6.3.2. Role of Histamine in Scratching Behaviour in Murine Allergic Dermatitis Models

Pruritus is a major symptom of allergic skin diseases like atopic dermatitis but often difficult to control. There is little information on the role of histamine in scratching behaviour and sensory transmission of atopic dermatitis and chronic eczema. Fukamachi et al. (2011) showed an increased expression of semaphorin 3A in normal human epidermal keratinocytes by histamine. Semaphorin 3A plays an inhibitory role for C-fiber elongation in upper layer of epidermis and decreased expression of semaphorin 3A has been found in lesional skin of AD (Fukamachi et al., 2011). In murine keratinocytes, the expression of semaphorin 3A mRNA is reduced by incubation with histamine and this reduction can be reversed by an \( H_1 \) receptor antagonist (olopatadine) but not by an \( H_4 \) receptor antagonist (JNJ7777120) (Ohsawa and Hirasawa, 2012). The efficacy of classical \( H_1 \) receptor antihistamines under clinical conditions is limited to a few diseases (like urticaria and insect bite reactions) and chronic pruritus as seen in atopic dermatitis patients does not respond to \( H_1 \) receptor blockade (Akdis et al., 2006b, Zuberbier et al., 2009). The discovery of the \( H_4 \) receptor has rekindled the interest in histamine receptors as antipruritic targets. The \( H_4 \) receptor seems to be a promising target for the treatment of pruritic skin diseases; \( H_4 \) receptor antagonists showed similar or even superior effects compared to traditional \( H_1 \) receptor antihistamines in the attenuation of experimental pruritus (Dunford et al., 2007, Rossbach et al., 2009b, Cowden et al., 2010). In two mouse models of allergen-mediated pruritus induced by repetitive administration of strong sensitizers (2,4-dinitrochlorobenzene or toluene-2,4-diisocyanate, respectively), a combination of the \( H_1 \) receptor antagonist cetirizine and the \( H_4 \) receptor antagonist JNJ7777120 led to a reduction of scratching bouts to up to 90%. According to Dunford et al. 2007, the antiprurinergic potential of the \( H_4 \) receptor antagonist JNJ7777120 because of any sedative properties can be excluded,
even though JNJ7777120 crosses the blood brain barrier. Moreover, in a mouse model of chronic dermatitis induced in NC/Nga mice by repeated challenge with the hapten picryl chloride, the combination of the H₄ receptor antagonist olopatadine and JNJ7777120 showed an antipruritic efficacy as potent as that of prednisolone (Ohsawa & Hirasawa, 2012). Thus, a combination of H₄ and H₁ receptor antagonism might be a new strategy to treat pruritus related to allergic diseases like atopic dermatitis. Since the H₃ receptor serves as an inhibitory receptor which possibly increases the threshold for histamine-induced itch, the addition of an H₃ receptor agonist might be even more beneficial. To date, there is only one study that analysed the involvement of H₃ receptor in allergen-induced pruritus, but this study revealed that the H₃ receptor inverse agonist pitolisant had no further effect on allergen-induced itch in a murine model of allergic contact dermatitis (Rossbach et al., 2011).

6.4. Summary

Taken together the aforementioned studies provide evidence for an immunomodulatory role of the H₄ receptor in allergic inflammatory skin diseases and pruritus. However, not all results confirm a pro-inflammatory role for H₄ receptor. In particular the modulation of cytokine secretion in antigen presenting cells favour an anti-inflammatory role mediated by the H₄ receptor. Nevertheless, pro-inflammatory outcomes in mouse models of allergic dermatitis treated with H₄ receptor antagonists or performed with H₄ receptor knockout-mice have not yet been reported. In most settings, a clear anti-inflammatory effect has been demonstrated by blocking the H₄ receptor. Also as far as pruritus is concerned, a blockade of H₄ receptor might be a new option for the treatment of pruritus associated with allergic skin diseases.
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