Eotaxins and CCR3 Receptor in Inflammatory and Allergic Skin Diseases: Therapeutical Implications

Paolo Amerio*1, Alessandra Frezzolini1, Claudio Feliciani2, Roberto Verdolini3, Patrizia Teofoli1, Ornella De Pità1 and Pietro Puddu1

1Department of Immunodermatology and Allergology, Istituto Dermopatico dell’ Immacolata - IRCCS, Rome, Italy, 2 Department of Dermatology, University of Chieti, Chieti, Italy, 3Department of Dermatology, The Princess Alexandra Hospital, Harlow, Essex, UK

Abstract: Cell migration is mediated by a group of chemotactic cytokines called chemokines: low molecular weight molecules that have been shown as important leukocyte chemical attractants to sites of inflammation and infection.

Eotaxin-1, also called CCL11, was first described in 1994, as a highly specific eosinophils chemokine. Many cell types including lymphocytes, macrophages, bronchial smooth muscle cells, endothelial cells and eosinophils, are able to produce this chemokine, predominantly after cytokine stimulation, however little is known about its expression in human skin in vivo.

Eotaxin-1 also regulates the chemotaxis and, in some conditions, activation of basophils, mast cells and T lymphocytes.

Chemokine receptors are named from their ligand families, thus the CC chemokine eotaxin-1 binds to the CCR3 receptor which is expressed on eosinophils, mast cells, Th2 type lymphocytes and even on keratinocytes.

It seems that eotaxin-1 is one of the most important cytokines involved in tissue inflammation playing a central role in the pathogenesis of allergic airway diseases (asthma and rhinitis), in inflammatory bowel disease and gastrointestinal allergic hypersensitivity and recently it has been proposed as a therapeutical target for these conditions.

Our group has studied the role of eotaxin-1 in the pathogenesis of two skin conditions: dermatitis herpetiformis and AIDS-associated eosinophilic folliculitis, demonstrating that this chemokine, together with Th2 type cytokines (IL-13 and IL-4) is important in cell recruitment, inflammation and tissue damage; moreover eotaxin has proven to play an important role in other skin conditions such as, bullous pemphigoid, pemphigoid gestationis, atopic dermatitis and allergic drug reactions

Recent advances in the understanding of eotaxin-1-mediated mechanisms of chemotaxis in allergic and inflammatory conditions may predict that therapeutic antagonism is achievable.

This paper will focus on the role that eotaxin and its receptor play in the pathogenetical mechanism in a number of dermatologic diseases, some of which, like atopic dermatitis, may benefit from the introduction of novel and more selective therapeutic options.

INTRODUCTION

Chemokines: Chemiotactic Cytokines

The skin is one of the interfaces through which the body interacts with the environment. For a long time skin has been considered just a passive barrier; in 1970, however, the discovery of T lymphocyte homing in the skin [1] gave the first clue that some interactions between skin and the immune system occur. In 1978 Streilein first recognized that the skin is a specialized organ from the immunological point of view [2]; today it is generally accepted that the skin possesses a specific immunological environment [3].

Almost all the cells, either resident and transient in the skin, (keratinocytes, fibroblasts, endothelial cells, lymphocytes, leukocytes, mast cells etc.) are components of the complex skin immunological network. This network acts through a variety of mediators responding to the various exogenous insults both with cutaneous inflammation (innate immunity) and recruitment of specialized and primed cells (acquired immunity), but also it mediates autoimmune processes.

A fundamental part of the mechanism of skin immune response is represented by cell migration, a multistep process characterized by adhesion molecules interactions allowing the rolling and arrest of leukocytes on the vessel
chemokines in CL, CCL, CXCL and CX
subfamily and adds an L for ligand, thus dividing proposed nomenclature gives every chemokine the name of neutrophil specific chemokines [10]. The divided in two different groups of neutrophil and non-
"CXC family" (CXCL). The prototype of this class of aminoacids isolated in a guinea pig model of airway tissue growth and differentiation [6,7]. Chemokines have been classified on the basis of four cystein residues position present in the N-terminal [8].Utilizing this method, four subclasses (α,β,γ and δ) have been described. A newly proposed nomenclature gives every chemokine the name of the subfamily and adds an L for ligand, thus dividing chemokines in CL, CCL, CXCL and CX3CL, or an R for receptors adding a number to identify the molecule [9].

In the α-chemokines the cysteine residues are separated by a single aminoacid thus this family is also known as the “CXC family” (CXCL). The prototype of this class of chemokine is Interleukin-8 (IL-8). This family is further divided in two different groups of neutrophil and non-neutrophil specific chemokines [10]. The β-chemokines have the first two cysteine residues adjacent to each other (CC family). The remaining two families are less defined and include: the γ-chemokine or “C chemokines” family, that has only one cysteine residue near the N-terminal (C), this family is composed by lymphotactin and SCM1β. The δ-
chemokines or CX3C family is characterized by the fact that the two cysteine residues are divided by three aminoacids whose unique component is fraktaline. A complete list of chemokines is available, together with other cytokines, on the following web site http://cytokine.medic.kumamoto-u.ac.jp/.

Table I. The CC chemokine family is a complex and large group of molecules, located mostly on chromosome 17, that comprises 28 ligands binding to 11 receptors. Its members are implicated, mainly, in mediating innate and adaptive immune responses acting through dendritic cells, basophils, mast cells, lymphocytes and eosinophils.

This review will focus on three members of CC chemokines, at first thought to be highly selective for eosinophils, so much so that they have been given the name “Eotaxins”. These molecules, which have been shown to play a pivotal role in allergic and inflammatory diseases, are also involved in a variety of skin physiological and pathological processes and may represent important targets for more selective immunomodulating therapies.

THE EOTAXIN FAMILY

Eotaxin-1 (CCL-11)

Eotaxin-1, also called CCL11, is a protein of 73 aminoacids isolated in a guinea pig model of airway inflammation [11] as an eosinophil-specific attractant. Subsequently it was cloned and sequenced first in the mouse [12] and then in man [13,14]. The sequence omology and the genomic localization adjacent to other molecules in the cromosome 17 chemokine cluster [15] characterized eotaxin-1 as a member of the CC family.

Many cell types have been found to produce eotaxin-1, including: eosinophils [16], macrophages [13], lymphocytes [17], endothelial cells and epithelial cells from gut and respiratory tract [18,19], chondrocytes, smooth muscle cells [20-23], fibroblasts[16,24] and even keratinocytes [25]. Eotaxin-1 could be constitutively expressed in skin and in many other tissues but its production is strongly induced by interleukin-4 (IL-4) [26], interleukin-13 (IL-13) [27] or tumor necrosis factor-α (TNF-α) [16,20], through the activation of the transcriptional factors NF-κB and STAT6 [28].

It was first thought, in fact, that eotaxin-1 could be a very selective and potent (with a chemotactic activity 100 times more powerful than released upon activation T cell expressed and secreted RANTES) chemokine for eosinophils [14], but it was later demonstrated that this molecule also exerted its function on many other cells involved in immunological reactions such as basophils [29], mast cells [31] and a subset of T lymphocytes with Th2 functional characteristics[30].

The activation and chemiotaxis of Th2 lymphocytes is a controversial argument. Some authors have suggested that eotaxin receptor CCR3 is expressed on a very small percentage of T cells in tissue (less than 3%) [32] and its finding on circulating cells has been debated; moreover, some recent experiments have demonstrated that CCR3 expression is induced after Interleukin-2 (IL-2) and IL-4 co-incubation [33]. These findings suggest that migration and chemiotaxis of lymphocytes during inflammation may finely be tuned by a collaboration between Th1 and Th2 cytokines.

Since eotaxin-1 production is upregulated by Th2 cytokines (IL-4 and IL-13) [34] and downregulated by cytokines such as Interleukin-10 (IL-10) and by Th1 cytokines such as Interferon-γ (IFN-γ) [24,35,36], it is generally accepted that the axis CCR3/eotaxin mediates a Th2 driven immunological response.

The exact mechanism of eotaxin-mediate recruitment of eosinophils into skin is not known. There are, however, experimental evidences that suggest that this chemokine, after induction by a Th2 or pro-inflammatory cytokine, may participate from the very beginning of the inflammatory response favoring the release of eosinophils from bone marrow [37]. In the tissues, instead, it seems that it is a gradient of eotaxin concentration that drives the extravasation of eosinophils [38]. Many adhesion molecules are involved in the process of transmigration of leukocytes from the blood flow to the tissues, through mechanisms of tethering/rolling (P-selectin and E-selectin) and of sticking/transmigration through Inter Cellular Adhesion Molecule (ICAM)-1 and Vascular cells Adhesion Molecule (VCAM)-1. Experiments have shown that pro-inflammatory cytokines such as IL-1 and TNF-α may induce the expression of...
### Table 1. Human CC Chemokine Family

<table>
<thead>
<tr>
<th>Systematic name</th>
<th>Ligand</th>
<th>Receptor (s)</th>
<th>Chemotactic activity</th>
</tr>
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<tbody>
<tr>
<td>CCL1</td>
<td>I-309</td>
<td>CCR8</td>
<td>Monocytes, T cells</td>
</tr>
<tr>
<td>CCL2</td>
<td>MCP-1, MCAF</td>
<td>CCR2</td>
<td>Monocytes, T cells, basophils, NK</td>
</tr>
<tr>
<td>CCL3</td>
<td>MIP-1α, LD78 αβγ</td>
<td>CCR1, CCR5</td>
<td>Monocytes, T cells, basophils, eosinophils, NK, dendritic cells, hematopoietic progenitors</td>
</tr>
<tr>
<td>CCL4</td>
<td>MIP-1β, LAG-1</td>
<td>CCR5</td>
<td>Monocytes, T cells, NK, dendritic cells</td>
</tr>
<tr>
<td>CCL5</td>
<td>RANTES</td>
<td>CCR1, 3, 5</td>
<td>T cells, basophils, eosinophils, NK, dendritic cells</td>
</tr>
<tr>
<td>(CCL6)</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>CCL7</td>
<td>MCP-3</td>
<td>CCR1, 2, 3</td>
<td>Monocytes, T cells, basophils, eosinophils, NK, dendritic cells</td>
</tr>
<tr>
<td>(CCL8)</td>
<td>MCP-2</td>
<td>CCR2, 3</td>
<td>Monocytes, T cells, basophils, eosinophils, NK</td>
</tr>
<tr>
<td>(CCL9)</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
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<tr>
<td>(CCL 10)</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>CCL11</td>
<td>Eotaxin-1</td>
<td>CCR3,5, 2</td>
<td>Eosinophils, T cells, mast cells, basophils</td>
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<td>CCL12</td>
<td>Unknown</td>
<td>CCR2</td>
<td></td>
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<tr>
<td>CCL13</td>
<td>MCP-4, NCC-1, CKβ10</td>
<td>CCR2, 3</td>
<td>Monocytes, T cells, eosinophils</td>
</tr>
<tr>
<td>CCL14</td>
<td>HCC-1, HCC-3, NCC-2</td>
<td>CCR1</td>
<td>Monocytes, hematopoietic progenitors</td>
</tr>
<tr>
<td>CCL15</td>
<td>HCC-2, MIP-1α, NCC-3, Lkn-1, MIP-5</td>
<td>CCR1, 3</td>
<td>Monocytes, T cells, eosinophils</td>
</tr>
<tr>
<td>CCL16</td>
<td>HCC-4, LEC, NCC-4, LMC</td>
<td>CCR1</td>
<td>T cells, neutrophils</td>
</tr>
<tr>
<td>CCL17</td>
<td>TARC</td>
<td>CCR4</td>
<td>T cells</td>
</tr>
<tr>
<td>CCL18</td>
<td>DC-CK1, PARC, AMAC-1, MIP-4</td>
<td>Unknown</td>
<td>Naive T cells</td>
</tr>
<tr>
<td>CCL19</td>
<td>MIP-3β, ELC, exodus-3</td>
<td>CCR7, 11</td>
<td>T cells, B cells, dendritic cells, activated NK</td>
</tr>
<tr>
<td>CCL20</td>
<td>MIP-3α, LARC, exodus-1</td>
<td>CCR6</td>
<td>T cells, B cells</td>
</tr>
<tr>
<td>CCL21</td>
<td>SLC, exodus-2, TCA4</td>
<td>CCR7, 11</td>
<td>T cells, B cells, dendritic cells, activated NK, macrophage progenitors</td>
</tr>
<tr>
<td>CCL22</td>
<td>MDC</td>
<td>CCR4</td>
<td>T cells, eosinophils</td>
</tr>
<tr>
<td>CCL23</td>
<td>MIP-3, MPIF-1</td>
<td>CCR1</td>
<td>Dendritic cells, osteoclasts</td>
</tr>
<tr>
<td>CCL24</td>
<td>MPIF-2, eotaxin-2</td>
<td>CCR3</td>
<td>Eosinophils, basophils, Th2 cells,</td>
</tr>
<tr>
<td>CCL25</td>
<td>TECK</td>
<td>CCR9, 11</td>
<td>Memory T cells, B cells, immature thymocytes</td>
</tr>
<tr>
<td>CCL26</td>
<td>Eotaxin-3, MIP-4α</td>
<td>CCR3</td>
<td>Eosinophils, T cells</td>
</tr>
<tr>
<td>CCL27</td>
<td>CTACK, skinkine</td>
<td>CCR10</td>
<td>CLA+ T cells</td>
</tr>
<tr>
<td>CCL28</td>
<td>MEC</td>
<td>CCR10</td>
<td>T cells</td>
</tr>
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Adhesion molecules on the endothelial cells in the inflammation sites slowing the circulating leukocytes by enhancing their adhesiveness to the vessel walls. In addition to pro-inflammatory cytokines it has been shown that eotaxin-1 itself may mediate tethering of eosinophils by modulating P-selectine expression [39] and then control transmigration through the upregulation of ICAM-1 and VCAM-1 on the endothelium[40,41] and of macrophage-1 antigen (Mac-1) and very late antigen-4 (VLA-4) [42] on eosinophils facilitating Mac-1/ICAM-1 and VLA-4/VCAM-1 [43] interactions.

Once the eosinophils are in the tissue, eotaxin-1 continues its activity directly participating in the inflammatory reaction by 1) enhancing the production by eosinophils of reactive oxygen species that are involved in tissue damage [44], 2) inducing the release of leukotrienes and histamine from basophils [29], 3) supporting the migration of mast cells and inducing the in situ differentiation of mast cell progenitors [45].

This last point seems to be very important because it has been demonstrated, in animal models, that eosinophil...
recruitment is dependent on the presence of resident mast cells [46] with a mechanism that has still to be determined. In vitro experiments have tried to elucidate such a mechanism showing that mast cells enhance eotaxin production in mast cells-fibroblasts co-culture [47], and that mast cells cytokine production (Interleukin-5 IL-5 and Granulocytes monocytes- colony stimulating factor GM-CSF) increases eosinophils survival and activation, suggesting that these cells may play a central role in eotaxin-mediated eosinophil immunological reactions also in humans [48].

As we have previously pointed out, other members of the chemokine family, constitutively expressed in the tissues, have a role in the tissue homeostasis [6,49]. It is, therefore, not surpring that eotaxin-1 has shown, in vitro, to represent a weak stimulus for keratinocytes growth [50] and for angiogenesis by endothelial cells [51]; the biological relevance of these actions in vivo is not known, nevertheless these findings mean that eotaxins may contribute significantly to skin physiology.

Other two components of the eotaxin-1 chemokine family, Eotaxin-2 and Eotaxin-3, have been characterized in recent years through genetic cloning of sequences adjacent to chemokines gene clusters.

**Eotaxin-2 (CCL24)**

Eotaxin-2, also called CCL24 or MPIF-2 (myeloid progenitor inhibitory factor 2) was cloned and characterized for the first time in 1997 [52,53] and located on chromosome 7q11.23 [54] one year later. Although it shows only a 39 % homology in the aminoacid composition with eotaxin this chemokine was named eotaxin-2 since it possesses incredibly similar effects to eotaxin-1 on human eosinophils and basophils [52]. It is constitutively produced by fibroblasts and many other cells have been shown to express it: epithelial cells, macrophages and airway epithelial cells [55-57], prevalently after stimulation with IL-4 and/or TNF-α. Some studies, however, demonstrated that IL-4 stimulus is less efficacious in inducing the expression of eotaxin-2 than in inducing eotaxin-1 or eotaxin-3 [57]. Eotaxin-2 exerts its activity on eosinophils [58], resting T cells [53] and basophils [59], in some conditions with a greater efficacy with respect to other CC chemokines, such as RANTES and monocyte chemiotactic protein-4 (MCP-4) [57], that also attract these cells.

Eotaxin-2 does not mimic the effects of eotaxin-1, but it seems to have its own role in immunological reactions. In vivo and in vitro experiments have demonstrated that there is some sort of temporal sequence in the expression of the various components of the eotaxin family in the skin during immune responses. Specifically eotaxin-1 seems to act early in cutaneous allergic reactions ( by mediating the chemotaxis of eosinophils which appear within 6 hours of the allergen challenge) while eotaxin-2 drives a late eosinophil influx almost 24 hour after the initial challenge Fig. (1).

The similarity of eotaxin-2 with eotaxin-1 is maintained in the molecular mechanism of eosinophils recruitment. Part of the eotaxin-2 chemotactic activity is, as for eotaxin-1, exerted through the modulation of adhesion molecules; this chemokine, in fact, stimulates eosinophils to detach from endothelial cells, through the down regulation of VCAM-1, thereby promoting their migration into the tissue [61]. Moreover there is experimental evidence that eotaxin-2, like eotaxin-1, participates directly in tissue inflammation, through the release of reactive oxygen species [62] and the induction of histamine and LTC-4 degranulation in basophils. All these effects are mediated through the binding to the CCR3 receptor.

**Eotaxin-3 (CCL26)**

Whilst scanning the DNA strand near the genetic locus for eotaxin-2, researchers in 1999 cloned [63] a novel CC chemokine named eotaxin-3 because its chromosomic locus

<table>
<thead>
<tr>
<th>Table 2. The Eotaxin Family</th>
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<tbody>
<tr>
<td><strong>Eotaxin type</strong></td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>Eotaxin-1 (CCL11)</td>
</tr>
<tr>
<td>Eotaxin-2 (MPIF-2) (CCL24)</td>
</tr>
<tr>
<td>Eotaxin-3 (CCL26)</td>
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</table>
Pro-inflammatory and Th2 cytokines, probably released by the mast cells, stimulate the production of eotaxin-1 in endothelial cells and dermal fibroblasts. Eotaxin-1 acts through 1) the promotion of the mobilization (together with IL-5) of the eosinophils from the bone marrow, 2) by mediating, the activation and differentiation of mast cells and infiltration of the tissue by eosinophils, basophils and T lymphocytes of the Th2 subset. Later in a Th2 cytokines rich environment the fibroblasts and endothelial cells express eotaxin-2 that continues the recruitment of eosinophils and other inflammatory cells in the tissue perpetuating the response.

and functions are strictly related to eotaxin-2. This chemokine expression was first found in human heart and ovarian tissue by northern blot, later on its expression was more precisely located in dermal fibroblast [64] and endothelial cells [65]. Eotaxin-3 exerts its chemiotactic activity on eosinophils and basophils [66]. Its production is mediated by Th2 cytokine type such as IL-4 and IL-13, through the activation of the transcription factor STAT6. Surprisingly TNF-α, a strong inducer of eotaxin-1 in dermal fibroblasts, has no effect on this cell type in eotaxin-3 induction.

Although little is known about the biological action and relevance of eotaxin-3, it is interesting to note that it has a very weak binding to the eotaxin receptor CCR3, compared to that of eotaxin-1, the difference is about 10-fold less [63].

The evidence that TNF-α does not induce eotaxin-3 and that this cytokine is strongly induced by Th2 cytokines has led to the hypothesis previously described for eotaxin-2, that eotaxin-3 may act in a time sequence with eotaxin-1, in response to a high levels of IL-4 and IL-13 [64]. However, unlike eotaxin-2, eotaxin-3 is less potent in inducing the release of ROS by eosinophils and thus may play a minor role in the tissue damage.

**EOTAXIN RECEPTORS**

Chemokines mediate cell migration and activation by binding to specific trans-membrane helix G protein-coupled cell surface receptors [67,68].

It was initially thought that eotaxin-1, eotaxin-2 and eotaxin-3 could only bind to CCR3. This fact was somehow unusual since many chemokines act on multiple receptors, for example RANTES, monocyte chemiotactic protein-3 (MCP-3) and MCP-4 (all CCL chemokines) bind to CCR3 but they can interact also with other receptors such as CCR1, CCR5 and CCR2 [69].

More indepth studies over the last two years have demonstrated that eotaxin also interacts with CCR2 [71], CCR5 [71] and CXCR3 [72].

Eotaxin-1 binds to CCR2 with a 5 fold lower affinity than to CCR3, this finding suggests that it may act, in vivo, as a partial antagonist counteracting CCR2-mediated monocyte chemiotactic protein-1 (MCP-1) cell recruitment. Moreover it has been hypothesized that eotaxin-1 antagonist effect on this receptor may induce Th2 responses, since
CCR2 is generally involved in Th1 type immunological reactions [73].

The functional significance of eotaxin-1/CCR5 binding is still not known and, moreover, its interaction is still controversial especially since the two different research teams has published contradicting results [70,71].

CCR3 has an high sequence homology with CCR1 and shares with this receptor many ligands such as RANTES, MCP-3 and macrophage inflammatory protein-18 (MIP-18). Other CC chemokines (monocyte chemiotactic protein-2 or MCP-2, MCP4) are also CCR3 ligands but eotaxin-1, eotaxin-2 and eotaxin-3 have the greatest affinity and the strongest activation proprieties [62].

CCR3 was initially clones from monocytes [74], eosinophils [75] and was then found to be expressed also on basophils [29], mast cells [32], a small number of peripheral memory T cells [76] as well as in the cells involved in Th2 cytokine driven inflammation such as Th2 lymphocytes [31]. The proof of the later has recently been refuted with the suggestions that this receptor may be invariably expressed on both Th2 anf Th1 lymphocytes [32,77].Serious studies have demonstrated that although this chemokine receptor is not present on circulating T cells, its expression could be induced on lymphocytes after IL-2 and IL-4 stimulation [33].

CCR3 also mediate other mechanisms different from cell chemiotaxis. It has been proven to act as a co-receptor for human immunodeficiency virus-1 (HIV-1) cell entry [78], with a mechanism similar to CCR5 [79] which is a more efficient HIV-1 coreceptor involving a two step binding.

Other chemokine receptors show a broad distribution in non-hematopoietic cells, CXCR receptors have, for example, been identified on endothelial cells and fibroblasts [80], so it was not surprising that CCR3 was also found on keratinocytes [50,81] and on endothelial cells [82]. The functional and physiological role of CCR3 on non-circulating cells is not totally clear. It could be that its presence on keratinocytes is necessary for skin inflammation modulation. On the other hand recent papers provide convincing evidence that CCR3 expression on endothelial cells and keratinocytes permits eotaxin-1 to directly mediate neoangiogenesis [83] and keratinocytes growth and differentiation [50] in the absence of eosinophilic infiltrate.

EOTAXINS AND SKIN DISEASES

There is a large body of evidence on the role of chemokines and their receptors in a variety of disease processes. The role of eotaxins as a major eosinophil-chemotactic factor, its role in allergic conditions (such as asthma and rhinitis) and other inflammatory disorders characterized by eosinophils accumulation (inflammatory bowel disease, eosinophilic gastroenteritis) has been well documented [84]. Recently, the demonstration of human dermal fibroblasts as a major source of biologically active eotaxins and the identification of eotaxins receptor CCR3 also on epidermal keratinocytes indicates the involvement of this chemokine in skin physiology and pathophysiology [85,57,50]. Studies directed at identifying a role for eotaxin in skin inflammation have focused expesially on atopic dermatitis, autoimmune bullous diseases and other skin conditions (AIDS-EF, Cutaneous drug reactions)

Atopic Dermatitis

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease mainly affecting flexural areas, with onset most commonly during infancy or childhood. It is a common disorder affecting about 1 to 2 % of the population [86]. Its distinctive skin lesion is an erythematous vesiculo-papular rash which gradually evolves into a lichenified chronic dermatitis. Histology shows an infiltration of activated T cells, predominantly CD4+ memory T cells, monocytes/macrophages and some eosinphils which though not present in elevated number, release high amounts of major basic protein [87]. Mast cells and basophils are also found in low number in different stages of degranulation.

The pathogenesis of this condition is still unclear, however the systemic activation of Th2 cells leading to an exaggerated cutaneous immune response to environmental antigens seems to be at the basis of atopic dermatitis [88]. Generally peripheral blood samples of atopic patients reveal a high number of T cells producing IL-4 and IL-13, a reduced number of T lymphocytes producing IFN-γ [89,90]. However both type 1 (IL-2,IFN-γ, IL-12) and type 2 (IL-4,IL-5, IL-13) interleukins levels have been found altered in skin samples of different clinical stages of atopic dermatitis [91].

Since Th2 cytokines such as IL-4 and IL-13 are eotaxin-1 major inducers it is likely that these and the other proinflammatory cytokines such as TNF-α may contribute, through eotaxin-1, to eosinophil infiltration in this disease.

It seems that in the initial phase, allergen specific T cells in AD patients preferentially express Th2-type interleukins; these molecules (particularly IL-4, IL-5 and IL-13) promote the growth and activation of eosinophils and eosinophilia which is a condition related to disease activity in AD patients. In AD eosinphils are important effector cells that promote inflammation and tissue damage [87]. Tissue eosinophilia has been correlated with eotaxin-1 and CCR-3 expression both at mRNA and protein level [92] and a higher blood eosinophil count has been found in atopic subjects with a marked eotaxin-1 skin expression. Some authors showed that eotaxin-1, together with other CC chemokines such as MCP-1 and RANTES, are elevated in plasma of atopic patients [93] without, however, any correlation to blood eosinophilia or to disease severity [94].

The relationship between eosinophilic inflammation and chemokines in AD was best elucidated by Taha et al.[38]. They confirmed that eotaxin-1 mRNA expression was increased in chronic and acute AD subepithelium skin samples demonstrating that its expression was significantly correlated with the number of epidermal eosinophils but not with other mononuclear inflammatory cells. This finding
suggested that there may be a gradient of eosinophilic-specific chemokines (i.e. eotaxin-1 or MCP-4) that favours the infiltration of eosinophils in the tissue.

Moreover there is experimental evidence that CCR3 expression is elevated also in non lesional skin of atopic subjects [92], thus suggesting that eotaxin-1 and other CCR3 binding chemokines may be involved in the very initial steps of AD inflammation mechanism.

Th2 cytokines not only contribute to tissue eosinophilia through the induction of eotaxin-1 [26], but also upregulate the expression of VCAM-1 on endothelial cells facilitating the chemiotactic process. Thus the pathway leading to the accumulation of eosinophils appears to be mediated by eotaxin-1 in a sort of short feed back amplification system.

Also the expression of CCR3 on keratinocytes [50] may suggest a role of eotaxin-1 not only in the triggering and maintenance of inflammation, but also in the epidermal changes typical of chronic AD.

AD treatment may sometimes be frustrating, the mainstay of therapy are generally immunosuppressive agents. However, the immunosuppressants currently available are not selective therefore the potential importance of eotaxin-1 in all phases of AD pathogenesis makes this molecule or its receptor CCR3 an optimal candidate for selective immunotherapy.

**Bullous Diseases**

Autoimmune bullous diseases of the skin are a wide group of skin disorders in which blistering may occur through the deposition of autoantibodies against desmosomes and hemidesmosomes which are skin cohesion structures. Classification of autoimmune blistering diseases depends on autoantibody deposition at different anatomic levels: between epithelial cells in pemphigus, at the basement membrane in both bullous pemphigoid and herpes gestationis or in the dermal papille in dermatitis herpetiformis.

Beside autoantibody deposition, also trafficking and activation of circulating leukocytes migrating into the sites of inflammation could be crucial events in the pathogenesis of autoimmune blistering disease. The histological picture of three autoimmune blistering diseases (namely dermatitis herpetiformis, bullous pemphigoid and pemphigoid gestationis), characterized by the presence of a tissue infiltrate mainly composed of lymphocytes, eosinophils and other leukocytes, and other experimental data support this hypothesis [95,96].

**Dermatitis Herpetiformis (DH)**

DH is a lifelong blistering skin disease with pathogenomic immunoglobulin (Ig)A deposits in the papillary dermis. It was the first autoimmune bullous disease to be directly associated with the presence of eotaxin-1 by our group. The clinical features of this disease consist mainly of urticarial plaques and blisters on the elbows, buttocks, and knees, although other sites may also be involved [97]. CD4+ T lymphocytes skin infiltration together with a varying number of neutrophils and eosinophils, is thought to be important in the pathogenesis of blister formation during DH, through the production and release of cytokines and inflammatory mediators. It has been demonstrated that T-cell lines from DH patients were predominantly CD4+, with a greater proportion of IL4 producing cells suggesting that a Th2 immunological response operates in this disease [98].

We detected eotaxin-1 immunoreactivity in DH active skin lesions, mainly in the dermis and in microabscesses of the papillae, by lympho-monocytic infiltrate and also by epidermal Langerhans cells [99]. Fig. (2c). Furthermore eotaxin-1 expression was strictly related to the presence of dermal mast cells and to the expression of IL-13, a Th2 cytokine, and TNF-α, both of which are potent inducers of eotaxin-1. Thus we have added new insight into the pathogenesis of DH, suggesting that in a Th-2 cytokine rich microenvironment, TNF-α may induce eotaxin-1 production supporting both eosinophil recruitment and persistence in damaged tissue. Moreover the correlation of eotaxin-1 expression and mast cell localization demonstrated in our study, supports the hypothesis, as already shown in live animal models, that this cell type is very important for eosinophil recruitment [46].

**Bullous Pemphigoid (BP)**

BP is an acquired sub-epidermal blistering disease of the elderly characterized by tense blisters resulting from the dermo-epidermal separation following the deposition of immunoglobulins and complement along the Basement Membrane Zone (BMZ) [100].

Early cutaneous infiltration of activated CD4+ Th2 cells and eosinophils seems to be a crucial event in the development of bullous lesions; several cytokines and mediators released from these cells have largely been detected in the skin, the blood and blister fluids of BP patients and have been related to disease activity and cutaneous involvement [101-103].

There is experimental evidence that the T cells population isolated from in skin blisters and in BP peripheral blood produce IL-4 and IL-13, confirming that skin-infiltrating lymphocytes in BP belong to the Th2 subset [104]. In the light of this, chemokines and their receptors were presumed to have a role in the multi-step process of leukocyte extravasation in BP inflamed skin. Eotaxin-1 production in this disease was first reported by Wakugama M et al. [105]. These researchers demonstrated eotaxin-1 expression in the epidermal keratinocytes of lesional skin; eotaxin-1 levels in BP blister fluid were significantly related to the number of dermal infiltrating eosinophils, thus explaining the role of this chemokine in eosinophil skin migration during BP. Immunoreactivity for eotaxin-1 was further observed by Shrikhande M. et al. in the mononuclear cell infiltrate seen in BP acute skin lesions, indicating that macrophages and T-cells are also able
to produce eotaxin-1 [106]. High levels of eotaxin-1 and IL-5 were also found in blister fluids and the acute phase sera of BP patients and were parallel to the increased number of eosinophils in the blood and the skin, proving that cooperation between eotaxin-1 and IL-5 plays a central part in eosinophil migration and activation during BP. In a recent study our group reported that eotaxin-1 expression is predominantly associated with the infiltration of eosinophils and T lymphocytes, and also documented the presence of CCR3 on CD3+ T cells in skin active lesions in support to the concept that Th2 cells are involved in the pathogenesis of BP [107]. Fig. (2a), (2b). We confirmed that BP blister fluids contain significantly higher amounts of eotaxin-1 with respect to suction blister fluids from healthy individuals, indicating a substantial production of eotaxin-1 at sites of tissue damage. Thus we suggest a further mechanism in the pathogenesis of BP: that from the early stages of the disease, eotaxin-1 is responsible for the chemotaxis of both eosinophils and Th2 cells that, through local production and release of cytokines (such as IL-4, IL-5) and eotaxin-1 itself, are able to maintain and amplify immunological processes underlying blister formation.

**Herpes Gestationis (HG)**

HG is a pregnancy associated autoimmune blistering disease belonging to the pemphigoid group of diseases. It occurs in the last trimester of pregnancy as a result of the interaction of circulating autoantibodies with the hemidesmosomal protein BP180. The histological picture of HG is characterized by the infiltration of lymphocytes and eosinophils. Some members of our group (Teofoli P. and Amerio P. personal communication) have evaluated the presence of eotaxin-1 and of CCR3 and CCR5 in this disease. It was found that eotaxin-1 was strongly expressed in skin cellular infiltrates of HG Fig. (2d). Moreover we were able to demonstrate a higher expression of CCR3 and CCR5 among the lymphocytic infiltrate and in particular, CCR3 seemed to co-localize with eotaxin-1 in eosinophils and CD4+ T cells.

**Drug-induced Cutaneous Reactions**

Skin disorders are the most common adverse reactions attributed to drugs. In spite of the great variability of their pathophysiological pathways, clinical signs of symptoms, severity, and eliciting drugs, it is now well accepted that skin infiltration of drug-specific cytokine-producing T cell clones is a common immunopathological feature [108]. In drug-induced skin eruptions there is a predominant CD8+ T cell activation that leads to more severe (bullous) skin symptoms or liver involvement, while a predominant activation of CD4+ lymphocytes may elicit mainly maculopapular reactions. A potential role of eotaxin-1 in inducing skin inflammation was recently investigated by Yawalkar N and the co-workers [109] in drug-induced maculopapular exanthems, histologically characterized by infiltration of T lymphocytes and, to a lesser extent, dendritic cells, macrophages, neutrophils and eosinophils. Parallel to blood and tissue eosinophilia, a significant enhancement of eotaxin-1 immunoreactivity was found in the mononuclear cells of the drug-induced exanthem skin samples, together

Fig. (2). Eotaxin-1 and CCR3 expression in skin diseases.

a-b) Bullous Pemphigoid: immunoreactivity for (a) CCR3 and (b) eotaxin in consecutive lesional skin sections, showing a similar pattern of distribution on inflammatory infiltrate, mostly in the upper and middle dermis of lesional site. (HRP two-steps amplified method, DAB chromogen substrate, hematoxylin counterstaining, X250); c) Dermatitis Herpetiformis: strong eotaxin expression in subepidermal microabscesses and in the perivascular lymphomonocytic infiltrate. (APAAP method, new fuchsin chromogen substrate, hematoxylin counterstaining, X250); d) Pemphigoid gestationis: Strong immunoreactivity for eotaxin in lymphomonocytic infiltrate wide spread throughout the upper and middle dermis. (HRP two-steps amplified method, DAB chromogen substrate, hematoxylin counterstaining, X400); e) Atopic Dermatitis: eotaxin immunoreactivity is demonstrated in both epidermis and dermis particularly in perivascular areas. (APAAP method, new fuchsin chromogen substrate, hematoxylin counterstaining, X250).
with the presence of IL-5, providing evidence that these factors may play a part in the pathogenesis of the disease by generating eosinophilic infiltration.

**Eotaxins and CCR3 Receptor in Inflammatory and Allergic Skin Diseases**

**AIDS-Associated Eosinophilic Folliculitis**

Our group also studied eotaxin-1 expression and immuno-localization in AIDS-Associated Eosinophilic Folliculitis (AIDS-EF) [110]. AIDS-EF is a pruritic dermatosis occurring in HIV infected individuals characterized by a chronic pustular-papular eruption on the face, scalp and upper trunk. The histological picture of this disease is predominated by a folliculocentric inflammatory infiltrate mainly composed of eosinophils and lymphocytes. Diagnosis is based on clinical findings, histological examination and negative cultures. Although little is known about AIDS-EF pathogenic mechanism we have demonstrated that there is a co-expression of Th2 cytokines such as IL-4 and IL-5 and the chemokines eotaxin-1 and RANTES [110]. We hypothesize that the immune disregulation in the advanced stages of AIDS (a shift towards a Th2 immunological response), may participate in the aberrant Th2 response to an unknown triggering agent [111]. There is a high expression of IL-4 in the skin of these subjects which could be a strong stimulus for the production of eotaxin-1 and other chemokines which act together with IL-5 in the selective recruitment of eosinophils around the follicles. The mechanism of eosinophil recruitment could be maintained even after the triggering agent is cleared, with the autocrine production of eotaxin-1 leading to the chronic perpetuation of the disease.

**Toxic Erythema Of The Newborn**

Toxic erythema of the newborn is a common, self-limiting neonatal dermatosis of unknown etiology affecting about 70% of healthy full-term newborns during the first days of life. Affected infants often have sterile papulo-pustules on the body surface (with the exception of the palms and soles), histology is characterized by a massive dermal infiltration of eosinophils [112]. A recent study of the immuno histological features of toxic erythema of the newborn, revealed the presence of numerous inflammatory cells identified as dendritic cells, eosinophils, neutrophils and macrophages in lesional skin [113]. In the same study the authors demonstrated that the accumulation and activation of immune cells in toxic erythema lesions is strictly related to the presence of a strong staining for IL-8 in the epidermal and dermal layers and of eotaxin-1 in the epidermis and in dermal infiltrate, suggesting that eotaxin-1 may be essential for the activation and recruitment of both eosinophils and dendritic cells in this condition.

**EOTAXINS AND CCR3 AS DRUG TARGETS**

The central role of eotaxins in the eosinophil and Th2 driven inflammatory and allergic diseases suggest that eotaxin receptor binding and the eotaxin molecule itself might be a promising drug target.

In fact early in vitro studies on CCR3 receptor blocking with monoclonal antibodies lead to the development of novel anti-inflammatory drugs which target eosinophils recruitment through receptor antagonism [114].

Unlike the current drugs which act on the cells after their migration into the inflammation site, these new drugs act upstream in the inflammation mechanism and inhibit the recruitment and activation of inflammatory cells before they enter the tissues. Until now drug development has concentrated on the interference of eosinophils recruitment in the treatment of asthma, it is conceivable that these novel drugs may also be applied to the treatment of other Th2 driven diseases [115].

The two main approaches developed are: eotaxin blockage with monoclonal antibodies and CCR3 signal transduction blocking through the development of receptor antagonists [116] Fig. (3).

Cambridge Antibody Technology group has announced a phase I/Ia clinical trials in humans with a human monoclonal antibody against eotaxin. Previously the phase I clinical trials had already proven the safety of this new drug and the developers believe it might be a good candidate for an efficacious treatment of allergic conditions.

Eotaxin receptor, CCR3, belongs to the class of seven transmembrane spanning receptors, which have proven to be the excellent targets for many diseases (anti-histamines, β-agonists and others). Interest in chemokines receptors as potential drug targets has increased in the last few years with the proof that many of these receptors (in particular CXCR4, CCR5 and CCR3) may inhibit HIV entry into cells.

The major types of CCR3 antagonists manufactured are chemically modified chemokines or small molecule antagonists.

The chemokines NH₂-terminal region of CCRs is the reason for their specific biological activity, therefore its modification profoundly alters the chemokine activity on leukocytes. The prototype of these structurally modified compounds is Met-RANTES. Met-RANTES is the result of RANTES expression by biologically engineered E. coli strains. This modified chemokine is CCR5 and CCR3 selective and can inhibit eosinophils chemotaxis in animal models [117]. With this same model the authors have shown that Met-RANTES was even more effective than monoclonal antibodies against eotaxin in inhibiting eosinophil recruitment because it acts on many receptors (CCR3, CCR5) resulting in a more complete action [118,119]. Another modified chemokine, AOP-RANTES [120], inhibits CCR3 mediated HIV-1 cells entry, moreover it down regulates the expression of CCR3 and activates the eosinophil respiratory burst [121,122] by acting as a partial agonist. More recently another protein antagonist for CCR3 has been developed. Met-chemokine beta 7 (Met-ckβ7) is a modified form of MCP-4, very specific for CCR3 and more
Two strategies are currently available: blocking the ligand with antibodies against eotaxin or interfering with eotaxin receptor with modified chemokines, small molecules specific antagonists for CCR3, and anti CCR3 antibodies (7B11).

Proteins, however, have some limitation as therapeutical agents: their development and production cost is high, their bioavailability is poor and their delivery route is limited since they cannot be administered orally. These disadvantages have increased the research for smaller non peptide molecules capable of blocking or antagonizing CCR3.

Many CCR3 receptor small non-peptide molecule antagonists have been developed [124-126]. The main features of these compounds are: the very high selectivity for CCR3 receptor and the capacity in in vitro models to inhibit the mechanism of eosinophils migration [125]; blocking important steps such as shape change [126] and chemokine dependent intracellular calcium increase [127].

Research on CCR3 inhibitors or antagonists is the most promising since it is unlikely that a single chemokine could be responsible for complex pathogenic mechanisms such as those occurring in allergic and autoimmune skin disease. The advantage of CCR3 antagonists is that they may block a combination of chemokines that act through the same receptor in sequence.

Research in this field will undoubtedly find novel therapies which could be applicable not only to the allergic diseases (where most research efforts are made), but also in all the other diseases where the eotaxins play a major role.

**ABBREVIATIONS**

- ICAM-1 = Inter cellular adhesion molecule-1
- VCAM-1 = Vascular adhesion molecule-1
- ROS = Reactive oxygen species
- RANTES = Regulated on activation normal T cell expressed and secreted
- VLA-4 = Very late antigen-4
- Mac-1 = Macrophage antigen-1
- kDa = Kilodalton
- IL-2 = Interleukin2
- IL-4 = Interleukin-4
- IL-5 = Interleukin-5
- IL-10 = Interleukin-10
- IL-13 = Interleukin-13
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