



Chemokines in allergic conjunctivitis

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Purpose of review

Chemokines are a large group of low molecular weight cytokines that attract and activate leukocytes throughout the body and therefore have a key role in the framework of late-phase allergic responses. The purpose of this article is to provide an overview of the main chemokines involved in allergic conjunctivitis, their primary functions and their physiological roles, and therapies targeted at chemokines and their receptors for ocular allergic diseases.

Recent findings

In recent years, there have been considerable advances in the understanding of ocular pathophysiology of ocular surface inflammatory diseases including both allergic eye diseases and dry eye syndrome. Several therapies being developed for dry eye inflammation are recognized as possible therapies for ocular allergic diseases as there are often common chemokines involved in both disease spectra.

Summary

Chemokines represent an integral part of the late-phase cascade of ocular allergic inflammation. A deep understanding of specific chemokines and their interactions will help in targeting therapies to effectively manage ocular clinical findings and symptoms of allergic eye disease.

Keywords

allergic conjunctivitis, allergic eye diseases, chemokines, late-phase response

INTRODUCTION

Allergic eye disease describes a category of disorders that adversely affect the entire ocular surface from the outer lids inward to include the conjunctiva, cornea, lacrimal glands, and tear film [1–4]. The ocular surface is responsive to multiple environmental, cosmetic and pharmacological antigens that may cause allergic eye diseases. Syndromes have been classified as a variety of ocular surface allergic eye diseases [2,5–7] including the most common which are seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC), as well as atopic keratoconjunctivitis (AKC), vernal keratoconjunctivitis (VKC), contact lens-associated acute allergic conjunctivitis and giant papillary conjunctivitis (GPC) [1,2]. SAC and PAC are considered a mild form of allergic conjunctivitis while VKC and AKC are regarded as severe diseases [1,2].

Many inflammatory agents are involved in ocular diseases. Of these, the role of chemokines as players of allergic inflammation is not well known and scarcely reviewed. This review will focus on the roles of chemokines in allergic eye disorders and will focus on CXCL8 (IL8), CCL11, eotaxins, chemokines and mast cells, T lymphocytes and keratocytes, the roles of chemokines in VKC, the chemokines

in tears, as well as therapies targeting chemokine activity.

CHEMOKINES

Allergic eye diseases are a result of reactions to antigens characterized by an incursion of eosinophils, Th2 T lymphocytes, basophils and mast cells to the invaded tissue, be it lung, skin or nasal mucosa [1,8–10]. Chemokines are potent, small peptide chemoattractants typically 8–10 kDa in size that moderate the migration and location of action of all immune cells, but specifically of leukocyte subpopulations, by binding to specific receptors on their cell surface [11,12,13[■]]. They usually function relatively locally rather than systemically. When originally discovered, chemokines were

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KEY POINTS

- Chemokines are small, potent, peptide chemoattractants that moderate the migration and activation of cells that participate in allergic inflammation, by binding to specific receptors thus playing a central role in the progression of the late-phase allergic response.
- Chemokines are present at low levels in healthy eyes in the tears, conjunctiva, limbal area and corneal keratocytes, and their levels in the tears increase during late-phase allergy response. Defining this tear composition may promote detection of tear mediators as possible disease markers.
- Drug research focus is in developing chemokine receptor targeted therapies through various avenues such as peptide nanoparticle inhibitors, amino terminus modification, peptide-based and nonpeptide-based antagonists and mAbs. Part of the challenge is to adequately individualize the antagonist drug to overcome the redundancy issue, as multiple chemokines can have multiple receptors.

divided into two groups based on functionality [1,11,12,13¹¹,14,15]. Elicited chemokines support the stem cells and navigate the passage of immature lymphocytes from the bone marrow to the thymus [4,8,11]. Inflammatory chemokines are the principal regulators of recruitment and activation of leukocytes during the late-phase of allergic responses [9–12,13¹¹,14,15] to the injured or infected sites to stimulate wound healing and pathogen eradication [1,11,12,13¹¹,14]. Classification has evolved to structural characteristics of which the majority of chemokines reside within two major classes categorized as the CXC chemokines where an amino acid separates the two cysteine residues, and the CC chemokines where the two amino cysteine residues are adjacent [11,12,13¹¹]. The different structure affects receptor activation. We now know of the existence of over forty such molecules in humans correlated with the inflammatory process, each categorized mainly into either the CC (receptors CCR1–10), or CXC (receptors CXR1–6), or C and CX3C (receptors XCR1 and CX3CR1). Each chemokine is accorded two names and many perform a number of functions (Table 1) [15]. All these molecules are mediated by seven transmembrane-crossing G-protein-coupled receptors [9–12,13¹¹,14]. The chemokine complex displays a type of indiscrimination, where some can stimulate more than one receptor and many of the receptors can be activated by more than one chemokine (Table 2) [1,11,12,13¹¹,14]. Chemokines participate in many additional processes other than simple chemoattraction [16–22], including hematopoiesis,

angiogenesis, respiratory burst, degranulation, phagocytosis, as well as growth regulation and development mechanisms [16–23]. Some chemokines are produced from only one type of cell while others have multiple sites of origin (Table 3) [16–22]. Advanced technologies can distinguish a wide range of bioactive trace proteins in tears. Chemokines, cytokines growth factors, angiogenic modulators, enzymes and inhibitors have all been identified in small tear samples using these techniques [24–26]. Even in healthy patients, significant amounts of cytokines are secreted in tears [24–26].

CHEMOKINES AND THE ALLERGIC RESPONSE

Under nonthreatening circumstances, the conjunctiva contains an extensive population of mast cells when compared with other tissues that are considered more prone to allergic hypersensitivity [24–26,69,70]. The ocular response begins with the introduction of an allergen to mast cells through the early phase, including the release of inflammatory mediators, chemokines and initiating the recruitment of inflammatory cells [71–74]. The late phase begins with an influx of inflammatory cells into the conjunctiva, a process which takes approximately 20–30 min [71–73]. Then follows the activation and expression of various proteins and chemoreactors of the mobilized leukocytes which eventually evolves to the chronic phase of allergic reaction (Fig. 1a) [71–74]. Conjunctival mast cells express tryptase, chymase, IL-4 and other cytokines [71–74]. IL-4 promotes T-cell growth, IgE production from B cells, promotes and mediates adhesion molecules and chemokine secretion and monitors Th2 lymphocyte differentiation [71–74]. Eosinophils are central players at different stages of the allergic cascade [18,75,76]. When activated, eosinophils release chemokines, cytokines and leukotrienes [18,77,78]. In addition, they discharge eosinophil major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil neurotoxin [71,72,74]. Many of these products are epitheliotoxic and can cause corneal complications such as corneal ulcers and infiltrates [79–81]. Expressions from corneal keratocytes, conjunctival fibroblasts and epithelial cells all contribute to inflammatory cell recruitment and initiation of the late phase reaction.

CXCL8

CXCL8, also known as IL-8 [22], plays an important role in a high number of processes including both primary and secondary ocular inflammation, allergy and angiogenesis and is present in many ocular

Table 1. Primary CC and CXC chemokines involved in allergic conjunctivitis, their names and their function

Chemokine class	Chemokine name	Other names	Function
CXC chemokines	CXCL1	GRO α	Neutrophil chemoattractant [27,81,87]
	CXCL2	GRO β	Neutrophil chemoattractant [27,87] Basophil chemotaxis and degranulation [81]
	CXCL3	GRO γ	Neutrophil trafficking [27,87] Induces chemotactic activity in neutrophils [81]
	CXCL4	PF4	Procoagulant [27,87] Strong chemoattractant for neutrophils and fibroblasts [81]
	CXCL5	ENA-78	Neutrophil chemoattractant [27,81,87] Granulocyte chemotactic protein [81]
	CXCL6	GCP-2	Neutrophil chemoattractant [27,81,87] Granulocyte chemotactic protein [81]
	CXCL7	NAP-2	Neutrophil trafficking [27,81,87] Potent chemoattractant and activator of neutrophils [81]
	CXCL8	IL-8	Neutrophil chemoattractant [27,81,87] Granulocyte chemotactic protein [79–81] Attracts neutrophils, basophils and T cells [81]
	CXCL9	Mig	Th1 immune response [27,81,87] T-cell chemoattractants [28,79]
	CXCL10	IP-10	Th1 immune response [27,81,87] T-cell chemoattractants [28] Chemoattractant for CD4+ T cells and inhibits early normal and leukemic hematopoietic progenitor proliferation [79–81]
	CXCL11	ITAC	Th1 immune response [27,81,87] Chemoattractant for interleukin-activated T cells [79–81]
	CXCL12	SDF-1 α , SDF-1 β	Myelopoiesis, B lymphopoiesis [27,81,87] haematopoietic progenitor cell (HPC), neutrophil homing to marrow [27,81,87] A strong chemoattractant for lymphocytes [79–81]
	CXCL13	BCA-1	B and T-cell trafficking [27,81,87] Promotes the migration of B lymphocytes [79–81]
	CXCL14	BRAX	Macrophage migration [27,87] Chemoattractant for monocytes can stimulate the migration of activated NK cells [80,81,87]
CC chemokines	CCL1	I-309 309A-3	Th2 response [27,81,87] and Treg trafficking [27,87] Attracts monocytes, NK cells, immature B cells and dendritic cells [80,81,87]
	CCL2	MCP-1	Chemoattractant for monocytes [28,79,80], T cells, eosinophils and basophils [28,79,80]
	CCL3	MIP-1 α	T cell and monocyte [27,87] Macrophage trafficking [27,81,87] Expressed primarily in T cells, B cells, and monocytes after antigen or mitogen stimulation [80,81,87]
	CCL4	MIP-1 β	Macrophage-NK migration [27,87] T-cell/dendritic cell interaction [27,81,87]
	CCL5	RANTES	Macrophage-NK migration and T-cell/dendritic cell interaction [80,81,87] Chemoattractant for monocytes, memory T-helper cells and eosinophils. Release of histamine from basophils and activates eosinophils [28,79,80]
	CCL6	C10	Chemoattractant that only expressed in rodents expressed in cells from neutrophil and macrophage lineages [27,87]
	CCL7	MCP-3	Th2 immune response [28,79,80] Activate monocytes [27,29,80,87] Basophils and eosinophils [28,79,80]
	CCL8	MCP-2	Th2 immune response [27,87]
	CCL9	MIP-1 γ	Mediates hepatobiliary excretion of numerous organic anions and may function as a cellular cisplatin transporter [27,29,80,87]

Table 1 (Continued)

Chemokine class	Chemokine name	Other names	Function
	CCL10	IP-10	Chemoattractant for CD4+ T cells and inhibits early normal and leukemic hematopoietic progenitor proliferation [27,29,80]
	CCL11	Eotaxin-1	Th2 immune response [27,87] Eosinophil infiltration [27,29,80], basophil migration [27,29,80]. Regulates the recruitment and activation of inflammatory leukocytes, particularly eosinophils [27,29,80]
	CCL12	MCP-5	Monocyte, eosinophil, basophil trafficking and degranulation [81,87] A strong chemoattractant for human monocytes [81,87]
	CCL13	MCP-4	Th2 response [81,87] Eosinophil chemoattractant. Induces chemotaxis of monocytes and T lymphocytes [27,81,87]
	CCL14	HCC-1	Produced as a precursor which is processed to generate a mature active protein exhibiting 46% amino acid homology to CCL3 and CCL4
	CCL15	HCC-2, MIP-1δ	Induces changes in intracellular calcium concentration in monocytes [27,81,87]. CCL15 is thought to act through the CCR1 receptor but also binds to CCR3 [27,81,87]
	CCL16	HCC-4	Dendritic cell maturation factor [27,81,87] Chemoattractant for lymphocytes [27,81,87]. Potent myelosuppressive activity and suppresses proliferation of myeloid progenitor cells [27,81,87]
	CCL17	TARC	Th2 immune response [27,80,81,87] Chemoattractant for T lymphocytes but not monocytes or granulocytes plays important roles in T-cell trafficking and activation of mature T cells [27,80,81,87]
	CCL18	PARC	Th2 response [87] Dendritic cell attraction of T, B cells and hematopoiesis [27,87] A chemoattractant for lymphocytes attracts naive T cells, involved in B-cell migration [27,87]
	CCL19	ELC	T-cell and dendritic cell homing [27,29,80,87] Constitutively expressed in various lymphoid tissues [27,81,87]. Attracts lymphocytes, dendritic cells, human T and hematopoietic progenitor cells [27,29,80]
	CCL20	MIG3α LARC	Th2 immune response [27,81,87] Th17 response [27,87] B and dendritic cell homing [81,87] Chemoattractant that attracts lymphocytes and neutrophils [81,87]
	CCL21	SLC	T-cell and dendritic cell homing to lymph node [27,29,80,87] Lymphocyte homing to secondary lymphoid organs, chemoattractant for thymocytes and activated T cells [81,87]
	CCL22	MDC	Th2 immune response [27,29,80,87] Chemoattractant for activated T lymphocytes, monocytes and dendritic cells trafficking of activated T lymphocytes [81,87]
	CCL23	MIP1-1	Chemoattractant for monocytes, resting T lymphocytes and neutrophils [87]
	CCL24	Eotaxin-2	Basophil migration [81,87] Eosinophil infiltration [27,29,80,87]
	CCL25	TECK	T-cell homing to gut, thymocyte migration [27,87] Chemoattractant for dendritic cells, thymocytes and activated macrophages [81,87]
	CCL26	Eotaxin-3	Th2 immune response [27] Eosinophil infiltration [29,80,87] Chemoattractant for eosinophils and basophils [27]
	CCL27	CTACK	Homing of T cells to skin [27,87] Attracts T lymphocytes. Homing of lymphocytes to cutaneous sites [27,81,87]
	CCL28	MEC	Homing of T cells to mucosal surface [27,87] Chemoattractant for resting CD4 or CD8 T cells and Eosinophils [27,87]

NK, natural killer.

Table 2. Chemokine receptors and their corresponding ligands

Chemokine class	Chemokine receptors	Ligands/Chemokines
CXC chemokines	CXCR1	CXCL6, CXCL8 [27,81,87]
	CXCR2	CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8 [27,81,87]
	CXCR3	CXCL4, CXCL9, CXCL10, CXCL11, CCL10
	CXCR4	CXCL12, CXCL14 [27,79–81,87]
	CXCR5	CXCL13 [27,79–81,87]
	CXCR6	CXCL16 [27,79–81,87]
	CX3CR1	CX3CL1 [27,79–81,87]
	CXCR3B	CXCL4 [27,79–81,87]
CC chemokines	CCR1	CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9, CCL10, CCL13, CCL14, CCL15, CCL16, CCL18, CCL23 [28,79,80]
	CCR2	CCL2, CCL6, CCL7, CCL8, CCL11, CCL12, CCL13, CCL16, CCL26, CCL27 [27,28,30,79–81]
	CCR3	CCL5, CCL6, CCL7, CCL8, CCL11, CCL13, CCL15, CCL24, CCL26, CCL27, CCL28, CXCL9, CXCL10, CXCL11 [27,28,30,79–81]
	CCR4	CCL5, CCL17, CCL22 [27,28,30,79–81]
	CCR5	CCL3, CCL4, CCL5, CCL8, CCL11, CCL16, CXCL11 [27,28,30,79–81]
	CCR6	CCL20 [28,79,80]
	CCR7	CCL19, CCL21 [28,79,80]
	CCR8	CCL1, CCL16, CCL18 [27,28,30,79–81]
	CCR9	CCL25 [28,79,80]
	CCR10	CCL27, CCL28 [27,28,30,79–81]
	CCR11	CCL8 [28,79,80]

conditions [22,82]. The severity of the resulting effect of CXCL8 activity depends on the source of the secretion as well as the site of action [22,83]. It can be detected in the conjunctiva, cornea, iris, retina and orbit [22] and has been found in significantly increased levels in tears of patients both with dry eye disease as well as in patients with ocular mucous membrane pemphigoid [83]. In ocular allergy CXCL8 is the most potent chemotactic factor for neutrophils, attracts subpopulations of T cells and upregulates neutrophil adhesion. This diversity of responses encourages development of anti-IL-8-targeted drugs targeting CXCL8 signal blocking in desired areas and tissues [22].

CCL11

Chemokines CCL11 (eotaxin-1), CCL5 (RANTES) and CCL7 (MCP-3) have been discovered to be imperative for tissue infiltration. Of the three, CCL11 has been acknowledged as the primary mediator as its ligand, CCR3, is a dominant receptor on eosinophils [12,13²²,84–87]. CCR3 reacts to chemotactic stimuli as well as to CCL5, CCL7, CCL8 (MCP-2), CCL11, CCL13 (MCP-4), CCL15 (leukotactin-1), CCL24 (eotaxin-2), CCL26 (eotaxin-3) and CCL28 (MEC) [12,13²²,84–87]. As a consequence of the

simultaneous activity, blocking a single chemokine often results in partial inhibition of eosinophil attraction [12,13²²,87]. One study demonstrated that in CCL11-deficient mice the eosinophilic response in their airway was partially inhibited but may have been compensated by other CCR3 ligand activity [88]. CCL11 is found in low quantities in tears of healthy individuals [70,89]. Patients with a known SAC have significantly higher levels of CCL11 than newly diagnosed or healthy patients [70,89–92]. CCL11 is central to the inflammatory cell invasion, propelling the late phase of allergy and therefore is not yet present in newly diagnosed SAC patients [70,89,91,92]. CCL11 has a critical role in ocular allergy, specifically regarding mast cell regulation, promoting mast cell differentiation but not degranulation [93]. Evidence of a correlation between the quantity of eosinophils or eosinophil-related chemokines and the severity of VKC indicates they have a direct role in the progression of this disease [16–18,94,95].

CHEMOKINES AND MAST CELLS

Mast cells moderate or exacerbate allergic disease. Mediators released by mast cells stimulate eosinophil degranulation as well as eosinophil adhesion to

Table 3. Chemokines and their physiological cells of origin

Chemokine class	Chemokine	Receptor activity	Elicited by
CXC chemokines	CXCL1	CXCR2	Fibroblasts, endothelial cells, activated monocytes, epithelial cells, macrophages, neutrophils and stromal cells [31,33]
	CXCL2	CXCR2	Neutrophils, macrophages, T lymphocytes [31,32], macrophages and stromal cells [33,34]
	CXCL3	CXCR2	Neutrophils, macrophages, epithelial cells and stromal cells [31,33]
	CXCL4	CXCR3	Platelets [35,36]
	CXCL5	CXCR2	Neutrophils and fibroblasts [31,37]
	CXCL6	CXCR1, CXCR2	Neutrophils and macrophages [31]
	CXCL7	CXCR2	Neutrophils, macrophages, monocytes and megakaryocyte [31,38]
	CXCL8	CXCR1, CXCR2	Neutrophils, macrophages, T cells, endothelial, epithelial, fibroblasts and dendritic cells [31,38,39]
	CXCL9	CXCR3, CCR3	Keratinocytes, neutrophils, macrophages and monocytes [27,40,41,81,87]
	CXCL10	CXCR3, CCR3	Keratinocytes, neutrophils, macrophages and endothelial cells [40,41,79–81]
	CXCL11	CXCR3, CCR3, CCR5	Monocytes [27,81,87], macrophages and neutrophils [40,41,79–81]
	CXCL12	CXCR4	Bone marrow stromal cells [40,41,79–81]
	CXCL13	CXCR5	Dendritic cells, monocytes and macrophages [42]
	CXCL14	Still remains obscure	Fibroblasts [43]
CC chemokines	CCL1	CCR8	Monocytes and T cells [27,29,44,80,87,117]
	CCL2	CCR2	Fibroblasts and neutrophils [37,131]
	CCL3	CCR1, CCR5	Neutrophils [131]
	CCL4	CCR1, CCR5	Neutrophils [131]
	CCL5	CCR1, CCR3, CCR4, CCR5	Endothelial cells, macrophages, T cells, natural killer cells, dendritic cells, basophils, eosinophils, fibroblasts, bone marrow stromal cells and macrophages [31,37,45,46]
	CCL6	CCR1, CCR2, CCR3	Neutrophils and macrophages [47]
	CCL7	CCR1, CCR2, CCR3	Fibroblasts [48]
	CCL8	CCR1, CCR2, CCR5, CCR11	Fibroblasts and macrophages [31,49,50]
	CCL9	CCR1	Osteoclasts and epithelial cells [51,52]
	CCL10	CCR1	Monocytes, endothelial cells and fibroblasts [53]
	CCL11	CCR3, CCR2, CCR5	Epithelial cells, endothelial cells, T cells, fibroblasts, macrophages and eosinophils [27,29,44,80,87,117]
	CCL12	CCR2	Macrophages [54]
	CCL13	CCR2, CCR3	Epithelial cells [29,44,80,87,117]
	CCL14	CCR1	Circulates in high concentrations in plasma [55]
	CCL15	CCR1, CCR3	Basophils and endothelial cells [56,57]
	CCL16	CCR1	Monocytes [58]
	CCL17	CCR4	Epithelial cells, dendritic cells and monocytes [29,44,59,80,87,117]
	CCL18	CCR8	Monocytes, dendritic cells and macrophages [60–62]
	CCL19	CCR7	Stromal cells [63]
	CCL20	CCR6	Epithelial cells, stromal cells and macrophages [64–66]
	CCL21	CCR7	Stromal cells [63]
	CCL22	CCR4	Epithelial cells, langerhans cells and monocytes [27,29,80,87]
	CCL23	CCR1	Epithelial cells and neutrophils [131,132]
	CCL24	CCR3	Epithelial cells and fibroblasts [29,44,80,87,117]
	CCL26	CCR3, CCR2	Epithelial cells and fibroblasts [27,29,44,80,87,117]
	CCL27	CCR3, CCR2, CCR10	Keratinocytes [67]
	CCL28	CCR10, CCR3	Epithelial cells [68]

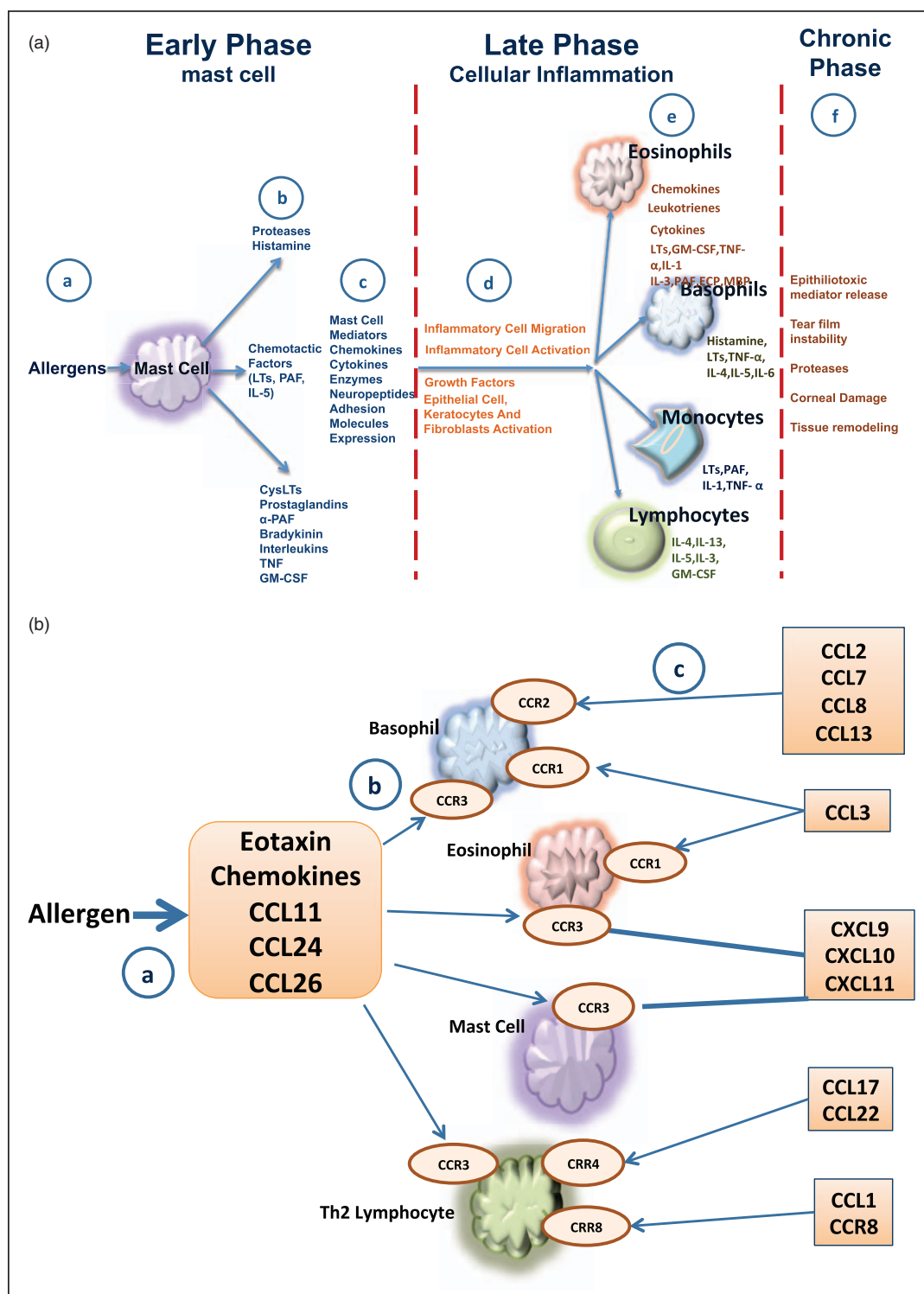


FIGURE 1. (Part a) The stages of ocular allergy response from the introduction of allergy to mast cell through early phase, late phase and chronic phase. (a) An allergen presents itself to a mast cell (b) IgE on the mast cell causes release of inflammatory mediators which triggers clinical manifestations and (c) mast cell degranulation inducing expression of chemokines, adhesion molecules, promoting the recruitment of inflammatory cells beginning (d) the late phase (e) with activity of leukocytes releasing proteins and chemoreactors, which over time evolves to the (f) chronic stage of the disease with its clinical manifestations. (Part b) Primary chemokines and their corresponding receptors on leukocytes elicited in vernal keratoconjunctivitis. The eotaxin chemokine family is elicited primarily from fibroblasts (a) after allergen presentation (b) mobilizing several subgroups of leukocytes (c) along with other chemokines. Note the identical receptors on several of the leukocyte cell walls, an example of the partial redundancy of the complex chemokine framework.

conjunctival epithelial cells, but it seems not to be the only factors involved [19–21,75,96–98]. Activated mast cells release histamine, leukotriene c4 and tryptase into the tears. This level of tryptase seems to correlate with the severity of VKC [99,100].

Conjunctival mast cells express cytokines (especially IL-4 and TNF- α) after meeting an allergen which promote chemokine expression (primarily eotaxins) from corneal epithelial cells, keratocytes and conjunctival fibroblasts [22,33]. These chemokines, each with their own specific attractant or multiple attractants and receptors, mobilize additional chemokines (most common CCL2,3,5,7,8, 11,24,26) [12,87,93,95,99,101–103], which in turn flood the area with additional proinflammatory cells and their products [12,87], causing the classic clinical signs and symptoms of this disease [18,104–109].

In AKC and in VKC, mast cell migration and activation occurs in and around the conjunctival epithelium [16–18,94,95]. Their presence is confirmed when inspecting tissue samples from giant papillae retrieved from these patients [16–18,94,110]. Immunohistological analysis of these giant papillae also shows that mast cells that interact with conjunctival epithelial cell cause CCL2 expression [95] as well as a significant amount of CCR3 [16–18,94,95].

CHEMOKINES IN VERNAL KERATOCONJUNCTIVITIS

Primary chemokines including the eotaxin family and their corresponding receptors on leukocytes in VKC exhibit a number of redundant connections displaying the complexity of the allergic reaction network (Fig. 1b).

VKC is an extreme expression of the late phase allergic eye response where chemokines play major roles [80,101,111,112]. This is a debilitating, chronic disease demanding continuous and diverse therapy based on understanding the mediators involved [80,101,111,112]. Patients with VKC have elevated levels of T lymphocytes, whose activation play a central role in the pathogenesis of the chronic allergic inflammatory response [113]. Immunohistochemical studies of conjunctival specimens from VKC patients demonstrated increased expression of inflammatory cells PARC, MDC and I-309, and CCL18 in inflammatory cells was elevated compared with CCL22 and CCL1 [114]. The amount of PARC(+) inflammatory cells was strongly correlated with the numbers of CD3(+) T lymphocytes [114]. Another study confirmed that VKC specimens have significantly more inflammatory cells expressing CXCR3 and most of the infiltrating T lymphocytes were also reactive for CXCR3 [115].

Apparently CXCR3 is the predominant chemokine receptor and is expressed copiously in the conjunctiva of patients with active VKC [115]. Other chemokines that have been found on the ocular surface in VKC are from the CC family, namely CCL11, CCL24, CCL26 (eotaxin-3) with chemokine receptor CCR3, of which CCL24 seems to have the highest expression [93,112,116,117]. These chemokines, predominantly secreted by fibroblasts, play a significant role in mobilizing various subgroups of leukocytes following an allergen challenge [93]. Their common receptor CCR3 can be detected on the surface of eosinophils, Th2 lymphocytes, basophils and mast cells [71,93,116]. Another study found CXCL8 and CCL24 to be associated with the exacerbation of chronic allergic conjunctival disease, and CCL24 mRNA levels were associated with disease severity [118]. Other studies found that CCL20 and its receptor CCR6 assist in the recruitment of memory B cells, memory T cells, Th17 cells as well as dendritic cells [89,93,112], and regulates various immune responses, homeostasis and inflammation of some mucosal tissue [70,88,119,120]. Another study found that VKC specimens exhibited a much higher level of CCL5, CCL2, CCL7 as well as the eotaxins (CCL11, CCL24, CCL26) than the healthy tissue [93].

A study found elevated amounts of the chemokines CCL5, CCL11,24,26 (eotaxin), CCL2 (MCP-1) and CCL7 (MCP-3) in the conjunctiva of patients with VKC in comparison with a control group [119]. Chemokines CCL11, CCL5, CCL2 (MCP-1) and CCL7 [119] were expressed at the limbal area in active VKC. In addition, the chemokine receptor CXCR3 and the ligand Mig were found to affect the mobilization of activated T lymphocytes in VKC [121].

VKC can also significantly promote VCAM-1 in vessels. Its expression correlates with eosinophil infiltration [122]. During inflammation, eosinophil activation and migration to the conjunctival epithelium is mediated locally by upregulated ICAM-1 [69]. Both ICAM-1 and E-selectin, which correspond with granulocyte or lymphocyte infiltration, are induced in the vessels of patients with severe allergic inflammation [69,102,123–125]. ICAM-1 and HLA-DR are expressed by conjunctival epithelial cells which attract leukocytes to the tissue and present them with antigens as antigen presenting cells. This infiltration is achieved by a chemotactic gradient provided by chemokines [69,102,123–125].

CHEMOKINES IN TEARS

Tear levels of cytokine, chemokine levels and their receptor expression is not generally used when

trying to determine a diagnosis but they do assist in evaluating the efficacy of antiallergic medications [24–26,126–129]. Tear compositions are helpful when trying to understand ocular allergy, but the various mediators in tears are not yet useful as specific disease biomarkers and are not used in clinical practice.

Many studies have revealed consistent differences in cytokine/chemokine tear concentrations between the various ocular allergy diseases [24–26, 126–129]. SAC and GPC characteristically lack cytokine changes [25,26,126–129]. VKC and AKC tend to manifest increased levels of Th1, Th2, proinflammatory cytokines and chemokines [24–26,126–129]. Eosinophils in tears during an allergic conjunctivitis express CCR1 and CCR3 [130]. Anti-CCR3 treatment markedly suppressed the chemotactic responses of eosinophils, whereas anti-CCR1 was less effective, showing that the primary receptor of chemokines on eosinophils is CCR3 [130]. Treatment with anti-CCR-1 and anti-CCR-3 antibodies, induced by a supernatant culture, inhibited up to 75.2 and 94.6% of eosinophil chemotaxis, respectively, and inhibited 27.8 and 74.5% of chemotaxis when induced with tear samples [130]. Studies have shown high levels of CCL11 and CCL24 in mucus of VKC patients [89]. Both CCL11 and CCL24, are potent eosinophil chemotactic and activating peptides, and are involved in eosinophil recruitment and in the pathogenesis of VKC and AKC [89,93].

Tear levels of CCL24 has been identified as a possible biomarker for the severity of VKC when mast-cell tryptase exhibited partial suppression of CCL11 and CCL26 to accumulate eosinophils but not on CCL24 [93]. CCL23 expression has been observed in eosinophils, monocytes and dendritic cells [93,111,112,116,131]. The tear levels of CCL23 in AKC or VKC were correlated with clinical severity scores of these diseases [111,132].

A study examined the clinical accuracy of a multiple tear cytokine and chemokine test by determining tear levels of CCL17, CCL24 and IL-16 [80]. They found consistently elevated tear levels of all three in patients with allergic conjunctivitis, AKC and VKC compared with the control group and determined these chemokines can be considered markers and the test useful when assessing acute and chronic allergic inflammation [80].

CHEMOKINES AND KERATOCYTES

Keratocytes, after stimulation of TNF- α and IL-4, are a source of CCL11 [93,119,120]. They are implicated as being the primary culprit when there is corneal involvement in ocular allergic responses [93,119, 120]. Stimulation of human keratocytes with a

combination of TNF- α and either IL-4 or IL-13 resulted in elevation in both the amount of CCL11 mRNA and the release of CCL11 protein [120]. This process may induce eosinophil recruitment to corneal ulcers in allergic ocular disease underscoring the severity of allergic disease when the corneal stroma is involved [119,120].

CHEMOKINES: THERAPY PERSPECTIVE

The redundancy of chemokines and their receptors makes it difficult to develop targeted therapies. Over the past decade, various attempts including amino terminus modification, peptide-based and nonpeptide-based antagonists and mAbs have been explored. Attempts to block subsets of chemokines have revealed unexpected responses of these mediators [79,133[■]].

As previously discussed, CCL11 is one of the central mediators in allergic eye diseases. In ocular allergy, the primary function of this chemokine is modulation of mast cell activation or differentiation [11,79,93,133[■],134,135]. With this understanding, new modalities of drugs specifically developed to target CCL11/CCR3 interaction have been investigated [11,79,93,133[■],134,135]. CCR3 is a receptor that binds to a number of chemokines, especially of the CC family of which many are associated with eosinophil recruitment during allergic inflammation [76–78,87]. The ability of this receptor to connect with so many chemokines may present an ideal target for a single therapy able to block their function [11,93,134]. The goal of one study was to develop an antagonist, peptide nanoparticle CCR3 inhibitor which has been called (R321) with the ability of blocking G-protein signaling [12,76–78,87,136]. CCR3 inhibitor (R321) can self-assemble into nanoparticles which then bind to the receptor, thereby interfering with its function [12,78,87,136].

Development of highly specific targeting drugs that affect a minimal number of genes is a way to achieve desired results with minimal side effects. Mapracorat, a nonsteroidal selective glucocorticoid receptor agonist, is one such drug targeted and proved effective at reducing clinical signs, eosinophil infiltration and eosinophil peroxidase activity in the late phase response of the conjunctiva of treated guinea pigs [137,138]. In addition, it decreased protein expression of both CCL5 and CCL11 [137,138]. Mapracorat proved to be even more effective than dexamethasone at increasing apoptotic eosinophils in the conjunctiva and is a promising topical treatment for allergic eye disease [137,138].

Rebamipide is known as a dry-eye therapy drug with anti-inflammatory effects on the corneal epithelium and ability to promote mucin secretion.

This drug suppressed the release of CXCL8 (IL-8) and inhibited CCL11 (eotaxin-1) expression [92]. By inhibiting the corneal fibroblasts from synthesizing chemokines, rebamipide seems to be an effective drug to treat corneal stromal inflammation as a result of allergy or bacterial infection [92].

Dendritic cells are potent contributors to the pathogenesis of corneal and ocular surface inflammatory diseases. There are two types of dendritic cells, lymphoid and nonlymphoid. The nonlymphoid dendritic cells originate from the conjunctiva, express the chemokine receptor CCR7 [139]. They then migrate to the lymph node and activate T cells [139]. The ligand CCR7 links with chemokines CCL19 and CCL21 and has surfaced as the central mediator of this intricate migratory mechanism [139]. Topical antagonists of the CCR7 receptor were found to be effective in suppressing allergic inflammatory responses in a mouse model of allergic conjunctivitis [139].

CONCLUSION

Multiple chemokines and their receptors play various roles in allergic inflammation. They are secreted by most of the cells that participate in allergic inflammation in the ocular surface, and are expressed in the cornea, conjunctiva and in tears. Knowledge regarding the relative importance of some of these chemokines and their receptors may promote more targeted and effective therapies.

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Conflicts of interest

There are no conflicts of interest.

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Biomarkers are measurable indicators in normal or pathological processes. They are a powerful tool to assist in diagnosis, disease regulation and help predict response to treatment. This article outlines the known ocular biomarkers in the tears, conjunctiva, aqueous humor and vitreous and their current uses.

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