

REVIEW ARTICLE

CX3CL1 in allergic diseases: not just a chemotactic molecule

V. Julia*

Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Institut de Pharmacologie Moléculaire et Cellulaire, Université de Nice-Sophia Antipolis, Valbonne, France

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Correspondence

Dr. Valerie Julia, Institut de Pharmacologie Moléculaire et Cellulaire, 660 route des lucioles, 06560 Valbonne, France.
Tel.: +33 (0) 4 93 95 77 82
Fax: +33 (0) 4 93 95 77 08
E-mail: julia@ipmc.cnrs.fr

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Abstract

Allergic asthma and atopic dermatitis (AD) are two allergic diseases that are primarily driven by the activation of T helper (Th)2 cells. Th2 cells produce cytokines that directly contribute to the symptoms of these diseases. The recruitment and maintenance of Th2 cells into the target tissues are two key events in the pathogenesis of allergic asthma and AD. While migration is mediated by both chemokines and lipid mediators such as leukotrienes and prostaglandins, very little is known about the molecules involved in lymphocyte survival and maintenance in inflamed tissues. However, chemokines could also play a role in this phenomenon. An example of this could be illustrated by CX3CL1, also known as fractalkine. CX3CL1 is a chemokine that is upregulated in some inflammatory diseases including allergic pathologies and that was recently demonstrated to provide a survival signal upon binding to its unique receptor CX3CR1.

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Atopic sensitization is defined by a genetic predisposition to the production of immunoglobulin E (IgE) against otherwise innocuous environmental antigens such as proteins from house dust mite, grass pollens, animal dander, and food. It can lead to the development of clinical symptoms such as dermatitis, rhinitis, and asthma. Allergic diseases affect up to 30% of the population, and their prevalence has steadily increased in the recent decades in part due to numerous changes in the environment (1). These pathologies mainly differ by their target organ. However, they may share a common etiology and are considered as a single but evolving physio-pathological entity: the atopic march. Atopic dermatitis (AD) is an inflammatory, chronically relapsing, pruritic skin disease that usually occurs during early infancy and childhood, but that can persist or start in adulthood. Atopic dermatitis results from complex interactions between susceptibility genes, environment, defective skin barrier function, and immunologic responses. It is believed to be the cutaneous manifestation of a systemic disorder that also leads to allergic asthma, allergic conjunctivitis, and allergic rhinitis (2). At least two forms of AD have been described, including an extrinsic form associated with IgE-mediated sensitization

exhibited by 70–80% of the patients. The prevalence of AD is 10–20% in children and 1–3% in adults in industrialized countries (3). More than 50% of children with AD may develop asthma, and approximately 75% suffer from allergic rhinitis during the first 6 years of life (4). AD prevalence has increased two- to threefold during the past three decades in industrialized countries. When severe, AD can be extremely disabling and AD management is complex. Avoiding or minimizing contact with known allergens is the primary treatment for AD. Topical treatments aimed at reducing both xerosis (dry skin) and inflammation are also administered. Corticosteroids are considered the most effective treatment against AD. However, steroid-containing creams induce stretch marks and thinning and cannot be used on the face or other areas where the skin is naturally thin. In addition, severe AD requires systemic administration of steroids that is not devoid of important side-effects in children. Allergic asthma is a chronic inflammatory lung disease characterized by airway hyper-responsiveness (AHR), excessive mucus production, lung eosinophilia, and airway remodeling (5). Affected individuals may suffer significant morbidity, and asthma causes death in some patients. Asthma currently affects 300 million

children and adults worldwide (6). As observed for AD, its prevalence has steadily increased during the last decades. The major therapies used to control asthma are glucocorticoids, in addition to β 2-adrenergic receptors agonists. These have broad-ranging anti-inflammatory actions in controlling disease symptoms but fail to provide a cure. Importantly, they do not prevent long-term decline in lung function.

In both humans and mice, allergic inflammation is primarily driven by the activation of T helper (Th)2 cells. These cells produce cytokines that directly contribute to the symptoms of the disease. Both recruitment and maintenance of Th2 cells into the airways or the skin are key events in the pathogenesis of asthma and AD. While the role of chemokines in T-cell recruitment has been well documented, very few studies have reported their implication in survival. This mechanism has probably often been neglected at least in part because it could easily be confused with migration. However, deficiency or neutralization of chemokines can alter accumulation of cells within the inflamed tissue by other ways than attracting them. Indeed, decreased cell numbers in the target tissue can be due to an impaired survival or retention within this tissue. In most cases, the action mechanisms of chemokines remain to be re-explored, as well as the absolute requirement for chemokine receptors to specific cell types.

CX3CL1

CX3CL1, also known as fractalkine or neurotactin, is a type I transmembrane protein with the CX3C chemokine domain present on an extended glycosylated stalk (7). Until present, CX3CL1 shares this unique membrane anchorage with the CXCR6 ligand, CXCL16. CX3CL1 is constitutively shed from the cell surface by the disintegrin and metalloproteinase (ADAM) 10. CX3CL1 cleavage is enhanced upon activation of the closely related protease tumor necrosis factor (TNF)- α -converting enzyme, TACE (ADAM-17) (7). TACE activity is induced *in vitro* upon IL-13 stimulation of human pulmonary epithelial cells (8) and in the inflamed lungs of mice that have been sensitized and challenged with OVA (9). CX3CL1 binds to only one receptor called CX3CR1. In humans, CX3CR1 has two known ligands: CX3CL1 and CCL26 (eotaxin-3) (10). In mice, CCL26 gene is however a pseudogene, and CX3CL1 is therefore the only known ligand of CX3CR1.

Distribution of CX3CL1-expressing cells

Studies in humans have shown that CX3CL1 is expressed in a wide variety of cell types including neurons (11), intestinal (12), airway (13), skin (14) epithelium cells, airway smooth muscle cells (15), and endothelium (7). Because of the lack of specific staining reagents, very little information on CX3CL1 expression in mice was available until Jung and his coworkers generated a mouse strain in which a red fluorescent reporter protein could be used as a surrogate marker for CX3CL1 expression (16). An exhaustive characterization of these mice confirms the previous findings in humans and delineates more precisely the CX3CL1-expressing cell types. CX3CL1 was expressed in intestinal epithelial cells by goblet cells, in both

alveolar and bronchial epithelial cells including Clara cells, and in tubular epithelial cells and glomeruli. Of note, CX3CL1 expression could not be detected on the vascular endothelium of naïve mice.

Distribution of CX3CR1-expressing cells in homeostasis

CX3CR1 expression in humans can easily be monitored by flow cytometry and immunochemistry using commercially available antibodies. CX3CR1 is expressed in neurons (17), microglial cells (17), monocytes (18, 19), CD56^{dim}CD16⁺ natural killer (NK) cells, and subsets of T lymphocytes (18, 20). Although no CX3CR1-specific staining reagents are available in mice, CX3CR1 expression has been studied using hemizygote mice in which one CX3CR1 allele has been replaced by the eGFP gene (21). As observed in humans, CX3CR1 is expressed at high levels in microglial cells. It is also expressed in blood monocytes, various dendritic cell (DC) progenitors, plasmacytoid DC, cytotoxic NK cells, macrophages in the intestine lamina propria, and CD8⁺ T lymphocytes (16, 21, 22).

CX3CL1–CX3CR1 axis in allergic diseases

A role for CX3CL1 and CX3CR1 has been postulated in many inflammatory diseases in which CX3CL1 expression was found to increase, such as psoriasis (23–25), chronic obstructive pulmonary disease (26, 27), pulmonary hypertension (28, 29), and pulmonary fibrosis (30). It has been proposed that CX3CR1 contributes to the pathogenesis of these diseases by promoting the transmigration of CX3CR1-expressing monocytes or lymphocytes. In contrast, the role of CX3CR1 has been clearly demonstrated in rheumatoid arthritis [see for review (31)] and atherosclerosis (32, 33).

A large body of evidence suggests that CX3CL1–CX3CR1 interactions play a role in allergic diseases. Genetic analyses show that two CX3CR1 single nucleotide polymorphisms were associated with asthma and atopy in French-Canadian populations (34) and German children (35), respectively. Patients with allergic rhinitis or allergic asthma exhibit increased levels of soluble CX3CL1 in serum and bronchoalveolar lavage fluids (BALF). This latter increases even more following segmental allergen challenge in allergic asthmatic patients (13). CX3CL1 expression was also increased in airway smooth muscle, lung endothelium, and airway epithelium upon allergen challenge in asthmatic patients (15). Altogether, these results are consistent with the activation of CX3CL1 cleavage in allergic asthma most likely as a result of ADAM17 activation. Furthermore, CD4⁺ T lymphocytes from patients with allergic rhinitis and allergic asthma exhibit increased CX3CR1 function in response to CX3CL1 as compared to those of healthy individuals. Likewise, patients with allergic rhinitis or asthma exhibit a higher fraction of memory/effector peripheral CD4⁺ T expressing CX3CR1 (13). In agreement with a critical role of CX3CL1–CX3CR1 interaction in airway allergic diseases, we have recently demonstrated that both CX3CR1-deficient mice and wild-type mice treated with CX3CR1-blocking reagents exhibited reduced

allergic asthma (36). Transfer of wild-type CD4⁺ T cells into CX3CR1-deficient mice restored asthma, and CX3CR1-blocking reagents prevented airway inflammation in CX3CR1-deficient recipients injected with wild-type Th2 cells. Altogether, these results demonstrated that the expression of CX3CR1 by effector CD4⁺ T cells was required for asthma development.

Much little information is available on the role of CX3CL1–CX3CR1 interactions in AD. CX3CL1 was found to be upregulated in both endothelium cells and skin lesions in patients with AD (14). In that latter study, serum CX3CL1 levels were positively associated with disease severity and inversely correlated with improvement in skin lesions. More recently, a study reported that while CX3CR1 mRNA expression was consistently increased in AD skin, CX3CL1 mRNA levels were only increased in some patients with a significant correlation with the disease severity (10), a result that may explain why Fraticelli and coworkers have failed to detect CX3CL1 in skin lesions of their four patients with AD (23). Our data using a mouse model of AD support a role of CX3CR1 in the development of the disease (unpublished observations).

Regulation of CX3CR1

While a subset of memory/effector peripheral CD4⁺ T cells were found to express CX3CR1 in asthmatic patients (13), the phenotype of these CX3CR1⁺ CD4⁺ T cells was not extensively characterized. In another study, CX3CR1-expressing CD4⁺ T cells were found to express CCR5, suggesting that they were Th1 cells (20). Likewise, cord blood T cells polarized under Th1 conditions exhibited high level of CX3CR1 mRNA, while polarized Th2 cells did not (23). In mice, we have found that both Th1 and Th2 cells that were generated *in vitro* did not express CX3CR1 unless they were incubated with lung cells or injected into sensitized mice exposed to aerosols of their cognate allergen. Therefore, CX3CR1 expression seems to be tightly regulated in T helper cells. The human CX3CR1 gene is controlled by three distinct promoter regions leading to the transcription of three mature mRNA that only differ by their untranslated regions (37). This highly complex regulation is likely to allow versatile and tightly regulated CX3CR1 expression in different cell types and micro-environment. Although the regulation of CX3CR1 expression has been poorly studied in hematopoietic cells, some studies have shown that IL-2 induced CX3CR1 on both human CD4 and CD8 T cells (18) and that IL-10 and IFN- γ increase CX3CR1 expression in monocytic cell lines (38). In contrast, IL-15 was reported to decrease CX3CR1 expression by human CD56⁺ NK cells, while IL-2 increased it (39, 40). It remains to be investigated whether any of these cytokines is responsible for the CX3CR1 expression that was evidenced in airway CD4⁺ T cells of asthmatic mice.

Migration, proliferation, or survival?

Although chemokines were originally defined as chemoattractants, a growing body of evidence suggests that they could

also promote survival. The anti-apoptotic role of CX3CL1 was first identified in neurons and microglia. CX3CL1 reduced neuronal apoptosis induced by the HIV-1 envelope protein gp120, and this phenomenon was blocked by anti-CX3CR1 antibodies (41). Likewise, CX3CL1 reduced cPAF- or Tat-induced neuronal apoptosis, further confirming a neuroprotective role of CX3CL1 in HIV infection (42). Furthermore, CX3CL1 inhibited both Fas-induced apoptosis (43) and LPS-induced activation of microglial cells (44). Altogether, these studies suggested that CX3CL1–CX3CR1 interactions could promote the survival of neurons and microglial cells in at least some pathogenic conditions. More recent studies have shown that CX3CL1–CX3CR1 interactions could promote monocyte survival. This was first demonstrated in mice both in steady-state conditions and in atherosclerosis (45). Further studies showed that this effect was mediated by the transmembrane form of CX3CL1 (16). However, soluble CX3CL1 was able to inhibit human monocytes from serum-deprivation-induced cell death *in vitro* (45). CX3CL1–CX3CR1 interactions can also promote lymphocyte survival. CX3CR1-induced survival was observed for both Th1 and Th2 cells upon airway inflammation in mice, but neither under homeostatic conditions nor upon lymph node inflammation (36). According to these findings, effector CD4⁺ T cells do not express CX3CR1 in the periphery but only in inflamed tissues. As observed with human monocytes, Th2 survival can be induced *in vitro* using soluble CX3CL1. However, it remains to be determined whether CX3CR1-induced *in vivo* survival requires the membrane-bound or the soluble form of CX3CL1.

CX3CR1 signaling pathway

Binding of chemokine receptors with their cognate ligand leads to signal transduction pathways controlling cytoskeletal reorganization and integrin activation, resulting in increased adhesion and migration of cells. As such, CX3CL1 binding to CX3CR1 leads to an increase in intracellular calcium, actin rearrangement, and activation of the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways. The regulation of these pathways results in the different effects induced by CX3CL1: cell survival, proliferation, and migration. CX3CR1 signaling pathways have been studied in rat neurons (46), rat microglial cells (47), CHO cells expressing a human CX3CR1 (48), and several human monocytic cell lines (49). The activation of CX3CR1 by CX3CL1 resulted in the stimulation of PI3K, MAPK, and Akt/PKB signaling cascades (41, 46–49). In contrast to its human counterpart, the mouse CX3CR1 does not activate the PI3K-dependent signaling pathway when expressed in CHO cells (50). The presence of a proline (instead of a serine) at position 326 in the carboxy-terminal region of the mouse CX3CR1 is responsible for this phenomenon. In another expression system, it was shown that CX3CL1 induced calcium flux in mouse CX3CR1-expressing HEK293 cells (51). It remains to be determined whether ERK and Akt are involved or whether this pathway is also defective.

Therapeutic applications

Allergic and nonallergic asthma affect 300 million people worldwide (6). Asthma has a tremendous impact on the healthcare system, the productivity and health-related quality of life of patients, and the quality of life of family members and caregivers. Although inhaled corticosteroids are very effective as anti-inflammatory treatment for persistent asthma, they do not cure asthma. Furthermore, 10% of asthmatic patients do not respond to this treatment (52). More specific therapeutic agents such as antibody to IL-5 have been tested in clinical trials, but the results have been rather disappointing (53). As corticosteroids and β_2 -agonist bronchodilators that constitute the standard therapy of asthma are given as inhaled drugs, our results showing that the local administration of CX3CL1 antagonist to antigen-sensitized mice resulted in reduced AHR and airway inflammation are very promising and should encourage preclinical studies to further explore the effect of CX3CL1 antagonist in human asthma.

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Concluding remarks

Chemokines were initially identified as small cytokines that induced directed chemotaxis in nearby responsive cells *in vitro*. However, chemokines have also been shown to play a role in other mechanisms such as survival, angiogenesis, tissue repair, tumor progression, hematopoiesis, antimicrobial mechanisms, and pain control. CX3CL1 that has been found to increase in allergic diseases was recently shown to play a major role in the development of asthma, not by directing the migration of immune cells into the airways but by promoting Th2 cell survival in the inflamed airways. This function not only provides crucial insight into the role CX3CL1 and CX3CR1 in allergic airway disease, but may also open the way for an alternative interpretation of the results of chemokine and chemokine receptor deficiencies.

Conflict of interest

The author has no conflict of interest to declare.

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