

## REVIEW ARTICLE

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# Chemokines in allergic asthma inflammation

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## ABSTRACT

Asthma is the most frequent noncommunicable disease and one of the leading causes of years lived with disability. Asthma has a severe impact on a patient's life, being able to disturb the activities of both children and adults. The morbidity and mortality of asthma may depend on the severity and progressiveness of the symptoms experienced by the patient. Different and complex pathomechanisms underline the pathology of asthma, in which the regulation of innate and adaptive immune responses plays a role. There is a complex interaction between immune cells including chemokines involved in the pathogenesis of asthma. Immune cell trafficking is orchestrated by a family of small proteins called chemokines. Leukocytes express cell-surface receptors that bind to chemokines and trigger transendothelial migration. This review article outlines the main role of chemokines in inflammatory reactions that occur in allergic asthma, based on the latest literature studies that have been published previously. The allergic reaction in asthma expresses various chemokines and their receptors. Chemokines including eotaxins (CCL11, CCL24, and CCL26), CCL2, CCL5, CCL17, and CCL22 regulate immune cells that under pathological conditions travel to the inflammatory site, mainly in the lung, to protect the body from pathogen invasion. Chemokines are released by a number of immune cells such as monocytes, dendritic cells, mast cells, and epithelial cells in the airway. The biological effects of chemokine production are enhanced by secreted cytokines when an allergic reaction occurs in asthma, such as IL-4, IL-5, and IL-13. Chemokines cause an accumulation of different inflammatory cells at the site of inflammation, which ultimately results in tissue damage to the airway. The inhibition of the reactions evoked by the interaction between chemokines and their receptors is considered a candidate for the development of potent therapeutic drugs for asthma in the future.

**Keywords:** Asthma, allergic inflammation, chemokines, immune cells

### Abbreviations:

ASM : airway smooth muscle cells; BA: bronchial asthma; BAMs: bronchoalveolar macrophages; CCL: chemokine (C-C motif) ligand; CCR: CC chemokine receptor; CD: cluster of differentiation 2; Cdc42 : cell division cycle 42; CRS: chemokine recognition site; CRTH2: chemoattractant receptor-homologous molecule expressed on Th2 cells; CXCL: chemokine (C-X-C motif) ligand; CXCR : CXC chemokine receptor; Cys-Lts: cysteinyl leukotrienes; DAG: 1,2-diacylglycerol; Dalys: disability-adjusted life years; DC: dendritic cell; EC: epithelial cell; ECL:

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extracellular loop; EGFR: epidermal growth factor receptor; ER: endoplasmic reticulum; FcεRI: high-affinity IgE receptor; FcεRII: low-affinity IgE receptor; FeNO: fraction of exhaled nitric oxide; GAG: glycosaminoglycan; GATA3: GATA binding protein 3; GDP: guanosine diphosphate; GEFs: guanine nucleotide exchange factors; GINA: global initiative for asthma; GM-CSF: granulocyte-macrophage colony-stimulating factor; GPCRs: G protein-coupled receptors; GTP: guanosine triphosphate; GTPases: guanosine triphosphatases; ICL: intracellular loop; ICOS: inducible co-stimulator; IFN $\gamma$ : interferon gamma; IgE: immunoglobulin E; IL: interleukin; ILC2: innate lymphoid cells 2; IP-10: interferon gamma-inducible protein 10; IP3: inositol 1,4,5-trisphosphate; ISAAC: international study of asthma and allergies in childhood; JAK: janus kinase; MAPK: mitogen-activated protein kinase; MBP: major basic protein; MCP-1: monocyte chemoattractant protein-1; MDC: macrophage-derived chemokine; MHC: major histocompatibility complex; NAP-1: neutrophil activating protein-1; NF- $\kappa$ B: nuclear factor  $\kappa$ B; NK: natural killer; Pak1: P21-activated kinase 1; PBMCs: peripheral blood mononuclear cells; PGA: paucigranulocytic asthma; PGD2: prostaglandin D2; PH: pleckstrin homology; PI3K $\gamma$ : phosphatidylinositol-3-kinase  $\gamma$ ; PIP2: phosphatidylinositol (4,5)-bisphosphate; PLC- $\beta$ : phospholipase C- $\beta$ ; PTKs: protein tyrosine kinases; PX: phox homology; RANTES: regulated upon activation normal T-cell expressed and presumably secreted; ROS: reactive oxygen species; STAT6: signal transducer and activator of transcription 6; TARC: thymus and activation-regulated chemokine; TGF- $\beta$ 1: transforming growth factor  $\beta$ 1; Th: T-helper; TLRs: toll-like receptors; TNF $\alpha$ : tumor necrosis factor- $\alpha$ ; TSLP: thymic stromal lymphopoietin; YLD: years lived with disability; 7TM: 7-transmembrane.

## INTRODUCTION

Allergic disorders are a significant public health concern. Ecological changes, industrialization, and immunologic associations increase the prevalence of allergic disease such as bronchial asthma.<sup>(1,2)</sup> Asthma is a chronic respiratory disease characterized by reversible airway obstruction, increasing mucus production, bronchospasm, and underlying airway inflammation.<sup>(3)</sup> The Global Initiative for Asthma (GINA) defined asthma as “a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough, that vary over time and intensity, together with variable expiratory airflow limitation”.<sup>(4)</sup> The prevalence of asthma has been increasing consistently for several decades.<sup>(5)</sup>

About 339 million individuals have asthma globally.<sup>(6)</sup> Asthma is positioned 16<sup>th</sup> among the leading causes of years lived with disability and 28<sup>th</sup> among the main causes of disease burden, as estimated by Disability-Adjusted Life Years (DALYs) universally. The percentage of total DALYs due to asthma in 0-9-year-olds ranked 19<sup>th</sup> in 2019.<sup>(7)</sup> Around 418,000 people die every year because of asthma, and DALYs attributable

to asthma account for 24.8 million.<sup>(1)</sup> The percentage of asthma in the U.S. population has increased over time. Current asthma prevalence increased from 7.3% in 2001 to 7.9% in 2017. In 2019, the total prevalence of asthma in the U.S was about 7.8%, while the percentage of asthma in the child population (<18 years) and in adults ( $\geq$ 18 years) was 7.0% and 8.0 %, respectively.<sup>(6)</sup> According to the 2018 Indonesian Basic Health Research, the prevalence of asthma at all ages in Indonesia is about 2.4%, while the highest percentage of asthma is for Yogyakarta (4.5%), followed by East Kalimantan (4.0%), Bali (3.9%), Central Kalimantan (3.4%), and North Kalimantan (3.3%).<sup>(8)</sup> Based on research utilizing the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire, the asthma prevalence in Indonesia varies by region, ranging from 4-11% in 6-7-year-olds and 6-13% in 13-14-year-olds.<sup>(9)</sup>

Under pathologic conditions, the immune responses of the individual require the coordination of various cells and secreted factors to defend the body against invasion by pathogens. Complex interactions between cells, cytokines, and chemokines are involved in asthma pathogenesis. Leukocytes express cell surface receptors (chemokine ligands) capable of binding to chemokines on endothelial cells, resulting in

transendothelial migration of the leukocytes.<sup>(10–12)</sup> Tissue-resident cells release inflammatory chemokines during an inflammatory response. Interaction between chemokines and types of lymphocytes in some allergic diseases has been presented in a previous literature study.<sup>(13)</sup> Here, this review aims to describe the role of chemokines especially in the allergic inflammation of asthma. An understanding of the cellular process of chemokines in asthma is essential for developing advanced asthma therapy. We used search engines including PubMed and Google Scholar to explore research articles, reviews, reports, e-books, and other data sources, published in 2013–2022. The search terms used include allergic inflammation, chemokine-receptor interactions, asthma endotypes, eosinophilic and non-eosinophilic asthma, and therapeutic strategies.

### **Classification of asthma and clinical manifestation**

Asthma is a common heterogeneous chronic respiratory disorder and has a variety of phenotypes. Classically, asthma has been divided into extrinsic and intrinsic asthma. Extrinsic asthma or atopic asthma is commonly associated with allergy, begins in childhood, the patient has relatives with allergy, and starts after an individual has been exposed to the allergen. In intrinsic asthma, there is no allergy or family history of asthma and the disorder begins in adulthood. The Global Initiative for Asthma has classified asthma according to the frequency and severity of symptoms into intermittent, mild persistent, moderate persistent, and severe persistent asthma.<sup>(14,15)</sup> However, based on the underlying pathological mechanism, asthma is classified as type 2 and non-type 2 asthma. This classification is widely accepted because it is considered to reflect better the endotypes.<sup>(16)</sup>

Type 2 is the most common type of asthma, in which T helper 2 (Th2) lymphocytes are related to airway inflammation. The involvement of Th2 lymphocytes causes eosinophilic inflammation of the airway by producing interleukin (IL)-4, IL-5,

IL-13, and immunoglobulin E (IgE). Innate lymphoid cells 2 (ILC2) also add to the airway inflammatory responses in type 2 asthma.<sup>(17)</sup> Frequently, patients with this type have a level of blood eosinophilia of  $\geq 150/\mu\text{L}$  and fraction of exhaled nitric oxide (FeNO) of  $\geq 20$  ppb. Type 2 asthma has been classified into early-onset allergic eosinophilic airway inflammation and late-onset nonallergic eosinophilic airway inflammation. The allergic form of type 2 asthma can be triggered by exposure to an allergen, but in late-onset nonallergic eosinophilic airway inflammation, there is no allergy involved. Microbes, viruses, air pollutants, glycolipids, and irritants can trigger this airway inflammation.<sup>(18,19)</sup>

Non-type 2 asthma is characterized as a non-eosinophilic asthma without the presence of type 2 inflammatory markers and consists of neutrophilic and paucigranulocytic inflammation, depending on the cellular findings in sputum specimens. Th1 and Th17 cells are involved in its pathobiology. Other cytokines implicated in this non-type 2 asthma include IL 1 $\beta$ , IL 6, IL 8, IL 17A/F, tumor necrosis factor- $\alpha$  (TNF  $\alpha$ ), and interferon-gamma (IFN  $\gamma$ ).<sup>(20)</sup> Neutrophilic inflammation is characterized by the proportion of neutrophils of  $\geq 40\%$ – $60\%$  in sputum specimens. Infection, tobacco smoke, and irritants can trigger this type of inflammation. Neutrophilic inflammation is often associated with Th1 and Th17-related cytokines, IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF) that induce neutrophils to migrate to the airways. Paucigranulocytic asthma (PGA) is the least common and milder disorder, in which the patients have normal eosinophils and neutrophils in the sputum.<sup>(18–20)</sup>

The frequency of asthma symptoms varies greatly, as various triggers can exacerbate the symptoms. Some patients have infrequent, brief attacks of asthma and some can suffer continuous symptoms. Asthma is characterized by excessive sputum production, shortness of breath, cough, and wheezing, sometimes accompanied by the inability to lie flat, insomnia, and fatigue. Symptoms often deteriorate at night.

Circadian rhythms of bronchomotor tone and bronchial reactivity reach the lowest point between 3-4 in the morning, increasing bronchoconstriction symptoms. The presence of tachypnea, wheezing, chest hyperinflation, tachycardia, diaphoresis, inability to speak, and difficulty lying are the signs that arise in severe asthma.<sup>(21,22)</sup>

### Pathomechanism of asthma

When the body is exposed to allergens, generally aeroallergens such as pollen and house dust mites, these attach to the Toll-like receptors (TLRs) and promote their activation. Activated TLRs induce epithelial cells to produce the cytokines thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, which are able to promote type-2 asthma adaptive immune responses. TLR activation also triggers the secretion of chemokines such as CCL2 and CCL20, that aim to promote the maturation of dendritic cells (DC). The dendritic cells then head to the lumen of the airway to take up aeroallergens and produce allergen peptide fragments to be served to type II major histocompatibility complex (MHC) proteins. Activation of Th2-mediated immune responses and inflammatory cytokine generation are induced by IL-33.<sup>(14,23,24)</sup>

Naive T cell stimulation requires costimulatory molecules such as CD28, inducible co-stimulator (ICOS), and OX40 with ligands that exist in DC (CD80/B7.1, CD86/B7.2, OX40 ligands, and ICOS ligands). Differentiation of T lymphocytes is also heavily influenced by the cytokine environment. Th2 polarization implies high amounts of IL-4 and low levels of IL-12.<sup>(14)</sup> Activation of Th2 cells through the main transcription factor, namely GATA binding protein 3 (GATA3), induces the secretion of IL-5, IL-4, and IL-13 by these cells. IL-5 plays a role in the maturation and survival of eosinophils. Furthermore, these cells will migrate to the epithelium of the bronchus and induce chemokines such as eotaxins (CCL11, CCL24, CCL26) and CCL5 which are paired with CCR3 receptors. Immunoglobulin isotypes changes in B cells are

mediated by IL-5, IL-4, and IL-13, the latter two promoting IgE synthesis through IL-4R $\alpha$ .<sup>(12,19)</sup> IgE recognizes two types of receptors, namely high-affinity receptors (Fc $\epsilon$ RI) and low-affinity receptors (Fc $\epsilon$ RII/CD23). Fc $\epsilon$ RI receptors are expressed by mast cells, basophils, dendritic cells, and eosinophils. In addition, Fc $\epsilon$ RI is found in other cells such as airway smooth muscle cells (ASM), and epithelial and endothelial cells. In antigen presentation, dendritic cells are induced by bonds between IgE and Fc $\epsilon$ RI receptors to activate Th2 cells. Through CD23, IgE receptors are activated on epithelial cells of the airway and are involved in the transportation of the IgE-allergen complex across the airway mucosal barrier. Eosinophils actively release mediators, such as histamine, lipid mediators, cysteinyl leukotrienes (cys-LTs), IL-8, major basic protein (MBP), GM-CSF, and reactive oxygen species (ROS). Besides eosinophils, histamine is also released by basophils and mast cells. The role of histamine in asthma is inducing bronchial smooth muscle contractions, increasing capillary permeability, disruption of the epithelial barrier, and hypersecretion of mucus. Cys-LTs and prostaglandin D2 (PGD2) are also produced by mast and eosinophil cells that can trigger eosinophil chemotaxis via the chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2), which are expressed in ILC2, Th2, eosinophils, and basophils. CRTH2 activation escalates airway inflammation. Epithelial damage in asthma is caused by MBP mediators. As for remodeling of the airway, this is mediated by leukotrienes. IL-8 plays a role in neutrophil recruitment.<sup>(12,24)</sup>

### Classification of chemokines and chemokine receptors

The purpose of the immune system is to protect the body against pathogens. Chemotactic trafficking of the immune response is governed by 20 G protein-coupled receptors (GPCRs) and more than 40 chemokines that escort the immune cells to the right site at the right time.<sup>(25)</sup> Chemokines are small soluble proteins (<10 kDa),

consisting of 70–80 amino acid residues with conserved sequence, are structurally related to cytokines, and play the main role in immunity and inflammation. Chemokines contain four conserved cysteine residues forming two disulfide bonds.<sup>(26,27)</sup> The term chemokine is derived from the word “chemotaxis”, meaning the cell movement triggered by a chemical stimulus. “Taxis” in classical Greek means “arrangement”. The stimulus will attract the cells towards chemoattractants including chemokines, which are arranged in concentration gradients in the microenvironment. Cells migrate from one location in the body to another, where they may invade tissues or recirculate through the blood or lymph. Chemokines are a broad category of molecules with similar fundamental structures and activities. Chemokines comprise almost 50 similar proteins with a molecular weight of 8 to 10 kDa.<sup>(28)</sup> Chemokines contain a three-stranded  $\beta$ -sheet and a C-terminal  $\alpha$ -helix domain lying across one face of the  $\beta$ -sheet, and flanked by an N-terminal domain (usually ~10 residues). Chemokines are classified into four subgroups based on the position in the molecule of the first two Cys residues, which may be adjacent (CC, n=28), separated by one amino acid (CXC, n=17), separated by three amino acids (CX3C, n=1), or only the first cysteine group is present (C, n=2). Most chemokines are chemotactic agonists.<sup>(28,29)</sup>

Chemokines are cytokines that stimulate cell migration toward sites with higher ligand concentrations. The ligands adhere to the extracellular matrix and generate a gradient of different expansion in tissues. Leukocytes that possess chemokine receptors, a class of G protein-coupled receptors expressed on the cell surface, can detect this concentration gradient.<sup>(28,30)</sup> The chemokine receptor consists of seven transmembrane helices (7TM) connected by 3 intracellular loops (ICL1-3) and 3 extracellular loops (ECL1-3), an extracellular N-terminal domain, and an intracellular C-terminal domain, which includes an  $\alpha$ -helix (Helix 8). A disulfide bond connects the N-terminus to ECL1 and ECL3 to ECL2. Chemokine receptors are

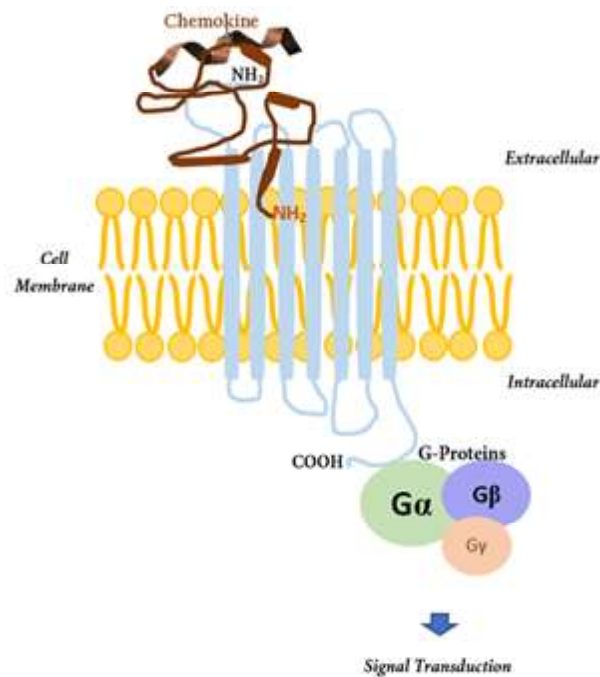
classified into 2 groups, namely the G protein-coupled chemotactic chemokine receptors (typical receptors) and the atypical chemokine receptors. Chemokine receptors are involved in biological processes, including cell adhesion and migration to inflammatory sites by binding to their ligands.<sup>(29)</sup> There are 19 chemokine receptors in humans, namely CCR1 – CCR10, CXCR1 – CXCR6, CXCR8, XCR1, and CX3CR1, that are activated by different chemokines.<sup>(31)</sup>

### Chemokine signaling pathway

All members of the chemokine family have the same tertiary structure, which consists of a flexible N-terminal and an irregular N-loop, followed by a three-stranded antiparallel  $\beta$ -sheet onto which a folded C-terminal  $\alpha$ -helix is attached. The N-terminal is widely recognized as being essential for receptor activation.<sup>(27,32)</sup> Chemokine receptors and their chemokine ligands interact via a two-step binding process. First, the chemokine's C-terminal domain binds to the receptor's N-terminal domain and extracellular loops (ECL)/CRS [chemokine recognition site 1 (CRS1)]. The unstructured N-terminus of the chemokine can then target the 7TM helical bundle (CRS2) and fix the receptor in an active conformation, thereby inducing intracellular signal transmission (Figure 1).<sup>(31)</sup>

The chemokine signaling pathway is transduced by G-protein coupled receptors expressed on the surface of immune cells. Interaction of the chemokine with the chemokine receptors which are expressed on the cell surface as 7-transmembrane proteins coupled with G-protein will promote cell signal transmission following ligand binding. After activation, GPCRs as guanine nucleotide exchange factors (GEFs) for the  $G\alpha$  subunit produce the exchange of guanosine diphosphate (GDP) to guanosine triphosphate (GTP), leading to dissociation of the GTP-bound  $G\alpha$  subunit from the  $G\beta\gamma$  heterodimers.<sup>(33)</sup>

Protein dissociation into subunits  $\alpha$  and  $\beta\gamma$  will initiate diverse downstream G protein-dependent effectors including phospholipases A2,



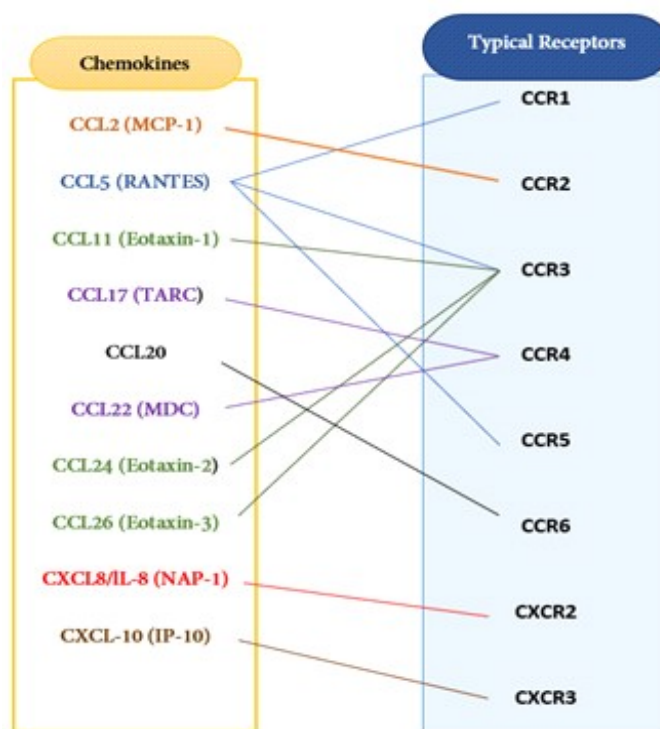
**Figure 1.** Chemokine–receptor interactions. The chemokine C-terminal domain ( $\alpha$ -helix) interacts with the  $\text{NH}_2$ -terminal domain and extracellular loops of the chemokine receptor (light blue). Following the targeting of the 7-TM helical bundle by the  $\text{NH}_2$ -terminus of the chemokine, the receptor is fixed in an active conformation, resulting in the induction of intracellular signal transmission. 7-TM (7-transmembrane)

C (subtypes  $\beta 2$  and  $\beta 3$ ) and D, protein tyrosine kinases (PTKs) and phosphatases, phosphatidylinositol-3-kinase  $\gamma$  (PI3K $\gamma$ ), low molecular weight guanosine triphosphatases (GTPases), and mitogen-activated protein kinases (MAPKs). Phospholipase C- $\beta$  (PLC- $\beta$ ) hydrolyzes phosphatidylinositol (4,5)-bisphosphate (PIP2) to form inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG). IP3 releases  $\text{Ca}^{2+}$  from intracellular stores in the endoplasmic reticulum (ER), which acts with DAG to activate protein kinase C (PKC), which involves regulating receptors via phosphorylation and desensitization. PI3K $\gamma$  phosphorylates PIP2 to form PIP3 at the cell membrane, which causes localized recruitment of proteins containing pleckstrin homology (PH) domains or PHOX (PX) domains, leading to actin polymerization and shape changes at the leading edge of the cell. These processes result in the movement toward the site of highest concentration of the chemokine. PH domain-containing targets (protein kinase B (Akt), RhoGEF, RacGEF, and cdc42GEF) modulate cell movement. Rho is involved in the regulation of

cell adhesion, chemotaxis, and myosin contraction, Rac controls the formation of lamellipodia, while cdc42 controls the formation of filopodia. P21-activated kinase 1 (Pak1) as downstream targets of a small guanosine triphosphatase also has a role in myosin contraction. Cellular regulatory mechanisms control the expression, activation, and signaling of GPCRs.<sup>(29,33)</sup>

### Role of chemokines in allergic asthma inflammation

The role of chemokines is critical in the immune system. The ability of dendritic cells to migrate to the lymph nodes is mediated by chemokines at the beginning of an adaptive immune response.<sup>(28)</sup> Chemokine receptors are activated when chemokines are secreted into blood vessels, where they promote leukocyte adhesion to vascular endothelium, reorganization of the actin cytoskeleton, and leukocyte extravasation or migration into the tissues. In the tissue extracellular matrix, chemokines form defined concentration gradients by binding glycosaminoglycans (GAG), which will eventually



**Figure 2.** Human chemokine and chemokine receptors are involved in the pathophysiology of asthma. CCL2 causes intracellular signal transduction by interacting with the CCR2 receptor. CCL5 interacts with CCR1, CCR3, and CCR5. CCL5 is a chemoattractant for a wide spectrum of immune cells (dendritic cells, T-cells, eosinophils, monocytes, basophils, NK cells), guiding them to the site of inflammation in the airway. Some chemokines have the same chemokine receptor, including eotaxin 1,2, and 3 (bind CCR3); CCL17 and CCL22 (bind CCR4). CXCL8 and CXCL10 bind CXCR2 and CXCR3, respectively

direct leukocyte migration to the location of tissue damage.<sup>(27)</sup>

Resident cells in the airway include epithelial cells and alveolar macrophages, and are cellular sources of chemokines.<sup>(34,35)</sup> The presence of a leaky epithelium due to damage to barrier integrity has been shown in asthma. Damage to the epithelial cells causes secretion of alarmins, chemokines, and other cytokines. The epithelial cell (EC)-derived cytokines such as TSLP, IL-5, IL-33, and GM-CSF can induce the differentiation and activation of Th2 cells, ILC2s, basophils, eosinophils, and alternatively activated macrophages.<sup>(35)</sup> Some chemokines have a crucial role in the pathophysiology of asthma. The chemokines and their receptors involved in asthma are shown in Figure 2.

### Eotaxin

Eotaxins are proteins that belong to the chemokine family, consisting of eotaxin-1

(CCL11), eotaxin-2 (CCL24), and eotaxin-3 (CCL26), each of which attracts and activates CCR3-bearing cells. Eotaxin-1 was the first eosinophil-specific chemoattractant to be discovered, followed by eotaxin-2 and eotaxin-3. According to a study, eotaxin-1 levels are correlated with the number of eosinophils found in samples of pig blood and lung, being crucial for eosinophil mobilization from the bone marrow. Children with stable asthma reported greater amounts of eotaxin-1 compared to controls, and they also had sputum eosinophilia. To respond to inflammation in the early stages, eotaxin-1 is required, while eotaxin-2 and eotaxin-3 are required later to keep eosinophils alive. Eotaxin-1 has also been found as a chemokine that has a central role in producing airway hyper-reactivity.<sup>(36,37)</sup> A recent study also showed that serum levels of eotaxin-2 and transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) were substantially greater in patients with severe eosinophilic

asthma, there being a significant association between these two cytokines.<sup>(35)</sup> After stimulation with IL-4 and IL-13, eotaxins are produced by lung epithelial cells, eosinophils, lymphocytes, mast cells, vascular endothelial cells, dermal fibroblasts, alveolar macrophages, and in smaller amounts by smooth muscle cells of the airways. Cutaneous and nasal epithelial cells, fibroblasts, and macrophages are the principal sources of eotaxin-2 (CCL24) in the human body, while eotaxin-3 is produced primarily by endothelial cells and dermal fibroblasts. When eotaxin activates the CCR3 receptor, the ligand is internalized, and chemotaxis is induced via calcium mobilization and actin polymerization. With IL-5, eotaxin primarily attracts eosinophils to the lungs.<sup>(36–38)</sup>

### CCL17 and CCL22

Epithelial cell-derived chemokines bind their receptors CCR3 and CCR4 in Th2 cells. The chemokine receptor CCR13 interacts with CCR3, while ligands for CCR4 (that is highly expressed by Th2 cells) are CCL17, also referred to as TARC (thymus and activation-regulated chemokine) and CCL22, a macrophage-derived chemokine. Both CCL17 and CCL22 are chemoattractants for Th2 cells, induce the differentiation of naive T cells into Th2 cells, and are considered a marker of human and murine M2a macrophages.<sup>(37,39)</sup>

CCL17 is one of the chemokines that has been linked to type 2 immunological responses. Human CCL17/TARC is an 8-kDa, 71-amino-acid protein that is encoded on chromosome 16q13. Mature myeloid dendritic cells and Langerhans cells are the main sources of this chemokine. CCL17 has a biological effect in the trafficking of Th2 cells in eosinophil-associated disorders, such as allergic asthma, by binding to CCR4. As a result, increased levels of CCL17 in serum and/or tissue, as well as cellular CCR4 expression, may serve as biomarkers for disease severity. CCL17 production is stimulated by IL-4 in immune cells, which synergizes with various cytokines. CCL17 production is initiated also by

IL-3, TNF- $\alpha$ , and IFN- $\gamma$ , which operate synergistically in an NF $\kappa$ B-dependent manner in bronchial and alveolar epithelial cells.<sup>(13,40)</sup> By connecting to the CCL17 gene promoter directly via two binding sites, activation of signal transducer and activator of transcription 6 (STAT6) is a crucial step in IL-4-induced CCL17 production. In research on asthma, the CCL17-CCR4 axis plays a role in Th2 cell migration to the lungs. In individuals with asthma, ex vivo allergen exposure of human bronchial explants has been found to stimulate the activation of CCL17.<sup>(40)</sup> A clinical study showed a significant association between sputum eosinophilia and overexpression of CCL17 mRNA in the epithelium and submucosa of bronchial biopsies of patients with asthma compared to controls.<sup>(13)</sup>

CCL22, also known as macrophage-derived chemokine (MDC), has a 37% amino acid sequence homology with CCL17/TARC, but has a greater affinity (2 to 3-fold) for its receptor (CCR4) than CCL17. In allergic asthma, pulmonary CCL22 level is elevated. CCL22 is produced by immune cells such as dendritic cells, monocytes, natural killer (NK) cells, and macrophages. It is more effective in inducing lymphocyte VCAM-1 integrin-dependent arrest. CCL22 stimulates activated CCR4-positive Th2 cells, which keep the allergic process going.<sup>(40,41)</sup> The interaction of dendritic cells with naive T cells involves a binding between OX40L on dendritic cells with OX40 on naive T cells. After activation and differentiation of T cells, Th2 releases cytokines TNF- $\alpha$ , IL 4, IL-5, and IL-13 which cause excessive mucus secretion, disruption of the epithelial barrier, inflammation, and airway hyperresponsiveness. Secretion of CCL17 by ECs is stimulated by IL-4 and IL-13. Expression of CCL17 in bronchial epithelial cells is mediated by metalloproteinase-dependent phosphorylation of epidermal growth factor receptor (EGFR), MAPK, and nuclear factor  $\kappa$ B (NF- $\kappa$ B). IL-13 also induces CCL22 secretion. Expression of CCL17 and CCL22 is upregulated together with CCR4 to recruit Th2 cells.<sup>(37,39)</sup>



## CCL2

CCL2, also called MCP-1 (monocyte chemoattractant protein-1), is an important 13 kDa protein with 76 amino acids that is encoded on chromosome 17q11.2 and is involved in monocyte and macrophage migration, particularly in the lungs, where these cells turn into bronchoalveolar macrophages (BAMs).<sup>(42,43)</sup> Monocytes and macrophages are the most common sources of CCL2, but it is also produced by endothelial cells, mast cells, fibroblasts, epithelial cells, and smooth muscle cells. CCL2 causes intracellular signal transduction by interacting with the CCR2 receptor (a G-protein-coupled receptor), which is expressed by immune cells such as T cells, B cells, monocytes, and NK cells.<sup>(42-44)</sup> CCL2 may play a role in allergic airway responses, resulting in airway hyper-responsiveness by promoting mast cell activation and the production of inflammatory mediators such as leukotriene C4. CCL2 that is released from bronchial epithelial cells via upregulated gene expression is enhanced by cytokines IL-4 and IL-13.<sup>(43,44)</sup> In the study by Velikova et al., MCP-1/CCL2 levels were shown to be considerably greater in children with bronchial asthma (BA) and cystic fibrosis than in controls.<sup>(42)</sup>

## CCL5

CCL5 from the CC chemokine family, with a molecular weight of 7.5 kDa, is a powerful leukocyte chemoattractant that plays a key function in inflammation. It is also known as regulated upon activation, normal T-cell expressed and presumably secreted (RANTES), that activates and trafficks a wide spectrum of immune cells, including T-cells, eosinophils, dendritic cells monocytes, basophils, NK cells, to the site of inflammation when it interacts with its receptors, CCR1, CCR3, and CCR5. CCL5 levels have been found to be higher in asthma patients. Furthermore, targeting CCL5 with antibodies in the allergic mouse model inhibited inflammation of the airway.<sup>(45)</sup> A study suggested that CCL5 is linked to allergic inflammation. CCL5 was significantly higher in patients with atopic asthma than in

controls, and it was positively correlated with absolute eosinophil counts and total serum IgE in juvenile asthma patients. In Asian and Caucasian populations, two CCL5 SNPs (located at -403G/A and -28C/G, respectively) were linked to the risk of asthma.<sup>(13)</sup>

Eosinophils are key effector cells in the airway inflammation of type 2 asthma,<sup>(37)</sup> and also has an important effect in airway remodeling. Interaction of eosinophils with mast cells in an in-vitro study showed that secretion of fibrogenic factors was followed by the release of TGF- $\beta$ 1 from eosinophils, while interaction of eosinophils with epithelial cells promoted the release of epithelial-derived cytokines such as CCL5, CXCL8, and CCL11. These chemokines will induce eosinophil infiltration into the airway tissues. Eosinophil granules contain a variety of cytokines, enzymes, extracellular matrix, growth factors, and chemokines, including CCL3, CCL5, CCL7, CCL8, CCL1, CCL13, CCL17, CCL22 and CXCL1, CXCL8, CXCL9, CXCL10, CXCL12,<sup>(46)</sup> among which CCL5 and CCL11 are potent eosinophil chemoattractants. CCL11 induces eosinophil chemotaxis through the MAPK and extracellular regulated protein kinase (ERK) pathways, while CXCL8 and CXCL10 induces eosinophil chemotaxis by binding to CXCR2 and CXCR3, respectively. The eosinophil invasion is associated with asthma severity.<sup>(37,47)</sup>

## CXCL8 and CXCL10

Neutrophils are involved in non-type 2 asthma, in which the patients show a neutrophil-predominant phenotype without evident Th2 cytokines. Potent chemoattractants for neutrophils are CXCL8 and CXCL10. A study by Takaku et al. identified that both CXCL8 and CXCL10 are increased in asthma phenotypes with increased neutrophils and eosinophils. CXCL8 binds to CXCR2, a specific surface receptor on neutrophils.<sup>(11,37)</sup> In addition, CXCL1 and CXCL5 also bind to CXCR2.

CXCL8/interleukin-8/neutrophil activating protein-1 (NAP-1), is a potent neutrophil chemoattractant and activator that is released by

neutrophils, macrophages, epithelial cells, and bronchial smooth muscle cells. Neutrophils can force their own recruitment by producing IL-8 when an inflammatory response occurs.<sup>(48,49)</sup> Like many other chemoattractants, CXCL8 causes cytoskeleton reorganization, intracellular  $Ca^{2+}$  alterations, integrin activation, granule protein exocytosis, and respiratory burst.<sup>(48)</sup> CXCL8 also attracts circulating monocytes, which develop into macrophages in the lungs and are assumed to be responsible for neutrophilic inflammation.<sup>(50)</sup>

The binding of CXCL1 and CXCL5 to CXCR2 activates neutrophils, stimulates adhesion molecule expression, and cell homing. The cytokine IL-17 induces CXCL1 in bronchial EC mainly via MPK and ERK pathways. IL-17 also promotes CXCL5 expression in EC. While CXCL10 secretion in EC cells is stimulated via the janus kinase (JAK) pathway in synergy with TNF- $\alpha$  and interferon- $\gamma$ , CCR6 is expressed by various cell subsets, including dendritic cells, T cells, and B cells. In the T-cell subset, the Th17 cell has the highest CCR6 expression. CCR6 binds to its receptor, CCL20, which results in Th17 migration to the lungs. A study in patients with asthma showed enhanced CCR6 expression on Th cells in peripheral blood mononuclear cells (PBMCs) compared to control subjects.<sup>(11,37)</sup>

Eosinophils are thought to have a role in asthma exacerbation through a variety of processes, including activation of CXCL10 and contact with neutrophils.<sup>(51)</sup> CXCL10 or interferon gamma-inducible protein (IP)-10 is produced by activated T cells, while its ligand (CXCR3) is highly expressed by Th1 cells. A study on virus-induced pediatric asthma showed elevated serum CXCL10 levels, implying that viral infections can trigger Th1-chemokine responses in asthmatic patients.<sup>(52)</sup> IP levels in pe-diatric asthma are positively correlated with the severity of airway obstruction. Thus, CXCL10 (IP-10) may be of benefit as a marker of inflammatory asthma severity.<sup>(53)</sup> CXCL10

with proinflammatory cytokines/chemokines such as TNF- $\alpha$ , IL-6, CXCL8, act together in the development and worsening of allergic asthma.<sup>(52)</sup> Through CXCR3, which is expressed on eosinophils, CXCL10 causes numerous effects including eosinophil adhesion,  $O_2^-$  generation, and in vitro cytokine production.<sup>(51)</sup>

A summary of the role of chemokines in allergic inflammation of asthma is listed in Table 1.

## CONCLUSION

Asthma is a common heterogeneous chronic respiratory disorder with a variety of phenotypes and endotypes. Chemokines have a prominent contribution to the process of allergic inflammation in asthma and their trafficking is driven by G protein-coupled receptors (GPCRs). The main sources of chemokines that are associated with allergic inflammation in the pathogenesis of asthma are epithelial cells, dendritic cells, eosinophils, and macrophages. Many chemokines are involved in the recruitment of inflammatory cells in the allergic reactions of asthma, particularly eotaxin, CCL17 (TARC), CCL22 (MDC), CCL2, and CCL5 (RANTES). Interaction of chemokines with chemokine receptors and their activation eventually direct leukocyte migration to the site of tissue inflammation. The inhibition of the interaction between chemokines and their receptors could be useful in the development of potent therapeutic drugs for asthma.

## CONFLICT OF INTEREST

All authors declare no conflict of interest.

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Table 1. The role of chemokines in allergic inflammation of asthma

References	Type of article	Chemokines	Mechanism of action
Zajkowska, et al. <sup>(36)</sup>	Literature review	Eotaxin 1, Eotaxin 2, Eotaxin 3	Mobilization of eosinophils, mast cells, and Th2 lymphocytes to the inflammation site.
Liu, et al. <sup>(37)</sup>	Literature review	Eotaxin 1	Crucial chemoattractant for eosinophils.
Bakakos, et al. <sup>(38)</sup>	Literature review	Eotaxin 1	Mobilization of eosinophils from the bone marrow.
Abdelaziz, et al. <sup>(39)</sup>	Literature review	Eotaxin 2, Eotaxin 3 CCL17, CCL22	Keeps eosinophils alive.
Catherine, et al. <sup>(40)</sup>	Literature review	CCL17	Induce the differentiation of naive T cells into Th2 cells and chemotaxis for Th2 cells to the lungs.
Rapp, et al. <sup>(41)</sup>	Original article	CCL22	Chemotaxis for Th2 cells to the lungs.
Velikova, et al. <sup>(42)</sup>	Original article; a cross-sectional study	CCL2	Maintains the allergic reaction by activating CCR4-positive Th2 cells.
Wang, et al. <sup>(43)</sup>	Literature review	CCL2	Attracts monocytes and macrophages to the lungs.
Bawazeer, et al. <sup>(44)</sup>	Original article	CCL2	Attracts monocytes and eosinophils, and induces mast cell activation and leukotriene C4 secretion.
Alturaiki, et al. <sup>(45)</sup>	Original article	CCL5	Promotes mast cell migration to the inflammation site.
Busse, et al. <sup>(46)</sup>	Literature review	CCL5	Activates and mobilizes T-cells, dendritic cells, monocytes, basophils, and NK cells, to the site of inflammation.
Kyriakopoulos, et al. <sup>(50)</sup>	Literature review	CXCL8 CXCL8	Induces infiltration of eosinophils, mast cells, Th2 lymphocytes, and basophils into the airway.
Nakagome & Nagata. <sup>(51)</sup>	Literature review	CXCL10	Promotes eosinophil infiltration into the airway.
Huoman. et al. <sup>(52)</sup>	Original article; a cross-sectional and longitudinal study	CXCL10	Attracts circulating monocytes into the airway.
			Stimulates eosinophil adhesion and O <sub>2</sub> <sup>-</sup> generation, resulting in worsening of allergic asthma.
			Associated with development of sensitization.

## CONTRIBUTORS

FC and SA contributed to conceptualization. SA contributed to drafting the article and the manuscript's revision. FC contributed to supervision and review. All authors have read and approved the final manuscript. ✚

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