Chapter

Chemokines Effective on Platelet Functions

Asuman Akkaya Fırat

Abstract

Chemokines or chemotactic cytokines are chemical signaling molecules that have a regulatory effect on the orientation of endothelial and epithelial cells, especially leukocytes, immune and inflammatory response, and cell regeneration. They are important in the management of endothelial damage, physical harm, atherosclerosis, vascular injury, bleeding, coagulation, interneuron transmission, and platelet functions. Chemokines are divided into four main subfamilies: CXC, CC, CX3C, and C. All of these proteins exert their biological effects by interacting with G-protein-coupled transmembrane receptors called chemokine receptors, which are selectively present on the surfaces of their target cells. Platelet chemokines increase the recruitment of various hematopoietic cells to the vascular wall by nurturing processes, such as neointima formation, atherosclerosis, and thrombosis, while also promoting vessel repair and regeneration after vascular injury. Regarding platelets, CXCL4 (platelet factor 4 and PF4) and the chemokine CXCL7, which is processed from platelet basic protein to connective tissue activating peptide-III and β-thrombomodulin, to its active form neutrophil-activating peptide-2, which are the most abundant. In this chapter, chemokines that are more effective on platelets will be discussed.

Keywords: chemokines, platelets, platelet-activating factor (PAF)

1. Introduction

Chemokines or chemotactic cytokines are chemical signaling molecules that have a regulatory effect on the orientation of endothelial and epithelial cells, especially leukocytes, immune and inflammatory response, and cell regeneration. They are important in the management of endothelial damage, physical damage, atherosclerosis, vascular injury, bleeding, coagulation, interneuron transmission, and platelet functions. Chemokines are divided into four main subfamilies: CXC, CC, CX3C, and C. All of these proteins exert their biological effects by interacting with G-protein-coupled transmembrane receptors called chemokine receptors, which are selectively present on the surfaces of their target cells. Platelet chemokines increase the recruitment of various hematopoietic cells to the vascular wall by nurturing processes, such as neointima formation, atherosclerosis, and thrombosis, while also promoting vessel repair and regeneration after vascular injury. Regarding platelets, CXCL4 (platelet factor 4 and PF4) and the chemokine CXCL7, which is processed from platelet basic protein to connective tissue activating peptide-III and β-thrombomodulin to its active

form neutrophil-activating peptide-2, which are the most abundant [1–5]. In this chapter, chemokines that are more effective on platelets will be discussed.

2. Chemokines

The word "chemokine" comes from the ancient Greek word "alchemy" and "kinesis." Cytokines are a family of small cytokines or signaling proteins that are secreted by different cells, especially immune system cells, and induce the movement, communication, and secretory functions of other cell types, including leukocytes as well as endothelial and epithelial cells [1, 2]. In addition to playing an important role in the activation of host immune responses, chemokines are important in biological processes, such as morphogenesis, hemostasis, wound healing, and also in the pathogenesis of diseases, such as cancer [1–3]. Chemokines are classified according to their behavioral and structural properties. All chemokines are small molecules, about 8–10 kDa by mass. The amino acid number and sequence of different chemokine molecules are 20–50% the same. It has four cysteine residues that are the basis for creating the Greek-key-like 3D shapes in basal positions. The first two cysteines in a chemokine are located near the N-terminal end of the protein, the third cysteines in the center of the molecule, and the fourth is near the C-terminal region. This is followed by a single-turn helix called a 3_{10}-helix, three β-strands, and a C-terminal α-helix. These helices and strands are connected by turns called the 30s, 40s, and 50s loops; the third and fourth cysteines are located in the 30s and 50s loops [4]. Chemokines are found in all vertebrates, some viruses, and some bacteria, but none have been found in other invertebrates [5].

Members of the chemokine family are divided into four groups based on the framework formed by the first two cysteine residues:

1. C chemokines

2. CXC chemokines

3. CC chemokines

4. CX_3C chemokines [4]

C chemokines (or γ-chemokines) differ from all other chemokines in that it contains only two cysteines; an N-terminal cysteine and a second cysteine downstream. Two chemokines have been identified for this subgroup and are designated XCL1 (lymphotactin-α) and XCL2 (lymphotactin-β). Thus, the terminology of chemokines is, for example, CCL1 for ligand 1 of the CC-family of chemokines and CCR1 for its corresponding receptor [4].

CXC chemokines the two N-terminal cysteines (or α-chemokines) are separated by an amino acid represented by an "X." There are 17 different CXC chemokines identified in mammals, with a specific amino acid sequence *glutamic acid-leucine-arginine (ELR domain)* just before the first cysteine of CXC. Chemokines with an ELR extension are called ELR-positive and those without an ELR extension are called ELR-negative. ELR-positive chemokines, specifically induce the migration of neutrophils. An example of an ELR-positive CXC chemokine is interleukin-8 (IL-8), which induces neutrophils to leave the bloodstream and enter surrounding tissue. Another

example is CXCL13, which is ELR negative and tends to be chemotactic for lymphocytes. Seven of the CXC chemokines have been discovered to date, and these ligands interact with their receptors, CXCRs. Receptors called CXCR1-7 belong to this group of chemokines [6].

CC chemokines (or β-chemokine) proteins have two adjacent cysteines (amino acids), near their amino terminus. There have been at least 27 distinct members of this subgroup reported for mammals, called CC chemokine ligands (CCL)-1 to –28; CCL10 is the same as CCL9. Chemokines of this subfamily usually contain four cysteines (C4-CC chemokines), but a small number of CC chemokines possess six cysteines (C6-CC chemokines). C6-CC chemokines include CCL1, CCL15, CCL21, CCL23, and CCL28 [4–7]. CC chemokines induce the migration of monocytes and other cell types, such as NK cells and dendritic cells. Examples of CC chemokine include monocyte chemoattractant protein-1 (MCP-1 or CCL2), which induces monocytes to leave the bloodstream and enter the surrounding tissue to become tissue macrophages. CCL5 (or RANTES) attracts cells, such as T cells, eosinophils, and basophils, that express the receptor CCR5. Increased CCL11 levels in blood plasma are associated with aging (and reduced neurogenesis) in mice and humans [7].

As a result, cell movement is achieved [1]. Chemokines, according to their amino acid composition, especially are grouped according to the first two cysteine residues of a conserved tetra-cysteine motif: the **CC** and **CXC** form of chemokines are the two largest groups. For example, CX3CL1, XCL1, and XCL2 are named. There are 47 known chemokines, and 19 chemokine receptors [8]. Chemokines that increase leukocyte migration include CCL14, CCL19, CCL20, CCL21, CCL25, CCL27, CXCL12, and CXCL13. Inflammatory provocateurs (such as IL-1 and TNF-alpha) include CXCL-8, CCL2, CCL3, CCL4, CCL5, CCL11, and CXCL10 [6]. This classification is not rigid; for example, CCL20 may also act as a proinflammatory chemokine [5].

3. Receptors

For the cell to respond to a chemokine, it must have a specific chemokine receptor "R." Following binding to the chemokine receptor, it associates with G proteins to transmit cell signals. Activation of G proteins by chemokine receptors then causes activation of an enzyme known as phospholipase C (PLC). PLC cleaves a molecule called phosphatidyl inositol (4,5)-biphosphate (PIP2) into two-second messenger molecules known as inositol triphosphate (IP3) and diacylglycerol (DAG), which trigger intracellular signaling; DAG activates another enzyme called protein kinase C (PKC) and IP3 initiates calcium release from intracellular stores. These reactions trigger multiple signaling cascades (such as the MAP kinase pathway) that produce responses, such as chemotaxis, degranulation, their lease of superoxide anions, and changes in the affinity of cell adhesion molecules called integrins, within the cell that host the chemokine receptor. Chemokine receptors usually belong to the broad group of receptors attached to G-protein (GPCRs). Related to chemokine a calcium signaling cascade is created by binding to its receptor and then causes the activation of small GTPases. This then has downstream activation of integrins (adhesion molecules in the cell) affects and promotes actin polymerization. A pseudopod (cellular projection) appears with polarized cell morphology [5–7].

Homeostatic chemokines are produced continuously in the thymus and lymphoid tissues. It is the chemokines CCL19 and CCL21 (expressed in lymph nodes and on lymphatic endothelial cells) that undertake homeostatic functions in homing, and

both have the same receptor CCR7. As a result of binding with these ligands, it is possible to direct antigen-presenting cells (APC) to lymph nodes during the adaptive immune response. Other homeostatic chemokine receptors include CCR9, CCR10, and CXCR5. CCR9 promotes the migration of leukocytes to the gut, CCR10 to the skin, and CXCR5 provokes the migration of B cells to the follicles of the lymph nodes. In addition, CXCL12 (SDF-1: stromal cell-derived factor 1), which is produced structurally in the bone marrow, supports the proliferation of progenitor B cells in the bone marrow microenvironment [7, 8]. The protein encoded by this gene is a member of the CXC chemokine family and is a majör mediator of the inflammatory response. The encoded protein is commonly referred to as interleukin-8 (C-X-C motif chemokine ligand 8). IL-8 is secreted by mononuclear macrophages, neutrophils, eosinophils, T lymphocytes, epithelial cells, and fibroblasts. Bacterial and viral products induce IL-8 expression. IL-8 also participates with other cytokines in the pro-inflammatory signaling cascade and plays a role in systemic inflammatory response syndrome (SIRS). This gene is believed to play a role in the pathogenesis of the lower respiratory tract infection bronchiolitis, a common respiratory tract disease caused by their respiratory syncytial virus (RSV) [8–10]. The overproduction of this pro-inflammatory protein is thought to cause lung inflammation associated with cystic fibrosis. This pro-inflammatory protein is also suspected of playing a role in coronary artery disease and endothelial dysfunction. This protein is also secreted by tumor cells and promotes tumor migration, invasion, angiogenesis, and metastasis. This chemokine is also a potent angiogenic factor [8–10]. The binding of IL-8 to one of its receptors (IL-8RB/CXCR2) increases the permeability of blood vessels and increasing levels of IL-8 are positively correlated with increase severity of multiple disease outcomes (e.g., sepsis). This gene and other members of the CXC chemokine gene family form a gene cluster in a region of chromosome 4q [7–11]. Chemokines, according to the types of cells affect monocytes/macrophages. Chemokines that mobilize these cells toward the site of inflammation include CCL2, CCL3, CCL5, CCL7, CCL8, CCL13, CCL17, and CCL22 [9, 12].

3.1 T-lymphocytes

Chemokines that attract T lymphocytes to the site of inflammation are: CCL2, CCL1, CCL22, and CCL17. In addition, CXCR3 expression is mediated by activated T cells. IFN-y-inducible chemokines are CXCL9, CXCL10, and CXCL11 [9, 12].

3.2 Mast cells

Express receptors for chemokines on their surface are: CCR1, CCR2, CCR3, CCR4, CCR5, CXCR2, and CXCR4. The ligands of these receptors CCL2 and CCL5 play an important role in mast cell recruitment and activation in the lung. There is also evidence that CXCL8 can inhibit mast cells [9–12].

3.3 Eosinophils

The migration of eosinophils various tissues is provoked by several chemokines of the CC family, which are as follows: CCL11, CCL24, CCL26, CCL5, CCL7, CCL13, and CCL3. The chemokines CCL11 (eotaxin) and CCL5 (RANTES) act via a specific CCR3 receptor on the surface of eosinophils, and eotaxin plays an important role in the initial recruitment of eosinophils to the lesion [9–12].

3.4 Neutrophils

Regulated primarily by CXC chemokines. CXCL8 (IL-8) is particularly chemotactic for neutrophils and activates their metabolism and degranulation [10–12].

Platelets are nuclear cell fragments derived from megakaryocytes that contain both pro-inflammatory and (anti-inflammatory) fragments in abundance. In particular, they play important roles in hemostasis. Angiogenic mediators physiological and pathological conditions in the vascular system, and immune cells are important in regulating platelet functions. Platelets as the main players in thrombosis and hemostasis. It is becoming a more interesting topic with increasing discoveries in the composition of inflammatory and immune-modulating molecules [10–12]. An important phenomenon also emerges in the atherosclerosis of platelets. It found an association between increased platelet concentration, aggregation, and the long-term incidence of fatal coronary heart disease in a population of apparently healthy middle-aged men [12]. Many platelet-derived substances and chemokines family of interesting proteins stored in it forms α-granules and exhibits numerous biological activities. In addition, chemokines are reexpressed by platelets [12, 13].

4. Platelet chemokines

The first recognized platelet chemokine PF-4 is now known as CXCL4. TGF beta-1 is also considered a chemokine. The NAP-2 fragment, now called CXCL7, is also a platelet chemokine. CCL3 (MIP-1), CCL5 (RANTES), CCL7 (MCP-3), and CXCL1 are also platelet-related chemokines. CCL17 (TARC) has recently been reported in platelets. CCL17 is an autocrine factor that increases platelet activation and its receptor CCR4 is also found in platelets. When platelets in plasma are activated *in vitro*, serum concentrations of these chemokines reach the range of 1–5 mol/L [13–17] **Table 1**.

CXCL4 was the first chemokine, whose effects on platelets were reported in 1985. Platelet factor 4 (PF4) is a minor cytokine belonging to the CXC family of chemokines, also known as chemokine ligand 4 (CXCL4). This chemokine is released from

Chemokine	Alternative name	Receptor
CXCL1	GRO-α	CXCR2 > CXCR1
CXCL4	PF4	CXCL3B, GAG
CXCL4L1	PF4alt	Unknown
CXCL5	ENA-78	CXCR2
CXCL7	PBP, βTG, CTAPIII, NAP-2	CXCR > CXCRI
CXCL8	IL-8	CXCRI, CXCR2
CXCL12	SDF-I α	CXCR4
CCL2	MCP-I	CCR2
CCL3	MIP-I α	CCRI, CCR2, CCR3
CCL5	RANTES	CCRI, CCR3, CCR4, CCR5
CCL17	TARC	CCR4, CCR8

Table 1.
The alternative names and receptors of chemokines.

the alpha granules of activated platelets during platelet aggregation and provokes blood coagulation by regulating the effects of heparin-like molecules. Due to these roles, it is predicted that they will also play a role in wound healing and vascular repair [5]. It is usually found in a complex with proteoglycan [18].

The human PF4 gene is located in human chromosome 4. Platelet factor 4 is a 70 amino acid-containing protein that is released from the alpha granules of activated platelet sand and binds to heparin with high affinity. Its main physiological role is thought to be the neutralization of heparin-like molecules on the endothelial surface of blood vessels, thereby inhibiting local antithrombin activity and provoking coagulation. As a potent chemotactic factor for neutrophil sand fibroblasts, PF4 probably has a role in inflammation and wound healing [19, 20]. PF4 is also a chemotactic factor for neutrophils, fibroblasts, and monocytes, and interacts with an additional counterpart of the chemokine receptor CXCR3, known as CXCR3-B [20].

PF4 complex is the antigen in heparin-induced thrombocytopenia (HIT), which is an autoimmune reaction specific to anticoagulant heparin administration [21]. PF4 autoantibodies have also been found in patients with thrombosis and similar to HIT, but who have not been given heparin before [22]. Antibodies against PF4 have been blamed in cases of thrombosis and thrombocytopenia after vaccination with the Oxford-Astra Zeneca or Janssen COVID-19 vaccine [23, 24]. This phenomenon was named vaccine-induced immune thrombotic thrombocytopenia (VITT) [25]. A relationship was also found in PF4 expression with long-term COVID symptoms [26]. It increases in patients with systemic sclerosis, who also have interstitial lung disease [27].

Human platelet factor 4 also specifically decomposes the digestive vacuole of the malaria parasite and neutralizes the malaria parasites in erythrocytes [28].

Transforming growth factor-beta 1 (TGF-beta1) is a polypeptide member of the transforming growth factor beta superfamily of cytokines. It is a secreted protein that performs many cellular functions, including the control of cell growth, proliferation, differentiation, and apoptosis. In humans, TGF-β1 is encoded on the TGFB1 gene [28, 29].

5. CXCL7

TGF-β Transforming growth factor-beta 1 (TGF-beta1) is a polypeptide member of the transforming growth factor beta superfamily of cytokines. It is a secreted protein that performs many cellular functions, including the control of cell growth, proliferation, differentiation, and apoptosis. In humans, TGF-β1 is encoded on the TGFB1 gene [28, 29].

A thrombomodulin (C-X-C motif) ligand 7, β-thrombomodulin (β-TG) or beta-thromboglobulin, is a chemokine protein secreted and stored by platelets [29–31]. Along with platelet factor 4 (PF4), β-TG is one of the themes specific platelet-specific proteins β-TG and PF4 are stored in platelet alpha granules and released during platelet activation [29, 32, 33]. In conclusion, it is a useful marker of platelet activation [29, 32]. β-TG also plays an important role in the maturation of megakaryocytes [34].

Among the chemokines stored and secreted by platelets, CXCL7 is the largest representative. β-TG levels are used as an index of platelet activation. It is measured in blood plasma or urine by the ELISA method and usually together with PF4. When platelets are active, CXCL4 is 0.4–1.9 μM in serum, while CXCL7 is 1.6–4.8 μM [35]. It is used as a measure for platelet activation [36].

It consists of proteolytic derivatives of 128 aa-precursor molecules called pre-PBP, the primary CXCL7 translation product. The primary sequence, pre-PBP is a 34 amino acid residue leader sequence [37]. β-TG is a molecule and N-terminal variant of pro CXCL7 containing 81 residue amino acids. Platelet basic protein (PBP; 94 aa), connective tissue activating peptide III (CTAP-III; 85aa), and neutrophil-activating neutrophil-activating (NAP-2, 70 aa), are CXCL7 N-terminal variants that diverge [38]. β-TG levels increase with age and in diabetes mellitus [39, 40]. β -TG levels were found to increase during treatment with synthetic ethinyl estradiol, but not significantly in that treated with the natural estradiol valerate [41–44]. β-TG levels were also found to be slightly increased or unchanged in an uncomplicated pregnancy [45].

Besides both CTAP-III and NAP-2o, the medium-size shortened CXCL7 variants, all its capacity to support various aspects of fibroblast metabolism has been demonstrated. For example, the synthesis of matrix components, such as hyaluronic acid and glycosaminoglycans (GAGs) [46]. Increased GLUT-1 glucose transporter expression and concomitant cellular glucose uptake are enhanced [47]. As a platelet-derived mediator, CXCL7 may also participate in reparative functions following vascular tissue injury. But the CXCL7, its role as a growth factor, is controversial. Thus, it is more likely that fibroblast mitogenic activity may be the task of full-length-PBP [48]. Like CXCL7, platelet factor 4 (PF4, CXCL4) and its closely related chemokine, platelet basic protein (PBP), are important in platelets. The role of PF4 in hemostasis/thrombosis *in vivo* has been demonstrated, PF4 plays a role in pathological thrombotic conditions, such as heparin-induced thrombocytopenia (HIT) and septic shock [48, 49].

The abundance of CXCL7 variants follows a specific sequence of proteolytic vents during platelet production and activation. Thus, the main player on megakaryocytes is PBP. A small amount of CTAP-III also provides stimulation. The proportion of the shorter variant CTAP-III as platelets mature increases, and the proportion of PBP drops to about 25% [49].

Removal of the inhibitory N-terminus to activate CXCL7 as a neutrophil-directed chemokine underlies its potential role for intravascular and extravascular. As we have shown, the translation of CXCL7 to NAP-2 is mainly catalyzed by NAP-2 target neutrophils. Neutrophil activation occurs via the serine protease cathepsin G-linked plasma inhibitors, which are not effective [50].

6. CXCL1

CXCL1 is a small peptide belonging to the CXC chemokine family that acts as a chemoattractant for several immune cells, especially neutrophils [51] or other non-hematopoietic cells, to the site of injury or infection and plays an important role in the regulation of immune and inflammatory responses. It was previously called the GRO1 oncogene, GROα, neutrophil-activating protein 3 (NAP-3), melanoma growth stimulating activity, alpha (MGSA-α). It is also known as keratinocytes-derived chemokine (KC) in mice or cytokine-induced neutrophil chemoattractant type-1 (CINC-1) in rats. In humans, this protein is encoded by the gene Cxcl1 [5] and is located on human chromosome 4 among genes for other CXC chemokines [52].

Under normal conditions, CXCL1 is not constitutively expressed. It is produced by activated macrophages, neutrophils, and epithelial cells, or by different immune cells, such as Th17. Moreover, its expression is indirectly provoked by IL-1, TNF-α, or IL-17 released by Th17 cells [11]. It plays a major role in inflammation [53, 54].

CXCL1 has a potentially similar effect as interleukin-8 (IL-8/CXCL8). Binds to the CXCR2, receptor CXCL1 triggers phosphatidyl inositol-4,5-biphosphate 3-kinase-y (PI3Kγ)/Akt, MAP kinases, such as ERK1/ERK2 or phospholipase-β (PLCβ) signaling pathways. CXCL1 increases the expression of inflammatory responses, and thus, contributes to the inflammation process [12]. CXCL1 is also involved in wound healing and oncogenesis processes [55–57].

CXCL1 has been shown to have roles in the development of breast cancer, gastric and colorectal carcinoma, or lung cancer tumors [58, 59]. In addition, it has been reported that CXCL1 is secreted by human melanoma cells and plays a role in mitogenic activity [60–62].

CXCL1 is expressed by neurons and oligodendrocytes in the brain and spinal cord and by microglia during pathologies, such as Alzheimer's disease, multiple sclerosis, and brain damage. A study in mice shows evidence that CXCL1 reduces the severity of multiple sclerosis [23]. In addition, CXCL1 contributes to CXCL1, playing a role in spinal cord development by acting on oligodendrocytes [7]. CXCR2 receptors for s to the release of prostaglandins, thereby resulting in increased sensitivity to pain. It initiates nonspecific sensitivity through the recruitment of neutrophils into the tissue. It increases the transcription of genes that induce chronic pain, such as cyclooxygenase-2 (COX-2) [12].

7. CCL 3

Chemokine (C-C motif) ligand 3 (CCL3) also known as macrophage inflammatory protein 1-alpha (MIP-1-alpha), is located on the CCL3 gene in humans[3]. By binding to all of the CCL3, CCR1, CCR4, and CCR5 receptors, it may play a role in the the recruitment and activation of polymorphonuclear leukocytes [63] in acute inflammatory conditions. Sherry et al. showed two protein subcomponents of MIP-1 called alpha (CCL3) and beta (CCL4) [64, 65]. CCL3 can produce rapid-onset symptoms of monophasic fever that are greater than or equal to fevers produced by recombinant human tumor necrosis factor or recombinant human interleukin-1. Moreover, unlike these two endogenous pyrogens, MIP-1-induced fever is capable of producing cyclooxygenase-induced fever. It is not inhibited by ibuprofen. CCL3 may participate in a type of febrile response that is not produced by prostaglandin and cannot be clinically inhibited by cyclooxygenase. CCL3 has been shown to interact with CCL4 to activate macrophages, monocytes, and neutrophils [66].

CCL4 also known as macrophage inflammatory protein-1β (MIP-1β), is a CC chemokine that specifically binds to CCR5 receptors. It is chemotactic for natural killer cells, monocytes, and various other immune cells [67] CCL4 is an important HIV suppressive factor released by CD8+ T cells [68]. Performance low-memory CD8+ T cells that normally express MIP-1-beta. The concentration of this chemokine is inversely proportional to micro RNA-125b. The concentration of CCL4 in the body increases with age, which can lead to chronic inflammation and liver damage and may be a marker [69].

8. CCL5

Chemokine C-C ligand 5 (CCL5) is a protein encoded on the CCL5 gene in humans [70]. The gene was discovered in situ hybridization in 1990 [71]. Also known as

RANTES (regulated by activation, normal T-cell expressed and secreted). CCL5 belongs to the CC subfamily of chemokines, due to its adjacent cysteines near the N terminus. It is an 8 kDa protein acting as a classical chemotactic cytokine or chemokine. It consists of 68 amino acids. CCL5 is a pro-inflammatory chemokine, that recruit's leukocytes to the site of inflammation. It is chemotactic for T cells, eosinophils, and basophils, but also monocytes, natural killer (NK) cells, dendritic cells, and mastocytes [72]. With the help of particular cytokines (i.e., IL-2 and IFN-γ) that are released by T cells, CCL5 also induces the proliferation and activation of certain NK cells to form CHAK (CC-chemokine-activated killer) cells. It is also an HIV-suppressive factor released from CD8+ T cells [72, 73].

The chemokine CCL5 is mainly expressed by T cells and monocytes, and it is not expressed by B cells. Moreover, it is abundantly expressed by epithelial cells, fibroblasts, and thrombocytes. Although it can bind to receptors CCR1, CCR3, CCR4, and CCR5 belonging to seven transmembrane G-protein-coupled receptor (GPCRs) family [8], it has the highest affinity to the CCR5. CCR5 is presented on the surface of T cells, smooth muscle endothelial cells, epithelial cells, parenchymal cells, and other cell types. After the binding of CCL5 to CCR5, phosphoinositide 3-kinase (PI3K) is phosphorylated and subsequently, the phosphorylated PI3K phosphorylates protein kinase B (PKB; also known as Akt) on the serine 473. Then, the Akt/PKB complex phosphorylates and inactivates a serine/threonine protein kinase GSK-3. After the CCL5/CCR5 binding, some other proteins are regulated as well. Bcl2 is more expressed and it induces apoptosis [74, 75].

RANTES acts as a typical chemokine causing chemotaxis of mononuclear cells at nanomolar concentrations. Transendothelial migration of monocytes and lymphocytes is integrin-dependent and requires adhesion molecules from molecules, such as ICAM-1 and ICAM-1 [76, 77].

Due to resting integrins, tissue needs activation signals confirmation and only has a low affinity for its ligands. Chemokines are important stimuli for integrin activation as they are released during inflammation and induce adhesion in all types of leukocyte subsets. It is phosphorylated shortly after exposure to RANTES [78].

Until now, the mechanism of integrin-dependent adhesion of RANTES has not been fully elucidated. In addition to initiating cell migration at high concentrations, RANTES also acts independently of its G-protein-coupled receptors. It induces the release of T cells and pro-inflammatory mediators [79, 80]. Its unique capacity to form homotypic clusters and its high affinity for GAGs on the surface of endothelial cells, basement membrane and extracellular matrix RANTES will be immobilized [79–82].

AGs heparin-binding site associated with residues located at the C-terminus of PF4 tetramer displays a band of positively charged residues [83]. In the RANTES molecule, two basic amino acid clusters present heparin-binding motifs in the 40s loop between this cond and the third β-strand only in the C-terminal α-helix. The essential remains in the 40s loop are specific and it shows the high affinity of RANTES to different GAG species. Effective leukocyte arrest in the endothelium, especially unlike chemotaxis appears to depend on the formation of RANTES oligomers to bridge the surface-bound RANTES and CCR1 [84]. Selective binding of chemokines to subgroups of glycosaminoglycans cell surface induces polymerization facilitating their attachment it binds to receptor and enhances their effects on high-affinity receptors in the local microenvironment [85, 86]. In addition, structural motifs required for oligomerization RANTES are important for heterophilic interaction. RANTES with PF4 increases surface immobility and enhances monocyte adhesion to endothelial cells under flow conditions [86, 87].

9. Chemokines and vascular biology

Data from animal experiments suggest that activated platelets are involved in the pathogenesis of atherosclerosis, which indicates that it is important in acute thromboembolism [88, 89]. Monocyte recruitment into the subendothelial artery space is an early step in pathogenesis. Platelets with an affinity for GAGs accumulate in the luminal endothelium, where chemokines, such as RANTES activate monocytes [83, 90]. The role of RANTES in humans is reported to be less. In one study group, serum levels of RANTES were found to be lower in atherosclerosis compared to healthy controls 92. PF4 and NAP-2 as well been shown to play a role in atherosclerosis. PF4, which can reflect platelet activity. Studies have been conducted on its importance in atherosclerosis [91–94]. PF4 and, to a lesser extent, NAP-2 are associated with human atherosclerotic plaques [94]. Besides, PF4 is involved in the metabolism of atherogenic lipids, for example, oxidized low-density lipoprotein (LDL). It is stated that LDL (oxLDL) plays a role in the atheroma plaque [94].

Patients with stable and especially unstable angina exhibit markedly elevated plasma NAP-2 levels. PF4 may also play an important role in the acute coronary syndrome that causes plaque formation. But "how does PF4 affect thrombosis?" has not been finally clarified [95]. Angiogenesis, which may be beneficial in wound healing, also it is effective in pathological conditions, such as cancer and atherosclerosis. Capillary sprouting and endothelial cell proliferation VEGF (vascular endothelial growth factor), bFGF (essential fibroblast growth factor) by *in vitro* platelet releases, and PDGF (platelet-derived growth factor) can be induced by isolated platelets [96, 97]. NAP-2 accelerates endothelial cell healing with CXCR2-dependent fibronectin, fibrinogen, and platelet-coated endothelial matrix of endothelial progenitor cells [98]. On the other hand, platelet products PF4 and PF4alt are potent inhibitors of angiogenesis. PF4 can exert its angiostatic activity via CXCR3B [99] (**Figure 1**).

The mechanism mediated by CXCR2 constitutes a very important area of research. CXCR2 binds to a G-protein. Multiple ligands are available. CXCR2 results in activation by binding of chemokines. NFκB, MAPK, PI3K, and Rac 1 are among other

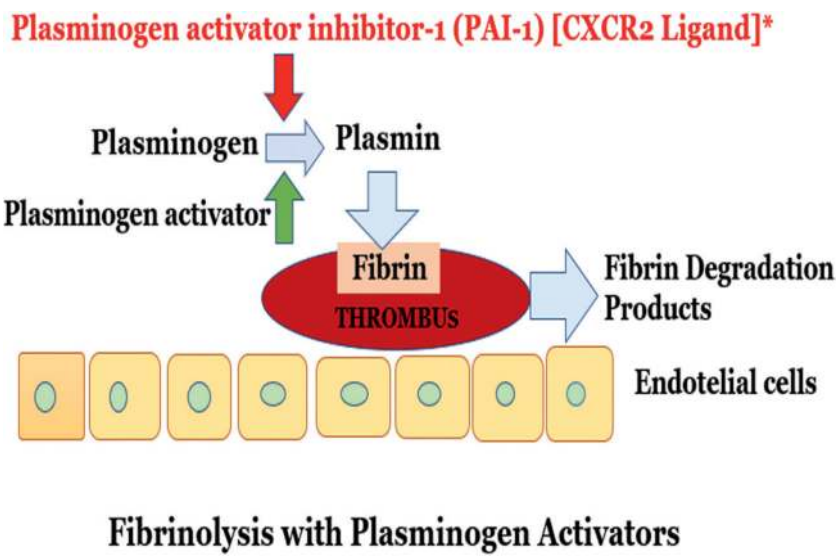

Figure 1.
Fibrinolysis with plasminogen activators.

signaling cascades. Activation by CXCR2 increases NAPDH oxidases, causing an explosion of reactive oxygen species (ROS). This ROS explosion plays a role in clearing pathogen infections by macrophages. It mediates the induction of apoptosis in cancer cells [100–102].

Congenital deficiency of PAI-1; since fibrinolysis is not sufficiently suppressed, it causes hemorrhagic diathesis. PAI-1 is found at increased levels in various disease states (such as several cancer types), as well as obesity and metabolic syndrome. It has been associated with increased thrombosis formation in patients with these conditions. PAI-1 can induce cellular senescence. PAI-1 appears to play an important role in the progression of fibrosis in inflammatory conditions, where fibrin accumulates in tissues. Possibly, lower PAI levels will lead to less suppression of fibrinolysis and conversely faster degradation of fibrin. Angiotensin II increases the synthesis of plasminogen activator inhibitor-1, thereby accelerating the development of atherosclerosis [103, 104].

Thrombotic complications are common in COVID-19 and contribute significantly to mortality and morbidity. Immune-mediated thrombotic mechanisms, complement activation, macrophage activation syndrome, antiphospholipid antibody syndrome, hyperferritinemia, and renin-angiotensin system dysregulation may be potential prognostic biomarkers in COVID-19. Recent studies are currently discussing the hypothetical benefits and anticipated challenges of therapeutic anticoagulation and fibrinolytic therapy in COVID-19 [105].

Abbreviations

CHAK	Chemokine-activated killer
COVID-19	Sars-Cov2 Virus 19
IP3	Inositol triphosphate3
LDL	Low-density lipoprotein
MCP- β	Monocyte chemoattractant protein-1 beta
MGSA	Melanoma growth stimulatingactivity alpha
MIP-1 β	Macrophage inflammatory protein 1beta
NAP3	Neutrophil activating protein-3
NK	Naturel killer
PAI1	Plasminogen activator inhibitor1
PIP2	Phosphatidyl inositol2
PF4	Platelet Factor4
PLC	Phospholipase C
RANTES	Regulated by activation normal Tcell expressed and secreted
ROS	Reactive oxygen species
RSV	Respiratory syncytial virus
SDF1	Stromal cell-derived factor1
SIRS	Systemic inflammatory response syndrome
TNFα	Tumor necrosis factor-alpha

Author details

Asuman Akkaya Fırat
Biochemistry Department, Fatih Sultan Mehmet (F.S.M) Training and Research Hospital, Health Sciences University (S.B.U), İstanbul, Turkey

*Address all correspondence to: asumanfirat44@gmail.com

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References

[1] Raman D, Sobolik-Delmaire T, Richmond A. Chemokines in health and disease. Experimental Cell Research. 2011;**317**(5):575-589

[2] Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. New England Journal of Medicine. 2006;**354**(6):610-621

[3] Davenport RD. An introduction to chemokines and theirroles in transfusion medicine. Vox Sanguinis. 2009;**96**(3):183-198

[4] Miller MC, Mayo KH. Chemokines form a structural perspective. International Journal of Molecular Sciences. 2017;**18**(10):2088

[5] Fernandez EJ, Lolis E. Structure, function, and inhibition of chemokines. Annual Review of Pharmacology and Toxicology. 2002;**42**:469-499

[6] Matsuo K, Yoshie O, Nakayama T. Multifaceted roles of chemokines and chemokine receptors in tumor immunity. Cancers (Basel). 2021;**13**(23):6132

[7] Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature. 2011;**477**(7362):90-94

[8] Laing KJ, Secombes CJ. Chemokines. Developmental and Comparative Immunology. 2004;**28**(5):443-460

[9] Xie JH, Nomura N, Lu M, Chen SL, Koch GE, Weng Y, et al. Antibody-mediated blockade of the CXCR3 chemokine receptor results in diminished recruitment of T helper 1cells into sites of inflammation. Journal of Leukocyte Biology. 2003;**73**(6):771-780

[10] Reeves EP, Williamson M, O'Neill SJ, Greally P, McElvaney NG. Nebulized hypertonic saline decreases IL-8 in sputum of patients with cystic fibrosis. American Journal of Respiratory and Critical Care Medicine. 2011;**183**(11):1517-1523

[11] Ang Z, Abdi GunawanKoen R, Er JZ, Lee LT, Tam Kit Chung J, Guo H, et al. Novel AU-richproximal UTR sequences (APS) enhance CXCL8 synthesisupon the induction of rpS6 phosphorylation. PLOS Genetics. 2019;**15**(4):e1008077

[12] Ono SJ, Nakamura T, Miyazaki D, Ohbayashi M, Dawson M, Toda M. Chemokines: Roles in leukocyte development, trafficking, and effector function. The Journal of Allergy and Clinical Immunology. 2003;**111**(6):1185-1199

[13] Deuel TF, Keim PS, Farmer M, Heinrikson RL. The amino acid sequence of human platelet factor 4. Proceedings of the National Academy of Sciences of the United States. 1977;**74**:2256-2258

[14] Dobroski DR, Rabbani LE, Loscalzo J. The relationship between thrombosis and atherosclerosis. In: Schafer JLAI, editor. Thrombosis and Hemorrhage. Baltimore, MD: Williams & Wilkins; 1998. pp. 837-861

[15] Dore M. Platelet-leukocyte interactions. The American Heart Journal. 1998;**135**:S146-S151

[16] Brandt E, Ludwig A, Petersen F, Flad HD. Platelet-derived CXC chemokines: Old players in new games. Immunological Reviews. 2000;**177**:204-216

[17] Hundelshausen P, Petersen F, Brandt E. Platelet-derived chemokines in vascular biology. Thrombosis and Hemostasis. 2007;**97**(05):704-713

[18] Eisman R, Surrey S, Ramachandran B, Schwartz E, Poncz M. Structural and functional comparison of the genes for human platelet factor 4 and PF4alt. Blood. 1990;**76**(2):336-344

[19] O'Donovan N, Galvin M, Morgan JG. Physical mapping of the CXC chemokine locus on human chromosome 4. Cytogenetics and Cell Genetics. 1999;**84**(1-2):39-42

[20] Lasagni L, Francalanci M, Annunziato F, Lazzeri E, Giannini S, Cosmi L, et al. An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as a functional receptor for platelet factor 4. The Journal of Experimental Medicine. 2003;**197**(11):1537-1549

[21] Warkentin TE. Drug-induced immune-mediated thrombocytopenia—from purpura to thrombosis. The New England Journal of Medicine. 2007;**356**(9):891-893

[22] Warkentin TE, Makris M, Jay RM, Kelton JG. A spontaneous prothrombotic disorders enabling heparin-induced thrombocytopenia. The American Journal of Medicine. 2008;**121**(7):632-636

[23] Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. The New England Journal of Medicine. 2021;**384**(22):2124-2130

[24] Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, EichingerS.Thromboticthrombocytopenia after ChAdOx1 nCov-19 vaccination. The New England Journal of Medicine. 2021;**384**(22):2092-2101

[25] Arepally GM, Ortel TL. Vaccine-induced immune thrombotic thrombocytopenia (VITT): What we know and don't know. Blood. 29 Jul 2021;**138**(4):293-298. DOI: 10.1182/blood.2021012152

[26] Ryan FJ, Hope CM, Masavuli MG, Lynn MA, Mekonnen ZA, Yeow AE, et al. Longterm perturbation of the peripheral immune system months after SARS-CoV-2 infection. BMC Medicine. 2022;**20**(1):26

[27] Volkmann ER, Tashkin DP, Roth MD, Clements PJ, Khanna D, Furst DE, et al. Changes in plasma CXCL4 levels are associated with improvements in lung function in patients receiving immunosuppressive therapy for systemic sclerosis-related interstitial lung disease. Arthritis Research & Therapy. 2016;**18**(1):305

[28] Love MS, Millholland MG, Mishra S, Kulkarni S, Freeman KB, Pan W, et al. Platelet factor 4 activity against P. Falciparum and its translation to nonpeptidic mimics as antimalarials. Cell Host & Microbe. 2012;**12**(6):815-823

[29] Sharma G, Berger JS. Platelet activity and cardiovascular risk healthy individuals: A review of the data. The Journal of Thrombosis and Thrombolysis. 2011;**32**(2):201-208

[30] Kaplan KL, Owen J. Plasma levels of beta-thromboglobulin and platelet factor 4 as indices of platelet activation in vivo. Blood. 1981;**57**(2):199-202

[31] Pillai MM, Iwata M, Awaya N, Graf L, Torok-Storb B. Monocyte-derived CXCL7 peptides in the marrow microenvironment. Blood. 2006;**107**(9):3520-3526

[32] Jumpupto Cella G, Scattolo N, Girolami A, Sasahara AA. Are platelet factor 4 and β-thromboglobulin markers of cardiovascular disorders? Ricerca in Clinic and Laboratory. 1984;**14**(1):9-18

[33] Jumpupto Cytokines and Cells Online Pathfinder Encyclopaedia. Beta-Thromboglobulin Retrieved on August 17, 2009

[34] Ha C-E, Bhagavan NV. Essentials of Medical Biochemistry: With Clinical Cases. Elsevier Inc.: All rights reserved Academic Press; 2015. p. 652 ISBN 978-0-12-095461-2. DOI: 10.1016/C2009-0-00064-6

[35] Loetscher P, Seitz M, Baggiolini M, Moser B. Interleukin-2 regulates CC chemokine receptor expression and chemotactic responsiveness in T lymphocytes. Journal Experimental Medicine. 1996;**184**:569-577

[36] Moore S, Pepper DS, Cash JD. The isolation and characterisation of a platelet-specific β-globulin (β-thromboglobulin) and the detection of antiurokinase and antiplasmin released from thrombin-aggregated washed human platelets. Biochimica et Biophysica Acta. 1975;**379**:360-369

[37] Begg GS, Pepper DS, Chesterman CN, et al. Complete covalent structure of human beta-thromboglobulin. Biochemistry. 1978;**17**:1739-1744

[38] Majumdar S, Gonder D, Koutsis B, et al. Characterization of the human beta-thromboglobulin gene. Comparison with the gene for platelet factor 4. The Journal of Biological Chemistry. 1991;**266**:5785-5789

[39] Martinelli I, Bucciarelli P, Mannucci PM. Thrombotic risk factors: Basic pathophysiology. Critical Care Medicine. 2010;**38**(2 Suppl):S3-S9

[40] Sterne J, Kirkwood BR. Essential Medical Statistics. Oxford: Blackwell Science; 2003

[41] Farris M, Bastianelli C, Rosato E, Brosens I, Benagiano G. Pharmaco dynamics of combined estrogen-progestin oral contraceptives: 2. effects on hemostasis. Expert Review of Clinical Pharmacology. 2017;**10**(10):1129-1144

[42] Kluft C, Lansink M. Effect of oral contraceptives on hemostasis variables. Thrombosis and Hemostasis. 1997;**78**(1):315-326

[43] Kuhl H. Adverse effects of estrogen treatment: Natural vs. synthetic estrogens. In: Lippert TH, Mueck AO, Ginsburg J, editors. Sex Steroid sand the Cardiovascular System: Proceedings of the 1st Interdisciplinary Workshop, Tuebingen, Germany, October 1996. London/New York: Parthenon; 1998. pp. 201-210

[44] Lindberg UB, Crona N, Stigendal L, Teger-Nilsson AC, Silfverstolpe G. A comparison between effects of estradiol valerate and low dose Ethinylestradiol on hemostasis parameters. Thrombosis and Hemostasis. 1989;**61**(1):65-69

[45] Hellgren M. Hemostasis during normal pregnancy and puerperium. Seminars in Thrombosis and Hemostasis. 2003;**29**(2):125-130

[46] Castor CW, Walz DA, Johnson PH, et al. Connective tissue activation. XXXIV: Effects of proteolytic processing on the biologic activities of CTAP-III. The Journal of Laboratory and Clinical Medicine. 1990;**116**:516-526

[47] Tai PK, Liao JF, Hossler PA, et al. Regulation of glucose transporters by connective tissue activating peptide-III isoforms. The Journal of Biological Chemistry. 1992;**267**:19579-19586

[48] Iida N, Haisa M, Igarashi A, et al. Leukocyte-derived growth factor links the PDGF and CXC chemokine families of peptides. The FASEB Journal. 1996;**10**:1336-1345

[49] Resmi KR, Krishnan LK. Proteaseaction and generation of beta-thromboglobulin-like protein followed by platelet activation. Thrombosis Research. 2002;**106**:229-236

[50] Harter L, Petersen F, Flad HD, et al. Connectivet issue-activating peptide III desensitizes chemokine receptors on neutrophils. Requirement forth proteolytic formation of the neutrophil-activating peptide 2. The Journal of Immunology. 1994;**153**:5698-5708

[51] Haskill S, Peace A, Morris J, Sporn SA, Anisowicz A, Lee SW, et al. Identification of three related human GRO gene encoding cytokine functions. USA: Proceedings of the National Academy of Sciences. Oct 1990;87(19):7732-7736. DOI: 10.1073/pnas.87.19.7732

[52] Ravindran A, Sawant KV, Sarmiento J, Navarro J, Rajarathnam K. Chemokine CXCL1 dimer is a potent agonist for the CXCR2 receptor. The Journal of Biological Chemistry. 2013;**288**(17):12244-12252

[53] Becker S, Quay J, Koren HS, Haskill JS. Constitutive and stimulated MCP-1, GRO alpha, beta, and gamma expression in human airway epithelium and bronchoalveolar macrophages. The American Journal of Physiology. 1994;**266**(3 Pt 1):L278-L286

[54] Ma K, Yang L, Shen R, Kong B, Chen W, Liang J, et al. Th17 cells regulate the production of CXCL1 in breast cancer. International Immunopharmacology. 2018;**56**:320-329

[55] Silva RL, Lopes AH, Guimarães RM, Cunha TM. CXCL1/CXCR2 signaling in pathological pain: Role in peripheral and central sensitization. Neurobiology of Disease. 2017;**105**:109-116

[56] Devalaraja RM, Nanney LB, Du J, Qian Q, Yu Y, Devalaraja MN, et al. Delayed wound healing in CXCR2 knockout mice. The Journal of Investigative Dermatology. 2000;**115**(2):234-244

[57] Reinecker HC, Loh EY, Ringler DJ, Mehta A, Rombeau JL. Mac Dermott RP. Monocyte-chemoattractant protein 1 gene expression in intestinal epithelial cells and inflammatory bowel disease mucosa. Gastroenterology 1995;**108**:40-50

[58] Chen X, Jin R, Chen R, Huang Z. Complementary action of CXCL1 and CXCL8 in the pathogenesis of gastric carcinoma. International Journal of Clinical and Experimental Pathology. 2018;**11**(2):1036-1045

[59] Hsu YL, Chen YJ, Chang WA, Jian SF, Fan HL, Wang JY, et al. Interaction between tumor-associated dendritic cells and colon cancer cells contributes to tumor progressionvia via CXCL1. International Journal of Molecular Sciences. 2018;**19**(8):2427

[60] Dhawan P, Richmond A. Role of CXCL1 in tumorigenesis of melanoma. Journal of Leukocyte Biology. 2002;**72**(1):9-18

[61] Tsai HH, Frost E, To V, Robinson S, Ffrench-Constant C, Geertman R, et al. The chemokine receptor CXCR2 controls the positioning of oligodendrocyte precursors in developing spinal cord by arresting their migration. Cell. 9 Aug 2002;**110**(3):373-383

[62] Omari KM, Lutz SE, Santambrogio L, Lira SA, Raine CS (2009). "Neuroprotection and remyelination after autoimmune demyelination in mice that inducibly overexpress CXCL1". The American Journal of Pathology. 174 (1):16476.

[63] Wolpe SD, Davatelis G, Sherry B, Beutler B, Hesse DG, Nguyen HT, et al. Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. The Journal of Experimental Medicine. 1988;**167**(2):570-581

[64] Sherry B, Tekamp-Olson P, Gallegos C, Bauer D, Davatelis G, Wolpe SD, et al. Resolution of the two components of macrophage inflammatory protein 1, and cloning and characterization of one of those components, macrophage inflammatory protein 1 beta. The Journal of Experimental Medicine. 1988;**168**(6):2251-2259

[65] Davatelis G, Wolpe SD, Sherry B, Dayer JM, Chicheportiche R, Cerami A. Macrophage inflammatory protein-1: A prostaglandin-independent endogenous pyrogen. Science. 1989;**243**(4894 Pt 1):1066-1068

[66] Guan E, Wang J, Norcross MA. Identification of human macrophage inflammatory proteins 1alpha and 1beta as a native secreted heterodimer. The Journal of Biological Chemistry. 2001;**276**(15):12404-12409

[67] Bystry RS, Aluvihare V, Welch KA, Kallikourdis M, Betz AG. B cells and professional APC srecruitregulatory T cells via CCL4. Nature Immunology. 2001;**2**(12):1126-1132

[68] Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factor sproducedby CD8+ T cells. Science. 1995;**270**(5243):1811-1815

[69] Kamin-Lewis R, Abdelwahab SF, Trang C, Baker A, DeVico AL, Gallo RC, et al. Perforin-low memory CD8+ cells are the predominant T cells in normal humans that synthesize the beta -chemokine macrophage inflammatory protein-1 beta. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**(16):9283-9288

[70] Donlon TA, Krensky AM, Wallace MR, Collins FS, Lovett M, Clayberger C. Localization of a human T-cell-specific gene, RANTES (D17S136E), to chromosome 17q11.2-q12. Genomics. 1990;**6**(3):548-553

[71] Jumpupto Chen L, Zhang Q, Yu C, Wang F, Kong X. Functional roles of CCL5/RANTES in liver disease. Liver Research. 2020;**4**(1):28-34

[72] Appay V, Rowland-Jones SL. RANTES: A versatile and controversial chemokine. Trends in Immunology. 2001;**22**(2):83-87

[73] Maghazachi AA, Al-Aoukaty A, Schall TJ. CC chemokines induce the generation of killer cells from CD56+ cells. European Journal of Immunology. 1996;**26**(2):315-319

[74] Zeng Z, Lan T, Wei Y, Wei X. CCL5/CCR5 axis in human diseases and related treatments. Genes & Diseases. 2022;**9**(1):12-27

[75] Krensky AM, Ahn YT. Mechanisms of disease: Regulation of RANTES (CCL5) in renal disease. Nature Clinical Practice Nephrology. 2007;**3**(3):164-170

[76] Nieto M, Frade JM, Sancho D, et al. Polarization of chemokine receptors to the leading edge during lymphocyte chemotaxis. Journal of Experimental Medicine. 1997;**186**:153-158

[77] Chuluyan HE, Schall TJ, Yoshimura T, et al. IL-1 activation of endothelium supports VLA-4 (CD49d/CD29)-mediated monocyte trans

endothelial migration to C5a, MIP-1 alpha, RANTES, and PAF but inhibitsmigrationto MCP-1: A regulatory role endothelium-derived MCP-1. Journal of Leukocyte Biology. 1995;**58**:71-79

[78] Szabo MC et al. RANTES stimulation of T lymphocyte adhesion and activation: A role for LFA-1 and ICAM-3. European Journal of Immunology. 1997;**27**

[79] Bacon KB, Premack BA, Gardner P, et al. T cell signaling pathways by the chemokine RANTES. Science. 1995;**269**:1727-1730

[80] Appay V, Dunbar PR, Cerundolo V, et al. RANTES activates antigen-specific cytotoxic lymphocytes in a mitogen-like manner through cell surface aggregation. International Immunology. 2000;**12**:1173-1182

[81] Gilat D, Hershkoviz R, Mekori YA, et al. Regulation of adhesion of CD4+ T lymphocytestoin tractor heparinase-treated subendothelial extracellular matrix by diffusible or anchored RANTES and MIP-1 beta. The Journal of Immunology. 1994;**153**:4899-4906

[82] Zhang X, Chen L, Bancroft DP, et al. Crystal structure of recombinant human platelet factor 4. Biochemistry. 1994;**33**:8361-8366

[83] Baltus T, Weber KS, Johnson Z, et al. Oligomerization of RANTES is required for CCR1-mediated arrest but not CCR5-mediated transmigration of leukocytes on inflamed endothelium. Blood. 2003;**102**:1985-1988

[84] Witt DP, Lander AD. Differential binding of chemokines to glucosamine glycan subpopulations. Current Biology. 1994;**4**:394-400

[85] Hoogewerf AJ, Kuschert GS, Proudfoot AE, et al. Glycosaminoglycans mediate cell surface oligomerization of chemokines. Biochemistry. 1997;**36**:13570-13578

[86] Ali S, Palmer AC, Banerjee B, et al. Examination of the function of RANTES, MIP-1alpha, and MIP1beta following interaction with heparin-like glucosamine glycans. The Journal of Biological Chemistry. 2000;**275**:11721-11727

[87] Martin L, Blanpain C, Garnier P, et al. Structural and functional analysis of the RANTES-glycosaminoglycans interactions. Biochemistry. 2001;**40**:6303-6318

[88] Huo Y, Schober A, Forlow SB, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. Nature Medicine. 2003;**9**:61-67

[89] Massberg S, Brand K, Gruner S, et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. Journal of Experimental Medicine. 2002;**196**:887-896

[90] Rothenbacher D, Muller-Scholze S, Herder C, et al. Differential expression of chemokines, risk of stable coronary heart disease, and correlation with established cardiovascular risk markers. Arteriosclerosis, Thrombosis, and Vascular Biology. 2006;**26**:194-199

[91] Muhlhauser I, Schernthaner G, Silberbauer K, et al. Platelet proteins (beta-TG and PF4) in atherosclerosis and related diseases. Artery. 1980;**8**:73-79

[92] Levine SP, Lindenfeld J, Ellis JB, et al. Increased plasma concentrations of platelet factor 4 in coronary artery disease: A measure of in vivo platelet activation and secretion. Circulation. 1981;**64**:626-632

[93] O'Brien JR, Etherington MD, Pashley M. Intraplatelet platelet factor 4 (IP.PF4) and the heparin-mobilisable pool of PF4 in health and atherosclerosis. Thrombosis and Haemostasis. 1984;**51**:354-357

[94] Pitsilos S, Hunt J, Mohler ER, et al. Platelet factor 4 localization in carotid atherosclerotic plaques: Correlation with clinical parameters. Thrombosis and Haemostasis. 2003;**90**:1112-1120

[95] Lambert MP, Sachais BS, Kowalska MA. Chemokines and thrombogenecity. Thrombosis and Haemostasis. 2007;**97**:722-729

[96] Rhee JS, Black M, Schubert U, et al. The functional role of blood platelet components in angiogenesis. Thrombosis and Haemostasis. 2004;**92**:394-402

[97] Brill A, Elinav H, Varon D. Differential role of platelet granular mediators in angiogenesis. Cardiovascular Research. 2004;**63**:226-235

[98] Hristov M, Zernecke A, Bidzhekov K, et al. Importance of CXC chemokine receptor 2 in the homing of human peripheral blood endothelial progenitor cells to sites of arterial injury. Circulation Research. 2009;**7**(Suppl. 1):31-34. DOI: 10.1161/01.RES.0000259043.42571.68

[99] Lasagni L, Francalanci M, Annunziato F, et al. An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as a functional receptor for platelet factor 4. Journal of Experimental Medicine. 2003;**197**:1537-1549

[100] Balkwill F. Cancer and the chemokine network. Nature Reviews Cancer. 2004;**4**:540-550

[101] Sallusto F, Mackay CR, Lanzavecchia A. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. Science. 1997;**277**:2005-2007

[102] Debidda M, Williams DA, Zheng Y. Rac1 GTPase regulates cell genomic stability and senescence. The Journal of Biological Chemistry. 2006;**281**:38519-38528

[103] Acosta JC, Loghlen A, Banito A, Raguz S, Gil J. Control of senescence by CXCR2 and its ligands. Cell Cycle. 2008;**7**:2956-2919, 2959

[104] Lijnen HR. Pleiotropic functions of plasminogen activator inhibitor-1. Journal of Thrombosis and Hemostasis. 2005;**3**(1):35-45

[105] Khan SS. The central role of PAI-1 in COVID-19: Thrombosis and beyond. The American Journal of Respiratory Cell and Molecular Biology. 2021;**65**(3):238-240