Chapter 4: Chemokines

1. General overview

Chemokines are cytokines whose main role is cellular activation and the stimulation of leukocyte migration. They exert their various functions by interacting with receptors expressed on the cell surface and coupled to G proteins (GPCRs). They are involved in the regulation of many biological processes such as apoptosis, proliferation, angiogenesis, hematopoiesis, and organogenesis. They maintain the homeostasis of the lymphocyte compartment and coordinate the functioning of the immune system. Moreover, chemokines and their receptors constitute ideal targets for certain viruses. It is noteworthy that many cancers are characterized by dysregulation of their expression or activity.

2. Structure and classification of chemokines

2.1. Classification by families

Chemokines are small proteins of 60 to 100 amino acids. About fifty chemokines have currently been identified and are classified into four major categories, according to the spacing between the cysteine amino acids located in the amino-terminal region of the molecule.

Depending on the number of amino acids present between the first two cysteines (located in the N-terminal region), chemokines are divided into four families.

These families are called **CXC, CC, C, and CX3C**. Each of them attracts very specific types of leukocytes.

1. CXC chemokines or CXC ligands (CXCL)

CXC chemokines (also called **α-chemokines**) are involved in acute inflammation and attract neutrophils, T and B lymphocytes, and killer cells.

This group comprises **16 members**, divided into two subgroups according to the presence or absence of an **ELR motif** (Glutamic acid–Leucine–Arginine) adjacent to the defining CXC motif.
Most **CXCL genes** are located in a cluster on **chromosome 4**, with the exception of **CXCL12, CXCL14 and CXCL16**, which are encoded on **chromosome 10**.

a) ELR$^+$ CXC chemokines

There are **eight ELR-containing chemokines**:
CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8 and CXCL15.

These chemokines preferentially attract **polymorphonuclear neutrophils**. They are produced by various cell types in response to **pro-inflammatory cytokine stimuli** (IL-1 and TNF-α). Their role is to promote **neutrophil adhesion to epithelial cells** and **migration across the**

endothelium.
These chemokines also have an **angiogenic role**, since they are chemoattractants for endothelial cells.

b) ELR⁻ CXC chemokines

This subgroup is composed of **CXCL4, CXCL9, CXCL10, CXCL12, CXCL13, CXCL14 and CXCL16**.
These chemokines preferentially attract **lymphocytes and monocytes**, with only weak activity on neutrophils.

2. CC chemokines or CCL

CC chemokines (also called **β-chemokines**) are involved in **chronic inflammation** and attract **monocytes, macrophages, T lymphocytes, as well as basophils and eosinophils**.

This group comprises **28 members**.
The locus of most CC chemokines is located on **chromosome 17**, with the exception of **CCL19**, which is located on **chromosome 9**, and **CCL24** and **CCL26**, which are located on **chromosome 7**.

The primary targets of these chemokines are **mononuclear cells**.
These chemokines are involved in both **homeostatic processes** and **inflammatory responses**.

Pro-inflammatory CC chemokines, which are involved in **allergy**, are generally **inducible**, whereas those involved in **homeostasis** are **constitutively expressed**.

3. C chemokines (CL or XCL family)

C chemokines (also called **γ-chemokines**) include only **two chemokines, XCL1 and XCL2**, which attract **T lymphocytes**.

This family comprises **two members: XCL1 and XCL2**.
XCL1 is able to induce chemotaxis of **$CD4^+$ and $CD8^+$ T lymphocytes**, as well as **NK cells**.
It does **not** show chemotactic activity toward **monocytes or neutrophils**.

In addition to its chemotactic function on these cell populations, **XCL1** can induce **suppressive and cytotoxic effects** against tumor cells or effector T cells.

4. CX3C chemokines (CX3CL family)

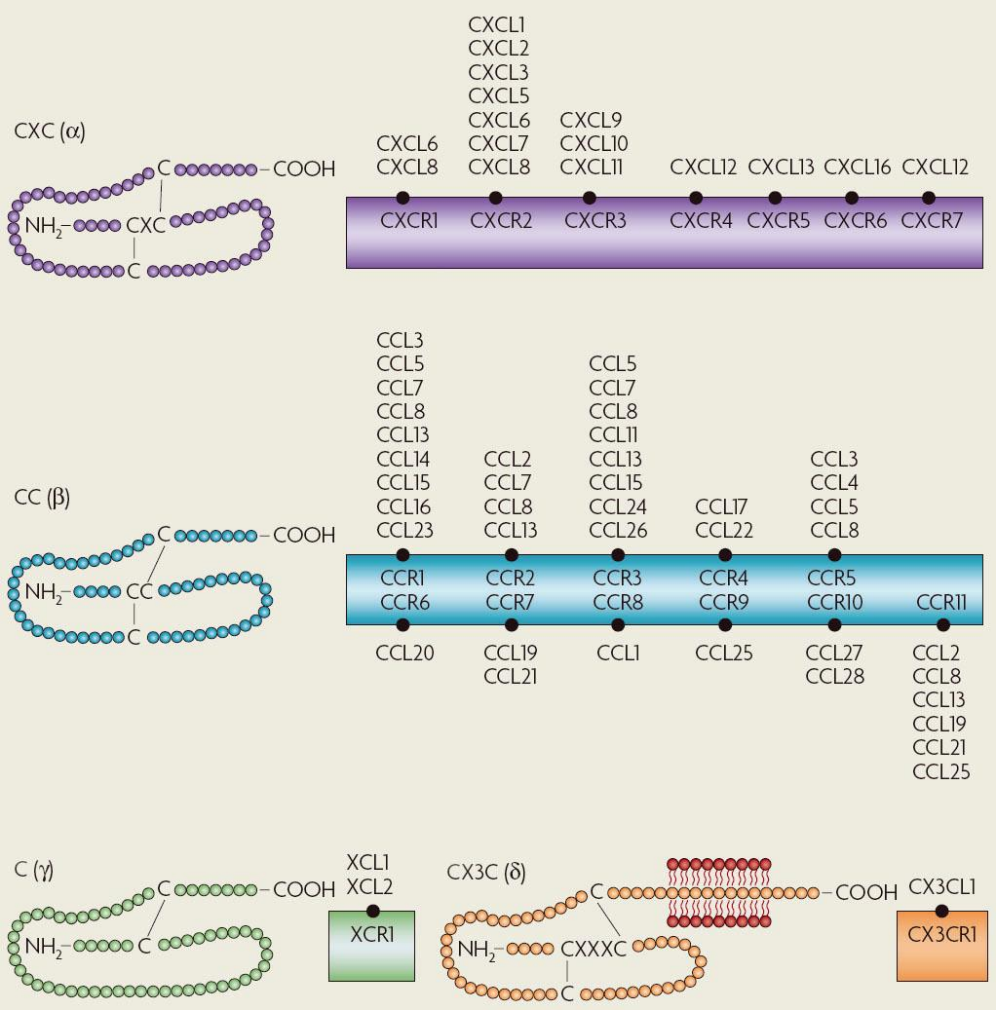
Finally, the **CX3C family** contains only **one ligand (CX3CL1, also called fractalkine)**, which is chemoattractant for **T lymphocytes**.

These chemokines exert their biological effects through **membrane receptors coupled to G proteins**, as is the case for most **neuropeptides and neurotransmitters** involved in neural transmission.

A particular feature of the chemokine system is the existence of a **high degree of redundancy between chemokines and their receptors**. Thus, **one chemokine can bind to several receptors**, and **one receptor can bind several chemokines of the same family**.

To date, **more than 50 chemokines and about twenty receptors** have been identified, as shown in the figure.
This redundancy likely has a physiological explanation, since it allows the effects of a given chemokine to be enhanced and, in the event of loss of activity (as in mice in which a gene has been deleted, i.e. **knock-out mice**), another chemokine can take over and exert similar effects.

This is also the reason why it is often difficult to demonstrate a specific phenotype in these transgenic mice.



2-2- Classification according to biological activity

Chemokines can also be classified on a functional basis. Thus, two main types of chemokines are distinguished: **pro-inflammatory chemokines** and **constitutive chemokines**.

a) Constitutive (or "homeostatic") chemokines

Constitutive (homeostatic) chemokines are produced in **lymphoid organs** and in certain **non-lymphoid tissues** such as the skin and mucosal tissues.
They regulate **lymphocyte trafficking and positioning** within these organs during lymphopoiesis, and also contribute to the maintenance of **immune surveillance**.

Examples include: **SDF-1, BCA-1, ELC and SLC**.
Some of these chemokines also appear to be **essential for the development of these organs**.

Examples of receptor/ligand pairs:

- **CCR7 / SLC and ELC**
- **CXCR5 / BCA-1**
- **CXCR4 / SDF-1**

b) Pro-inflammatory (or "inducible") chemokines

Pro-inflammatory (inducible) chemokines are produced at **sites of inflammation** by tissue cells or by **infiltrating leukocytes**, after activation by **pro-inflammatory cytokines** or following contact with a **pathogen**.

LPS, IL-1β and TNF-α (tumor necrosis factor) are among the molecules capable of inducing the expression of pro-inflammatory chemokines.
These chemokines recruit the different cell types involved in an **immune response**.

Examples of receptor/ligand pairs:

- **CXCR2 / IL-8 and GRO**
- **CCR5 / MIP-1**
- **CCR2 / MCP-1**
- **CCR3 / eotaxin and RANTES**
- **CX3CR1 / fractalkine**

c) Chemokines with dual function

However, the distinction between pro-inflammatory chemokines and constitutive chemokines is **not absolute**, since some chemokines belong to both functional categories.

Example:

- **CXCR3 / MIG, I-TAC and IP-10**

3. Chemokine receptors

3-1. Characteristics

Chemokines exert their activity by interacting with receptors expressed on the cell surface. The nomenclature of chemokine receptors is based on the chemokine group to which their ligand belongs.

All these receptors share a similar tertiary structure: they are **seven-transmembrane α-helix receptors**, with an **extracellular N-terminal end**, **three extracellular loops**, **three intracellular loops**, and an **intracellular C-terminal end**.
These receptors are coupled to a G protein and therefore belong to the **GPCR (G-protein-coupled receptor) superfamily**.

Each receptor family specifically interacts with a given chemokine family:

- **CCR** receptors bind **CC chemokines**: **11 receptors (CCR1 to CCR11)**
- **CXCR** receptors bind **CXC chemokines**: **5 receptors (CXCR1 to CXCR5)**
- **CX3CR** receptors bind **CX3C chemokines**: **one receptor (CX3CR1)**
- **XCR** receptors bind **XC chemokines**: **one receptor (XCR1)**

Therefore:

- The **same receptor can interact with several chemokines** (e.g. **CCR1** can bind **RANTES** and **MIP-1α**): this is referred to as **chemokine redundancy**.
- **Several receptors can bind the same chemokine** (e.g. **RANTES** binds **CCR1, CCR3 and CCR5**): this is referred to as **chemokine pleiotropy**.

3.2. Specificity of chemokine–receptor pairs

Chemokine–receptor pairs vary greatly in terms of selectivity.

Some chemokines bind to a single receptor, and vice versa, such as CXCR4 and SDF-1, CXCR5 and BCA-1, CCR6 and MIP-3α, or CCR10 and CTACK.

Some receptors, however, are able to bind two or three different chemokines. This is the case, for example, for CCR7, which binds SLC and ELC, and for CXCR3, which binds IP-10, MIG and I-TAC.

Many other receptors are less selective. For example, CCR3 binds eotaxin, eotaxin-2 and eotaxin-3, MCP-2, MCP-3 and MCP-4, CCL6, leukotactin-1, ESkine and RANTES.

This latter chemokine (RANTES) is itself able to bind CCR1, CCR2 and CCR3 with relatively high affinity.

Tableau 1. Nomenclature systématique des chémokines ainsi que leur(s) nom(s) commun(s) et le(s) récepteur(s) au(x)quel(s) elles sont associées — *Nomenclature of chemokines, their common names and their associated receptors* (Tanaka *et al.*, 2005).

	Nom systématique	**Nom(s) commun(s)**	**Récepteur(s)**
Chémokines CC	CCL1	I-309	CCR8
	CCL2	MCP-1	CCR2
	CCL3	MIP-1α	CCR1, CCR5
	CCL3L1	LD78β	CCR1, CCR5
	CCL4	MIP-1β	CCR5
	CCL5	RANTES	CCR1, CCR3, CCR5
	CCL6	Inconnu	CCR1, CCR2, CCR3
	CCL7	MCP-3	CCR1, CCR2, CCR3
	CCL8	MCP-2	CCR2, CCR3, CCR5
	CCL9/CCL10	Inconnu	CCR1
	CCL11	Eotaxin	CCR3
	CCL12	Inconnu	CCR2
	CCL13	MCP-4	CCR1, CCR2, CCR3
	CCL14	HCC-1	CCR1
	CCL15	HCC-2/Lkn-1/MIP-1δ	CCR1, CCR3
	CCL16	HCC-4/LEC/LCC-1	CCR1, CCR2
	CCL17	TARC	CCR4
	CCL18	DC-CK1/PARC	Inconnu
	CCL19	MIP-3β/ELC	CCR7
	CCL20	MIP-3α/LARC	CCR6
	CCL21	6Ckine/SLC	CCR7
	CCL22	MDC	CCR4
	CCL23	MPIF-1/CKb8	CCR1
	CCL24	MPIF-2/Eotaxin-2	CCR3
	CCL25	TECK	CCR9
	CCL26	Eotaxin-3	CCR3
	CCL27	CTACK/ESkine	CCR2, CCR3, CCR10
	CCL28	MEC	CCR3, CCR10
Chémokines CXC	CXCL1	GROα	CXCR2, CXCR1
	CXCL2	GROβ	CXCR2
	CXCL3	GROγ	CXCR2
	CXCL4	PF-4	Inconnu
	CXCL5	ENA-78	CXCR2
	CXCL6	GCP-2	CXCR1, CXCR2
	CXCL7	NAP-2	CXCR2
	CXCL8	IL-8	CXCR1, CXCR2
	CXCL9	Mig	CXCR3
	CXCL10	IP-10	CXCR3
	CXCL11	I-TAC	CXCR3
	CXCL12	SDF-1α/β	CXCR4
	CXCL13	BCA-1	CXCR5
	CXCL14	BRAK	Inconnu
	CXCL15	Inconnu	Inconnu
	CXCL16	SR-PSOX	CXCR6
Chémokines C	XCL1	Lymphotactin-α/SCM-1α	XCR1
	XCL2	Lymphotactin-β/SCM-1β	XCR1
Chémokines CXC	CX3CL1	Fractalkine	CX3CR1

4. Recruitment of immune cells by chemokines

4.1. Recruitment of lymphocytes

Four chemokines appear to be essential for the recruitment of T lymphocytes and for their polarization: **CCL2, CCL11, CCL22 and CCL17**.
In particular, **CCL2** induces **Th2 differentiation** of T lymphocytes, and mice in which the gene encoding the **CCL2 receptor (CCR2)** has been inactivated are unable to develop an **allergic response**.

CCL11, CCL22 and CCL17 selectively recruit **Th2 lymphocytes** to the inflammatory site. This effect results from the preferential expression of **CCR3 and CCR4** on Th2 lymphocytes.

4.2. Recruitment of eosinophils

Seven main chemokines are involved in eosinophil recruitment:
CCL11 (eotaxin-1), CCL24 (eotaxin-2), CCL26 (eotaxin-3), CCL5 (RANTES), CCL7 (MCP-3), CCL13 (MCP-4) and CCL3 (MIP-1α).
Eosinophils also respond to **CCL8** and **CCL28**.

CCR3 is a receptor for all of these ligands except **CCL3**.
Conversely, it is the **only known receptor** for the eotaxins **CCL11, CCL24 and CCL26**.

Consistently, eosinophils from most individuals express **high levels of CCR3**, whereas their expression of **CCR1** is lower.

4.3. Recruitment of mast cells

Mast cells are able to express **CCR1, CCR2, CCR3, CCR4, CCR5, CXCR2 and CXCR4**.
CCL2 and possibly **CCL5** appear to play an important role in mast cell recruitment.
In contrast, **CXCL8** seems to inhibit mast cell recruitment.

5. Involvement of chemokines in certain diseases

5.1. Chemokines and viruses

Given their role in lymphocyte homing, chemokines and their receptors represent privileged targets for viruses.
Interfering with chemokine–receptor interactions is an efficient way to escape immune surveillance.

Several examples are known in humans:

- The **p35 protein of poxviruses** binds CC-type chemokines with a higher affinity than their natural receptors. Similar examples have been described for **cytomegalovirus, herpesviruses**, and others.
- Some viruses modify the expression of chemokines or chemokine receptors. Thus, in patients infected with **HTLV-1**, plasma levels of **CCL2, CCL11, CCL24, CXCL10 and CXCL9** are markedly altered.
  It has also been shown that the expression of **MIP-3α/CCL20** is induced *in vitro* by the viral **Tax** protein.
- Another well-known example is **HIV**. The viral proteins **Tat** and **gp120** are able to interact with several chemokine receptors, in particular **CXCR4 and CCR5**.
  Ex vivo, **gp120** can inhibit the migration of B lymphocytes in response to the chemokines **SDF-1 (CXCL12), MIP-3α (CCL20) and SLC (CCL21)**.
  CXCR4 and CCR5 have also been identified as **co-receptors for HIV entry into $CD4^+$ T lymphocytes**.
- The **Kaposi's sarcoma–associated herpesvirus** synthesizes certain **virokines** (viral chemokines), including molecules similar to **MIP-2**, which block several CC- and CXC-type receptors and render infected cells insensitive to the chemokines that normally signal through these receptors.

5.2. Angiogenesis, cancer, and metastasis

Beyond their role in migration and organogenesis, chemokines are involved in various pathological mechanisms, including tumor development and metastasis.

Angiogenesis is a biological process by which new blood vessels are formed. The growth of a solid tumor requires adequate vascularization to ensure a continuous supply of oxygen and nutrients.

ELR^+ CXC chemokines act as potential angiogenic factors, capable of stimulating the chemotaxis of endothelial cells.

In contrast, most ELR^- CXC chemokines are strong angiostatic factors, inhibiting the chemotactic attraction of endothelial cells.

Chemokines are also widely implicated in metastasis development. Tumor cells often alter the expression of their chemokine receptors: some receptors are downregulated or inhibited, while others are overexpressed. Consequently, metastatic migration is not random, but determined by the receptors expressed and, therefore, by the type of cancer from which they originate.

In general, CXCR4 is the chemokine receptor most frequently expressed by cancer cells (e.g., small-cell lung cancer, pancreatic cancer, astrocytomas, myelomas, and B-cell lymphomas). Its ligand, SDF-1 (CXCL12), has a strong chemoattractant effect and is expressed in many tissues.

Additionally, SDF-1 promotes tumor cell survival and growth and induces cytokine secretion. Overall, lymphoproliferative diseases are characterized by alterations in the expression of one or more chemokine receptors.