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The role of chemokines in liver disease

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Abstract (abstract): Chemokines are involved in many processes, including the normal immune response to infection, as well as pathological processes such as carcinogenesis, autoimmunity and inappropriate inflammatory responses. These processes represent an important aspect in the context of liver disease, its progression and the possibility of inhibiting fibrosis. Chemokines belong to low molecular weight proteins with a wide range of functions affecting cell migration, involving different physiological and pathological processes. Depending on whether they participate in the maintenance of homeostasis during periods of well-being or represent a type of intervention of the organism to its disorders, their secretion by cells is constitutive or induced. This article focuses on the classification, structure and functions of these molecules and their possible involvement in the pathogenesis of liver diseases, as well as the possibilities of therapeutic application of this knowledge.

Keywords: chemokines, liver disease, alcoholic liver disease

Chemokines - structure and function

Chemokines are a special type of cytokines that are signaling molecules of the immune system. Their unique role is to control cell migration. Considering their structure, they are small-molecule proteins (8-14 kD, 66-111 amino acids) [3], with a highly homologous structure containing 20-70% of the amino acid sequence [4]. Even the smallest changes in structure involve large changes in biological activity. Chemokines are encoded by 48 different genes. However, due to separate post-translational processing and other modifications, the final number of functionally different molecules created is much larger. [1]

Classification and nomenclature of chemokines and their receptors

Prior to the development of a uniform chemokine naming system, chemokines were mainly named after their first recognized function. Often new, much more important functions of the same molecules were discovered, making their original name obsolete. As already mentioned, these molecules are characterized by high stability and homologous structure. Therefore, despite their diversity, it has become possible to divide them into 4 main groups based on structure.

4 cysteines:

- CC group (first two cysteines next to each other, not separated by other amino acids)
- CXC group (first two cysteines separated by one amino acid)
- CX3C group (first two cysteines separated by three amino acids)

2 cysteines:

- Group C

There is an additional division in the CXC group that is related to the presence of an ELR fragment located between the N-terminus and the first cysteine residue. This fragment consists of three amino acids (Glu-Leu-Arg) and is responsible for interaction with CXCR1 and CXCR2 receptors present on neutrophils, which also determines the role of ELR-positive chemokines as chemotactic factors for neutrophils. In some simplification, it can also be assumed that ELR-positive CXC chemokines have proangiogenic properties, in contrast to most ELR-negative chemokines.

Division of the CC group into MIP and MPC

The current classification of chemokines and regulation of their nomenclature were established at the 1999 Keystone Symposium on Chemotactic Cytokines.

At the same time, the names of receptors for chemokines were systematized to be analogous to each other (the letter L stands for ligand, the letter R for receptor for a given chemokine, and the number indicates the order of discovery) [3].

The only exception to this rule is group C (containing only one cysteine), in which an X is given at the beginning of the ligand and receptor name, e.g. CX1L, CX1R. This difference is due to the need to distinguish the CR1 receptor for complement proteins from the previously named receptor.

Structure and function of receptors for chemokines

Receptors for chemokines show great structural similarity among themselves. The vast majority of them consist of a transmembrane 7-helix (a helix that crosses the cell membrane 7 times) - the so-called 7TM - this abbreviation can be found in worldwide nomenclature.

Each receptor has a unique ligand interaction profile. It can be specific for several or even a dozen chemokines, but always within a single group. At the same time, one chemokine can interact with several different receptors. [4] These mechanisms determine certain properties of chemokines, i.e. pleiotropism - one chemokine can exert different effects on individual cells via different receptors, and redundancy - the same effect on a cell can be obtained by different chemokines acting on the same receptor. These interactions are very complex, complicating the possibilities of therapeutic use of chemokines or blocking their receptors.

Based on current knowledge, these receptors can be divided into two main groups [5]:

- 1) Typical, G-protein-coupled
- 2) Atypical, acting through different mechanisms

G protein-coupled receptors (GPCRs) are linked to G protein through the endocyttoplasmic portion of the second and third loops. [4] Atypical chemokine receptors (ACRs) are a heterogeneous group. Most of them have a standard structure like the other 7TMs, but lack signaling function or their signaling function is atypical (not involved in G-protein coupled transduction). This is usually due to the absence of the DRYLAIV sequence in the second loop of the helix, which deprives them of the ability to interact with the G protein. [Some of these receptors use their own alternative signal transduction pathways [6] [7], while others induce ligand internalization into the cell. [8]

Chemokine action profile and receptor expression

The aspect of action profile and receptor effectors of individual chemokines is closely related to the issue of expression of receptors for these molecules. The presence of particular receptors is closely related to the phenotype of immune cells.

To illustrate this situation, simplified relationships are shown in the following table [9],[10].

Type of effector cell	Receptor for chemokines
Neutrophils	CXCR1 CXCR2 CXCR4
Monocytes	CCR1 CCR2 CCR5 CXCR4
Eosinophils	CCR1 CCR3
Basophiles	CCR1 CCR2 CCR3
Th1 lymphocytes	CXCR3 CCR5
Th2 lymphocytes	CCR3 CCR4
B lymphocytes	CX3CR1 CXCR5

The described mechanism allows for the mobilization of those cells that can contribute to the restoration of homeostasis at a given time. Moreover, the receptors for chemokines may be constitutive (permanently bound to a specific cell type) or inducible (released on the cell surface under the influence of specific stimulatory factors). Therefore, the division of their corresponding ligands into permanently produced homing chemokines and inflammatory chemokines can be distinguished [9]. The first type of chemokines is responsible for proper distribution and localization of individual immune cells during their maturation and subsequent relocation to organs, as in the process of hematopoiesis. The second type of chemokines is involved in the inflammatory response activated when homeostasis is disturbed or in certain physiological processes, but they are not produced continuously. Their secretion is triggered by various signaling molecules, including proinflammatory cytokines. [50].

The role of chemokines in liver disease

The role of inflammation in the pathogenesis of liver disease

Inflammation plays a key role in the development of many liver diseases, despite diverse etiologies and many predisposing factors. It is known that uncontrolled activation of the inflammatory process can lead to progression of many liver diseases [12].

An individual's inflammatory response profile, which may be indicated by levels of certain chemokines and other signaling molecules, may define the pathway for further progression of certain minor disorders to advanced fibrosis and HCC.

Among all liver cells, several types play a unique role in the context of damage and inflammatory processes. These include Kupffer cells, a particular type of hepatic macrophage, liver sinusoidal endothelial cells (LCEs), and local NK cells (natural killers).

The role of chemokines in initiating inflammatory processes in the liver - cell recruitment.

The role of Kupffer cells

Kupffer cells are the largest population of organ-specific macrophages in the entire human body. They are located in the sinusoids of the liver and constitute the first line of response as part of the blood-gut barrier. In addition to their phagocytic role, they also have very important functions in innate immunity. Their activation is associated with the arrival of a signal - this signal are DAMP molecules or PAMPs (pathogen-associated molecular patterns PAMPs , danger-associated molecular patterns DAMPs - exogenous and endogenous activating factors), binding to receptors from the PRRs group (Multiple pathogen-recognition receptors), which include TLRs and NOD receptors. [49].

Kupffer cells have the ability to initiate a primary response to infectious and non-infectious damaging agents by promoting the production of cytokines (including the key TNF-alpha) via TLR receptors. The chemokines CXCL8 (IL-8), CXCL1 (KC), CXCL2 (MIP-2), CXCL3, CCL2, CCL3, and CCL4 produced by these cells call neutrophils and monocytes to the site of injury [13]. The chemokine CCL2 is actively involved in increasing the number of hepatic macrophages that derive directly from myeloid monocytes expressing the CCR2 receptor. [21].

Neutrophils are characterized by a strong cytotoxic effect. Their number must be particularly effectively controlled to prevent excessive inflammation in tissues [14]. Already in the initial phase of inflammation, excessive recruitment of these cells, also as a result of overexpression of the above-mentioned chemokines, can cause a lot of damage. Most neutrophil chemoattractants also play a role in stimulating the response of these cells, including enhancing oxidative stress [15].

In addition to the "calling" role itself, certain CXC chemokines are necessary for neutrophils recruited to sinusoidal vessels to internalize and pass from these vessels into the liver parenchyma. The recruitment and accumulation of neutrophils in liver sinusoidal vessels alone is not sufficient for them to cause damage [13]. The production of chemokines necessary to move cells out of the hepatic vasculature is involved not only in hepatic macrophages, but also in neutrophils already residing in the intercellular spaces of the liver. This demonstrates their sustaining role in this process [15]. Consequently, neutrophils generate intracellular oxidative stress, release proteases (e.g., cathepsin G and elastase) that lead to cell damage.

Neutrophil-dependent mechanisms play a central role in many types of liver injury, including alcoholic liver disease [16], endotoxemia [51], sepsis [19], ischemic injury and subsequent reperfusion [20].

Other cells that are involved in local defense mechanisms in the liver are NK cells. They are a subpopulation of lymphocytes, in the liver accounting for about 20-30% of the total residual leukocyte population.

NK cells are a very early response to inflammation. They appear in response to the chemokines CCL2, CCL5, CCL7, CCL8. [13]. CXCL16-CXCR6 axis-dependent accumulation of NK cells, associated with overexpression of these receptors, has been shown to be associated with an increased risk of developing chronic liver disease. Experimentally depriving mice of CXCR6 receptors had a protective effect on the development of organ fibrosis. [22].

As the disease process progresses, the initial role is played primarily by the recruited innate response cells described above. In subsequent stages, acquired response cells appear, which include CD8+ cytotoxic T cells, CD4+ helper T cells, and antibody-producing B cells. [21]. Helper T lymphocytes are further divided into subpopulations: Th1, Th2, the less numerous Th17, and regulatory T cells. This division is based on the profile of receptors present on the cells and the type of cytokines secreted, which implies functional differentiation [24].

Chemokines are also involved in the differentiation and recruitment of lymphocytes to the liver. This process is important because the balance between the type and subpopulation of T lymphocytes recruited to the area of inflammation is critical to the outcome of liver disease [23].

Dendritic cells present in the liver play an important role in lymphocyte differentiation. These phagocytic cells can originate from myeloid or plasma cells. Their main role is to recognize antigens through PRRs receptors and then transmit the information to peripheral lymphoid organs to stimulate T lymphocyte differentiation [13].

CXCR3 receptors are localized on T lymphocytes. The level of ligands for this receptor synthesized in the liver (CXCL9, CXCL10, CXCL11) has been shown to be responsible for enhancing Th1 responses. Production of these ligands is dependent on the presence of TNF-alpha and INF-gamma [23], which are released by numerous cells of the innate immune response, including Kupffer cells, other macrophages and infiltrating cells.

Role in inhibition of inflammation

In contrast to their huge role in enhancing inflammatory processes, some chemokines may also be involved in inhibiting or preventing inflammation. However, this role is less well known, having been demonstrated mainly for cytokines of the CXC group with an ELR motif.

In vivo, CXC ELR+ chemokines have already been shown to increase proliferation in many tissues, including liver cells [27], keratinocytes and epithelial cells.

Some of the CXC ELR+ chemokines show concentration-dependent effects: low concentrations are associated with repair and regenerative processes, high concentrations are hepatotoxic [25].

It has also been shown that ELR + CXC chemokines can improve liver regeneration under acute damage caused by paracetamol intoxication. [26]

Alcoholic liver disease

The immunological aspect of this disease is considered on many levels. The contribution of innate and acquired immunity is well known and fairly well understood. The involvement of individual cells, mechanisms and molecules has been known for many years. Chemokines are no exception. Interest in this topic has been ongoing since the publication shortly thereafter of two papers written in 1993 for the journal *Hepatology*, which demonstrated increased CXCL8 levels in patients with alcoholic liver disease [28] and alcoholic hepatitis[29]. A correlation was also found between the levels of this chemokine and the degree of neutrophilic infiltration of the organ [28]. Subsequent numerous studies have confirmed this correlation [30], [31], [32].

Subsequently, other multiple relationships between chemokine levels and expression of their receptors and the course of ALD were discovered. For example, comparing the expression of some CXC chemokines (CXCL8, CXCL5, CXCL1, CXCL6, CXCL10, CXCL4) and CCL2 in the liver and comparing it with a control group, increased secretion of these chemokines was found in ALD patients. The expression of CXC chemokines was associated with the degree of neutrophil infiltration and the risk of portal hypertension in patients. Moreover, higher expression of some of them (CXCL8, CXCL5, CXCL3, CXCL6) is associated with poor prognosis [33].

Among CC chemokines, elevated CCL2 levels in patient serum and its increased expression in the liver have been shown to correlate with disease severity, degree of neutrophil infiltration, and CXCL8 expression [34].

Also, hepatic CCL20 expression and serum levels were elevated in patients with alcoholic hepatitis and correlated with the degree of organ fibrosis, risk of portal hypertension, endotoxemia, overall disease severity and short-term mortality [35].

Non-alcoholic fatty liver disease (NAFLD)

This disease is the most common liver disorder in industrialized countries, affecting 20-30% of the population. The disease has a complex substrate consisting of genetic, metabolic and endocrine factors that modulate the immune response. Histologically, it encompasses a broad spectrum of lesions ranging from simple steatosis, steatohepatitis and cirrhosis to liver cancer [36]. When investigating the immunological basis of the disease, the chemokine CCL2, which is largely secreted by adipose tissue cells, was the first to be assigned an important role.

In obese mice, treatment with CCR2 receptor antagonists reduced macrophage accumulation in adipose tissue, improved insulin sensitivity, lowered body weight, and reduced fat accumulation in the liver [37]. This indicates a common role for CCL2 in the pathogenesis of both fatty liver disease and the metabolic changes leading to it - obesity and insulin resistance.

In humans, increased CCL2 expression has been shown in individuals with greater hepatic fat accumulation [38]. Serum CCL2 levels have also been shown to correlate with the degree of hepatic steatosis [39].

The chemokine CCL5 also plays a role in pathogenesis. Elevated expression of this chemokine has been shown in patients with simple steatosis and NASH. The role of this molecule in the development of liver fibrosis in this group of patients is also suspected, based in part on studies in which a CCL5 inhibitor effectively reduced the fibrotic process [40].

Viral hepatitis

This is one of the most thoroughly studied issues in terms of the involvement of chemokines, especially in hepatitis C. The number of publications and reports on this topic is so large that we give only brief mentions of the more important findings. For example, increased levels of CCR2 and its ligands (CCL2, CCL7, CCL8) have been demonstrated in HCV-infected liver tissue, which plays a role in cell recruitment to the site of infection [41]. Ligands for the CCR5 receptor (CCL3, CCL4, CCL5) are overexpressed, which has a role in Th1-type responses. [42]

Hepatic gene expression for CCL5 is increased in correlation with the severity of fibrosis and cirrhosis, inflammatory markers and liver damage in chronic HCV [43] and HBV infection [44].

Other chemokines shown to be involved in the pathogenesis of hepatotropic virus infection include CCL13-CCL16, CCL18-CCL21, CXCL8-CXCL13, CXCL16 [41].

Neoplastic liver tumors

The involvement of CXCL8 chemokine (IL-8) in the development of liver cancer has been repeatedly demonstrated, including the fact that serum CXCL8 levels have been shown to correlate with adverse prognosis in HCC patients [45] and with the degree of aggressiveness of the disease. A similar relationship has been observed for some other cancers, such as melanoma and renal cell carcinoma [46].

In HCC, increased tissue expression of CXCR4 is associated with risk of tissue metastasis and shorter survival [47]. The ligand for this receptor, CXCL12, and the entire CXCR4-CXCL12 axis have also been shown to play an important role in tumorigenesis, and blocking them with a synthetic antagonist is able to inhibit tumor cell migration and invasion [48].

Summary

The knowledge of chemokines is important because of their role in the pathogenesis of many diseases with diverse pathogenetic mechanisms. This allows for their potential use in diagnosis and therapy. Despite the discovery of many relationships between the concentrations of chemokines in various liver diseases, this topic seems to be still poorly studied and leaves much to be discovered.

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