Minireview

# The nuclear protein HMGB1, a new kind of chemokine?

Bernard Degryse, Maddalena de Virgilio*

*Department of Cell Biology, Division of Vascular Biology/VB5, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92130, USA*

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**Abstract The chromosomal protein HMGB1 is now regarded as a proinflammatory cytokine. Importantly, HMGB1 has chemotactic activity suggesting its involvement in the early and late events of the inflammatory reaction. Therefore, HMGB1 has all the hallmarks of a chemokine (chemotactic cytokine). We propose to classify HMGB1 into a new group of proteins unrelated structurally to chemokines but having chemokine-like functions, and to name this class CLF (chemokine-like functions). The CLF class should include other unrelated molecules such as urokinase and its receptor, cytokines macrophage migration inhibitory factor (MIF) and interleukin (IL)-6, anaphylatoxin C5a, ribosomal protein S19, and thioredoxin that have similar chemokine-like activities. This innovative concept may lead to the identification of new therapeutic targets.**


*Key words:* Chemokine; Inflammation; Cell migration; HMGB1; Urokinase; Urokinase receptor; Macrophage migration inhibitory factor; C5a; S19; Thioredoxin

## 1. Introduction

HMGB1 is a member of the high mobility group box (HMGB) family of chromosomal proteins, and is well known for its nuclear functions [1]. Recent studies have clearly demonstrated surprising cytokine-like roles [2–10]. Furthermore, HMGB1 is capable to induce chemotaxis of a variety of cells [11–15]. Therefore, we propose to consider HMGB1 as a new kind of chemokine (chemotactic cytokine). In this review, we discuss the recent data that support this view.

## 2. HMGB1 structure

The HMGB family consists of three proteins, HMGB1, HMGB2, and HMGB3 sharing a common structure. They are organized into three domains, two homologous DNA binding domains, boxes A and B, and an acidic C-terminal domain (Fig. 1A). This domain represents the main difference between the HMGBs. HMGB1 has the longest C-terminal domain while HMGB3 possesses the shortest. The HMG boxes, A and B, are similar 80 amino acid segments (29% identical, 65% similar) that form an L-shaped structure [1]. HMGB1 is a widely expressed and very abundant ($n > 1 \times 10^6$ copies/cell) 215-residue single chain, 25-kDa polypeptide [1].

## 3. Nuclear functions

HMGB1 binds to the minor groove of double-stranded DNA without apparent sequence specificity, but with high affinity for specific DNA structures such as kinked or bent DNA and four-way junctions (for a review see [16]). Moreover, various DNA binding proteins can recruit HMGB1 to DNA when in need of a local deformation of DNA in order to interact properly with their cognate binding site. In that case, HMGB1 can bend sharply the DNA, promoting the formation of nucleoprotein complexes and thus the interactions of DNA binding proteins with their respective DNA cognate sites [16]. This is exactly the task that HMGB1 performs on behalf of p53 transcription factor [17]. p53 binds inefficiently to linear DNA, HMGB1 bends the DNA, provides it to p53 that can thus bind efficiently to its sites on the DNA sequence, then HMGB1 dissociates from the complex [17]. HMGB1 exerts a similar role for other proteins interacting with DNA that are for instance, Hox, Pou, and TBP transcription factors, RAG1 site specific recombination protein [16]. The role of HMGB1 as a regulator of transcription is further supported by the phenotype of HMGB1$^{-/-}$ mice which have a defect in the activation of GR-responsive genes and die shortly after birth due to hypoglycemia [18].

## 4. Extracellular roles

Although HMGB1 (formerly HMG1 or differentiation enhancing factor, DEF, or amphoterin) does not harbor a signal sequence, it is released by various cells [2,3,8,15,19–22]. Its secretion does not occur through the classical ER-Golgi route [20,22]. In activated monocytes, HMGB1 is redistributed via a leaderless pathway from the nucleus into secretory lysosomes, and then released by exocytosis [22]. A similar mechanism may apply to murine erythroleukemia (MEL) cells [19].

*Corresponding author. Fax (1)-858-784 7422.
*E-mail address:* virgilio@scripps.edu (M. de Virgilio).

*Abbreviations:* 7TM-R, seven-transmembrane domain receptor; C5aR, C5a receptor; ECM, extracellular matrix; HMGB, high mobility group box; IL, interleukin; IL-6R, IL-6 α-receptor subunit; LPS, lipopolysaccharides; MEL, murine erythroleukemia; MIF, macrophage migration inhibitory factor; PA, plasminogen activator; PT, pertussis toxin; RAGE, receptor for advanced glycation endproducts; RP S19d, ribosomal protein S19 dimer; SMC, smooth muscle cells; Trx, thioredoxin; uPA, urokinase; uPAR, urokinase receptor

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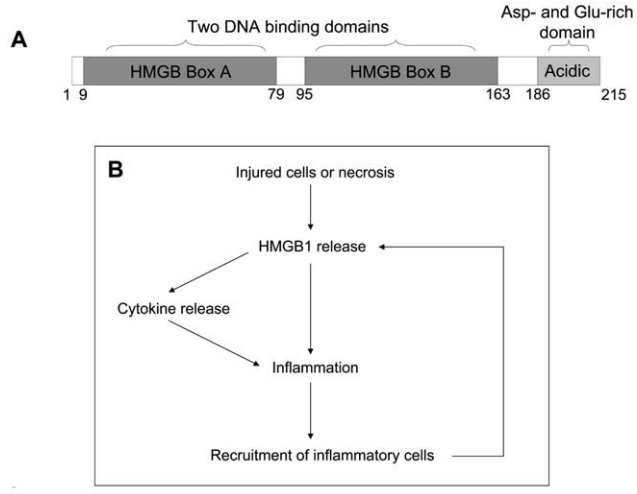


Fig. 1. A: Structure of HMGB1. The two HMG boxes harbor the chemotactic epitope(s). B: A very simplified model illustrating the role of HMGB1 in acute and chronic inflammation.

Once secreted, HMGB1 stimulates cell differentiation in an autocrine, paracrine or endocrine manner [19–21].

In the brain, HMGB1 is present in the nucleus and cytoplasm of neuronal cells, and extracellularly. Exogenous HMGB1 stimulates neurite outgrowth. Furthermore, anti-HMGB1 antibodies block laminin-dependent migration of neuroblastoma and glioma cells [11–14]. We showed that HMGB1 stimulates migration of smooth muscle cells (SMC) [15] and fibroblasts (Degryse and Bianchi, unpublished results). This effect is due to the presence of chemotactic epitope(s) within the HMG boxes. Both boxes A and B stimulate cell migration to a similar extent but box A requires a dose 10-fold lower than box B [15]. For the optimal concentration of HMGB1, boxes A and B are in the nanomolar range [15] comparable to optimal concentration of classical chemokines [23]. The sequences of the two boxes show low level of similarity (29%) but they have a very similar tertiary structure. Thus, it is most likely that boxes A and B exert their chemotactic effects through the same receptor and mechanism of signal transduction. This is further supported by a comparable sensitivity to Bordetella pertussis toxin (PT) [15]. HMGB1 or each of its boxes also induces similar reorganization of the actin cytoskeleton and changes of cell morphology typical of motile cells [15].

This effect of HMGB1 on cell motility may be of great importance for diseases such as cancers. Indeed, invasion of the surrounding tissue by tumors and metastasis require the coordination of cell signaling to induce cell motility, and pericellular proteolysis to degrade the extracellular matrix (ECM). Interestingly, HMGB1 has been shown to stimulate cell invasion, tumor growth and metastasis [24]. HMGB1 effect on cell invasion is blocked by the addition of anti-HMGB1 antibodies [24]. However, the complete mechanism of action is not known. Furthermore, HMGB1 is connected to an important system, the plasminogen activator (PA) system, involved in the regulation of pericellular proteolysis, cell migration, inflammation, fibrinolysis, wound healing, angiogenesis and cancer invasion [25]. Interaction between HMGB1 and tissue-type PA (tPA) stimulates plasmin formation [13,26]. HMGB1 also induces the activation of metalloproteases, MMP-2 and MMP-9 [24]. Thus, HMGB1 may directly induce cell motility, and indirectly promote the degradation of ECM proteins and facilitate cell invasion.

Binding of HMGB1 to the receptor for advanced glycation endproducts (RAGE) induces cell migration, cell invasion, tumor growth and metastasis [15,24]. However, other receptor(s) may exist. Indeed, HMGB1 interacts with a wide variety of molecules and RAGE has not been shown to interact directly with a heterotrimeric G protein [1,13,15,26–28]. Moreover, HMGB1 is internalized and can induce differentiation of MEL cells by binding to unknown receptor(s) [29,30].

HMGB1 triggers activation of the members of the mitogen-activated protein (MAP) kinase pathway, p38, JNK, and ERK 1/2, and subsequently of NF-κB [15,24,31]. In addition, both neurite extension and reorganization of actin cytoskeleton require the activity of small G proteins [15,31].

## 5. HMGB1 as a chemokine?

Cytokines are released at site of tissue injury and/or infection, and are part of the adaptive immune response as well as the inflammatory reaction. Chemokines are a subclass of cytokines that are chemotactic. There is presently an increasing confusion about cytokines classification created by the discovery of cytokines with various structures, effects and origins. In contrast, the chemokine classification is simpler and organized in four families, CXC, CC, C and CX3C (the letter C indicates a cysteine residue, and X an unconserved amino acid) based on the number and sequential relationship of the first two of four conserved cysteine residues. In addition, these small proteins share a common secondary structure exhibiting a flexible N-terminal portion, three antiparallel β-sheets, and a C-terminal α-helix [32]. Chemokine receptors are seven-transmembrane domain receptors (7TM-R) that belong to the rhodopsin family of G protein-coupled receptors (GPCRs) [32]. To date, about 50 chemokines and 20 receptors have been identified implying that a number of chemokines bind to the same receptor and that one chemokine can bind several receptors [32]. Their most characteristic function is the stimulation of cell migration that is inhibited by PT indicating a functional coupling between 7TM-R and G proteins [32]. Important downstream signaling molecules regulated by chemokines are for instance phospholipase C that leads to inositol-1,4,5-triphosphate generation, and the increase of cytosolic $Ca^{2+}$ concentrations. The MAP kinase pathway, which is an important regulatory pathway of cell migration, is also controlled by chemokines [32]. HMGB1 does not share any structural relationship with the chemokines described so far. However, HMGB1 exerts chemokine-like functions. We are going to discuss these points now.

The cytokine-like functions of HMGB1 are well documented. This protein is a late mediator of endotoxin lethality [2]. Lipopolysaccharide (LPS)-treated mice exhibit increased levels of HMGB1 in the circulation, and administration of anti-HMGB1 antibodies prevents LPS-induced mice death [2]. Increased serum levels of HMGB1 were also observed in human sepsis, and in one patient in hemorrhagic shock [2,33]. HMGB1 levels correlate with the severity of sepsis [2]. It has been proposed that $SPLI^{-/-}$ (secretory leukoprotease inhibitor) mice have a higher mortality from endotoxin shock than wild-type mice because $SPLI^{-/-}$ macrophages produce more HMGB1 and interleukin (IL)-6 [34]. It is striking that ethyl pyruvate, a lipophilic pyruvate derivative, administration pre-

vents lethal sepsis and probably hemorrhagic shock in mice by decreasing the levels of circulating HMGB1 [35]. In line with this observation, injection of HMGB1 is lethal and reproduces the signs of endotoxemia [2]. Moreover, HMGB1 can promote acute and chronic inflammation, inducing acute lung inflammation, synovitis and arthritis [4,6,10].

The role of HMGB1 in inflammatory diseases may be explained by its dual functions. HMGB1 can act directly by binding to the cell inducing signaling and chemotaxis, and indirectly by upregulating the expression and secretion of proinflammatory cytokines (Fig. 1B). HMGB1 is both a signal of tissue injury and a mediator of inflammation [2,4–6,8–10]. HMGB1 is passively released by injured or necrotic cells. In this way, HMGB1 acts as a signaling molecule, inducing local inflammatory responses [8,9]. HMGB1 knockout necrotic cells have a greatly decreased capacity to induce inflammation [8]. On one hand, this can benefit to the injured tissue. HMGB1 may initiate and modulate both early inflammatory steps, such as extravasation and chemotaxis of inflammatory cells, and late inflammatory events, such as the chemotaxis of cells, required to clear an infection and/or repair a tissue. On the other hand, the inflammatory response promoted by HMGB1 release may lead to pathological situations. We hypothesized that HMGB1 could be involved in vascular diseases such as atherosclerosis and restenosis [15]. Recent data further support our hypothesis. HMGB1 is secreted by activated monocytes and macrophages, and also released by damaged or necrotic cells, leading to inflammation [8]. In addition, HMGB1 induces the release by the injured endothelial wall of cytokines, chemokines and PAI-1 (PA inhibitor-1) that have chemotactic activities [9,36]. Thus, a damaged endothelium can induce the migration of SMC involved in vascular diseases. Furthermore, in diseases such as arthritis in which macrophages play a key role, chronic inflammation may be explained by the fact that HMGB1 is released by macrophages (Fig. 1B) [2,6].

HMGB1 may promote inflammation by binding to RAGE [1], but again other receptors cannot be excluded yet. For instance, syndecan-1 is a member of the syndecan family of transmembrane heparin sulfate proteoglycans, which play a role as co-receptors for primary signaling receptors such as growth factors, integrins and other adhesion receptors. Syndecans are involved in inflammation and are capable to regulate leukocyte trafficking [37].

We already mentioned the correlation between the inflammatory reaction and the expression of HMGB1. Cytokines and LPS induce HMGB1 release [2,3,38]. The opposite relationship has also been proven to be true. HMGB1 can regulate cytokine expression [4,5,7–9,10,39].

As a chemokine, HMGB1 should control the activation and chemotaxis of leukocytes. HMGB1 exerts these functions. HMGB1 expression is an activation event for neutrophils and monocytes/macrophages [5,10,39]. Recruitment of inflammatory cells through the endothelium and into the inflammatory foci requires adhesion and transmigration through the endothelium and the ECM. HMGB1 modulates the expression of adhesion molecules that facilitate the extravasation of monocytes/macrophages [9]. Importantly, HMGB1 generates a chemotactic signal by binding to the cell. HMGB1 promotes migration of adherent cells such as fibroblasts and SMC, and the recruitment of inflammatory cells such as neutrophils, monocytes and macrophages [4,6,8,10,15]. It has been suggested recently that the HMG boxes A and B have different properties, box A has an anti-inflammatory while box B has a proinflammatory activity [40]. In our hands, both boxes were equally chemotactic [15]. Last, HMGB1 can regulate the proteolytic activity needed for the degradation of the components of the ECM through its connection with the PA system and metalloproteases (see above).

In conclusion, like classic chemokines, HMGB1 is a chemotactic and immunoregulatory protein, which can promote an effective inflammatory immune response. The connection between HMGB1 and a 7TM-R is not proven yet. However, HMGB1-induced cell migration is achieved through a heterotrimeric G protein-dependent mechanism, and activation of the MAP kinase pathway, which are both generally activated by chemokine receptors. Therefore, HMGB1 has all the hallmarks of a proinflammatory chemokine. We propose to modify the chemokine classification based only on the presence of specific CXC, CC, C or CX3C (C indicates a cysteine residue) motifs in the sequence of the proteins. A new class of chemokines unrelated structurally to 'cysteine' chemokines but exerting chemokine-like functions should be created. We propose to name this new class of proteins CLF (chemokine-like functions). Another possible name for this class is HULCK (highly unconventional chemokine).

## 6. Other CLF chemokines

The CLF class should include other proteins that have also chemokine-like functions. We briefly present a few other CLF chemokines.

### *6.1. Urokinase (uPA) and its receptor (uPAR)*

uPA and uPAR are certainly among the best CLF candidates (Table 1). uPA is a serine protease that binds to cell surface through uPAR, a glycosyl-phosphatidyl-inositol (GPI)-anchored protein. By binding to uPAR, uPA induces a conformational change of uPAR resulting in the exposition of a chemotactic epitope located in the linker region of uPAR [25]. This change turns uPAR into a ligand of FPRL1, the low affinity 7TM-R for fMLP, which in turn stimulates cell migration [25]. Like chemokines, uPA/uPAR-dependent signaling is PT sensitive suggesting the involvement of a heterotrimeric G protein and leads to the activation of the MAP kinase pathway [25,41,42]. Both proteins play important roles in inflammation that have been documented by in vitro and in vivo studies (for a review see [25]). Recruitment of inflammatory cells is deficient in $uPA^{-/-}$ mice. In human, the expression of uPA correlates with the severity of inflammation in several diseases. uPA expression is also an activation event for macrophages and lymphocytes, regulating cytokine expression. Conversely, cytokines and LPS increase uPA expression. Furthermore, uPA is a strong chemoattractant promoting migration of adherent and non-adherent cells such as SMC, fibroblasts, macrophages and lymphocytes [25].

uPAR has already been proposed to be a membrane-associated chemokine [41,43]. uPAR possesses most of the chemokine-like properties of uPA (Table 1) [25]. uPAR is an activation antigen of T lymphocytes. uPAR expression is increased in several diseases such as acute myocardial infarction and high levels are found in the circulation of patients suffering of various cancers and HIV infections. In addition, uPAR is released during sepsis, endotoxemia, and rheumatoid

arthritis. uPAR is upregulated by LPS, formylated peptides and cytokines [25]. However, this receptor has other ligands, such as vitronectin, a protein of the ECM that promotes cell adhesion and migration, and high molecular kinin-free kininogen (HKa) that inhibits cell adhesion [25]. Thus, it is not surprising that uPAR$^{-/-}$ and uPA$^{-/-}$ mice have different phenotypes. For instance, leukocyte recruitment during acute inflammation, but not during long term inflammation, is drastically reduced in uPAR$^{-/-}$ mice, whereas it is completely abrogated in both cases in uPA$^{-/-}$ mice [25]. Finally, in contrast to classical chemokines, uPA/uPAR induce chemotaxis of all leukocytes.

### 6.2. Macrophage migration inhibitory factor (MIF)

MIF was first identified as a T cell-derived inhibitor of random cell migration of macrophages [44,45]. However, MIF has a unique characteristic, it is produced by the anterior pituitary gland and may be considered as a hormone that acts as a counterregulator of glucocorticoids [44,45]. MIF expression is induced by low dose of glucocorticoids and antagonizes their anti-inflammatory effects. Yet, MIF is also recognized as a proinflammatory cytokine and has chemokine-like functions (Table 1). As a cytokine, MIF can activate macrophages inducing the production of tumor necrosis factor (TNF)-α, IL-1, IL-6 and IL-8. Conversely, TNF-α, IL-1, IL-6, IL-8, and IL-12 induce MIF production by macrophages. MIF is also released by various cells including T cells, and endothelial cells. Surprisingly, MIF seems to play a role similar to HMGB1 in sepsis [44,45]. In mice, MIF exacerbates the lethal effect of LPS, whereas anti-MIF antibody protects against lethal peritonitis, exotoxemia, and endotoxemia [44,45]. Recruitment of neutrophils is reduced in LPS-treated rats by the administration of anti-MIF antibody [44] protecting against acute lung injuries. Similarly, anti-MIF antibody can also protect against LPS-induced acute liver injury [44]. Furthermore, MIF$^{-/-}$ mice are resistant to septic shock induced by high doses of LPS [44,45], and show decreased levels of TNF-α than their wild-type counterparts. Again surprisingly similar to HMGB1, MIF seems to be involved in wound healing, tumor growth, angiogenesis [44,45], and regulates chemotaxis [46,47]. MIF induces the production of MMPs enabling tumors growth and metastasis but also inducing tissue damages observed for instance in chronic inflammatory diseases such as arthritis. The mechanism of action of MIF is poorly understood. CD74 (or Ii, Iγ), MHC class II-associated invariant chain, has been recently identified as a cell receptor for MIF [48]. CD74 is a type 2 transmembrane receptor and so far, no interaction has been described between CD74 and an eventual 7TM-R and/or a G protein. MIF has been shown to activate p38, and p44/42 (ERK 1/2) MAP kinases, the latter in a CD74-dependent manner [48].

### 6.3. C5a anaphylatoxin and S19 ribosomal protein

The complement is a complex cascade of the immune system that leads to the elimination of the antigen and/or pathogen. Three pathways trigger complement activation. The classical pathway is activated by immune complex, the lectin pathway by the recognition of microbial carbohydrates by mannose binding lectin, and the alternative pathway by pattern recognition of foreign surfaces (for reviews see [45,49,50]). These pathways converge on C3, the central molecule of the complement. Cleavage of C3 leads through successive steps to the formation of C5b-9, the membrane attack complex [45,49,50]. Cleavage of C5 generates a byproduct, the anaphylatoxin C5a which promotes several proinflammatory effects such as SMC contraction, enhanced vascular permeability, vasodilatation, release of granular enzymes and secondary inflammatory mediators by phagocytic cells, production of oxidase activities in neutrophils and eosinophils [45,49,50]. C5a is also a strong chemoattractant (Table 1) that recruits a wide variety of leukocytes [45,49,50]. Furthermore, C5a induces the release of cytokines and chemokines including TNF-α, IL-1, IL-6, IL-8, IL-12 [50]. Thus, complement activation is associated with inflammatory disorders including sepsis. During sepsis, an overactivation of the complement generates an excess of C5a that results in explosive proinflammatory responses leading to tissue injury, multi-organ failure, and death [45,49,50]. In mice and rats, administration of antibodies against C5a or its receptor C5aR effectively protects against septic lethal shock [45]. This correlates with decreased levels of TNF-α and IL-6 [45]. C5a acts by binding to C5aR (CD88), a 7TM-R coupled to both PT-sen-

Table 1
Properties of CLF chemokines compared to a classic inducible chemokine

| | HMGB1 | uPA | uPAR | MIF | C5a | RP S19d | Trx | IL-6 | Inducible chemokine |
|---|---|---|---|---|---|---|---|---|---|
| Release under inflammatory conditions | yes | yes, release is increased, but secreted not only in inflammatory conditions | expression increased | yes | yes | yes | yes | yes | yes |
| Expression upregulated by cytokine | yes | yes | yes | yes | no | ? | no | yes | yes |
| Engage a specific subpopulation of leukocytes | yes | stimulate all leukocytes | stimulate all Leukocytes | yes | yes | yes | yes | yes | yes |
| Bind to a 7TM-R | no (?) | bind to uPAR which in turn bind to FPRL1, a 7TM-R | yes | no (?) | yes | yes | no | no | yes |
| Signal via a hetero-trimeric G protein | yes | yes | yes | no (?) | yes | yes | no | no | yes |
| Activate MAP kinase pathway | yes | yes | yes | yes | yes | ? | ? | yes | yes |
| Regulate cytokine expression | yes | yes | yes | yes | yes | ? | yes | yes | yes |
| Activate leukocytes | yes | yes | yes | yes | yes | ? | yes | yes | yes |
| Chemotactic | yes | yes | yes | yes | yes | yes | yes | yes | yes |

sitive and -insensitive G proteins [49]. By binding to C5aR, C5a activates p44/42 MAP kinases [51]. C5aR is expressed in leukocytes but also in other cells such as SMC, endothelial and epithelial cells.

The S19 ribosomal protein is a protein of the mRNA translation machinery leading to protein synthesis. However, as a cross-linked homodimer (RP S19d), it has a very different role [52,53]. RP S19d has been first purified from a rheumatoid arthritis synovial lesion suggesting a possible role in inflammation. This hypothesis was further supported by the finding that RP S19d is another ligand of C5aR. Furthermore, RP S19d is released by apoptotic cells [54], and is a chemoattractant for monocytes and macrophages [52,53]. In contrast to C5a, RP S19d is an antagonist of C5aR in polymorphonuclear leukocytes [53] inhibiting their chemotaxis. These data suggest that RP S19d might be involved in the recruitment of monocytes/macrophages into the apoptotic site, and promote in this way the clearance of apoptotic cells [52]. Thus, preventing tissue damage, inflammation, and autoimmune reactions. Too few data are presently available and further investigations are necessary to precise its role. Nevertheless, RP S19d represents a very good potential CLF chemokine (Table 1).

### 6.4. IL-6

IL-6 is a hematopoietin cytokine that exerts pleiotropic effects and plays a crucial role in cell growth, differentiation, survival, acute phase protein production, fever, inflammation, tumor growth and cell migration [55–57]. IL-6 is produced by a wide variety of cells including T cells, B cells, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells, SMC, and tumor cells. IL-6 induces also the production of cytokines and chemokines including IL-2, IL-8, MCP-1, and MCP-3, and depending on cell types, LPS, cytokines, IL-1, IL-4, TNF-α, interferon (IFN)-γ, tumor growth factor (TGF)-β, and chemokines such as MCP-1 (CCL2), and RANTES (CCL5) promote IL-6 synthesis (for a review see [56]). As a growth factor, IL-6 induces growth and/or differentiation of various cells, megakaryocytes, hematopoietic stem cells, osteoclasts, B cells, hepatocytes, T cells, PC-12 cells, myeloma cells, keratinocytes and mesangial cells. Importantly, IL-6 is a chemoattractant (Table 1) for SMC, osteoclasts, adherent lymphokine-activated killer cells, lymphocytes, and carcinoma cells [55,56]. In agreement with its growth factor and chemoattractant functions, the healing of skin wounds is dramatically delayed in IL-$6^{-/-}$ mice [57]. In addition, IL-6 enhances IL-1-induced production of MMPs and thus may promote tissue invasion. Due to decreased chemokine production, recruitment of neutrophils is defective in IL-6-deficient mice but can be restored by the administration of IL-6 [55]. IL-$6^{-/-}$ mice are also protected against joint inflammation and tissue damages resulting from arthritis [56]. In line with these data, in wild-type mice, antibodies against IL-6R (IL-6 α-receptor subunit) prevent these disease-induced damages [56]. On the other hand, IL-6 has also anti-inflammatory activity. In LPS-treated rats, injection of IL-6 decreased the levels of TNF-α and synthesis of IL-1. In an animal model of endotoxin lung injury and endotoxemia, levels of TNF-α were higher in IL-$6^{-/-}$ than in wild-type mice [56]. The mechanism of signal transduction is very particular, and initiated by the binding of IL-6 to IL-6R, which has no signaling activity. Then, the IL-6–IL-6R complex recruits the transducer gp130 [56]. The resulting IL-6–IL-6R–$(gp130)_2$ complex leads to the activation of the downstream MAP kinase signaling cascade and other pathways [56]. However, no link has been reported between IL-6 signaling and 7TM-R and/or G protein. Unlike gp130, the expression of IL-6R is limited. Nevertheless, a soluble form of IL-6R (sIL-6R) exists in the circulation and higher levels have been observed in chronic arthritis and other pathologic conditions [56]. Thus, IL-6–sIL-6R complex can act on cells devoid of IL-6R transforming them in IL-6-responsive cells [56]. A soluble form of gp130 has also been found [56]. In that case, interactions between sgp130 and IL-6–sIL-6R will certainly antagonize the effect of IL-6.

### 6.5. Thioredoxin (Trx)

Trx, or adult T cell leukemia-derived factor (ADF) is certainly a good example of CLF chemokine (Table 1). Indeed, Trx exhibits cytokine-like and chemokine-like activities ([23,58], for a review see [59]). It is striking that Trx shares several characteristics with HMGB1. Both are ubiquitously expressed, and their cellular distribution is also almost similar. Trx is present in the cell nucleus, cytoplasm, mitochondria, and also in the extracellular compartment [59]. In addition, the secreted Trx has no signal peptide, and does not follow the classical ER-Golgi route [59]. Furthermore, Trx also regulates the DNA binding activity of redox-sensitive transcription factors NF-κB, APC-1, p53, CREB, PEBP2/CBF, Myb, and both estrogen and glucocorticoid receptors [59]. However, Trx has specific characteristics. As a dithiol-disulfide oxidoreductase, Trx catalyzes the reduction of disulfides. Trx is a key player in keeping intracellular protein disulfides in their reduced state [59]. As a cytokine, Trx induces IL-2 receptor, and activates eosinophil functions, cytotoxicity and migration. Moreover, Trx exerts proinflammatory effects promoting cytokines, such as TNF, release by fibroblasts and monocytes [59]. The chemokine-like activities of Trx are well illustrated by its potent chemotactic effect on neutrophils, monocytes and T cells [23]. In addition, Trx has been found partially responsible for the chemotactic activity release by human T cell leukemia/lymphoma virus (HTLV)-1-infected cells [23]. The chemotactic activity is harbored by the CXXC active site of the enzyme that forms a disulfide in the oxidized form or a dithiol in the reduced form. Indeed, mutation of cysteines of the active site results in the loss of chemotactic activity [23]. The exact mechanism of signal transduction is not known. However, it has been shown that it is G protein independent and unlike classical chemokines does not require a 7TM-R [23]. Stress stimuli such as hypoxia, ultraviolet (UV) irradiation, X-ray radiation, oxidants such as hydrogen peroxide, phorbol ester, molecules increasing intracellular cAMP such as forskolin, infectious agents, hormones, chemicals, and LPS induce Trx production. Trx is released by various cells, for instance keratinocytes, melanocytes, monocytes, IFN-γ or LPS-activated macrophages and activated T lymphocytes as well as other normal and neoplastic cells [59]. Trx is upregulated in diseases associated with oxidative stress, for example in cerebral ischemia, brain injury, and open-heart surgery. In the circulation, high level of Trx is a marker of poor prognosis in HIV infection. Elevated levels of Trx are also observed in hepatitis C virus infection, synovial fluid of rheumatoid arthritis, and cancer [59]. Trx has also anti-inflammatory activities. Trx protects against hydrogen peroxide and hypoxia, and suppresses LPS-induced neutrophil adhesion and chemotaxis

Table 2
Primary and signal functions of CLF chemokines

| CLF chemokine | Characteristics | Primary functions | Signal |
|---|---|---|---|
| HMGB1 | 25 kDa, 215 amino acids | nuclear protein, regulation of transcription | cell injury, necrosis, sepsis |
| uPA | 54 kDa, 411 amino acids | protease | tissue remodeling |
| uPAR | 55–60 kDa, 283 amino acids | localization of protease activity | tissue remodeling |
| MIF | 12.5 kDa, 115 amino acids | cytokine, steroid resistance, regulation of both innate and adaptive immune responses | sepsis |
| C5a | 15 kDa, 74 amino acids | complement, innate immunity | infection, sepsis |
| RP S19d | 15.5 kDa, 145 amino acids (monomer) | ribosomal protein, regulation of translation | apoptosis |
| IL-6 | 21–28 kDa, 184 amino acids | cytokine, growth factor | wound (presence of fibrin clot) |
| Trx | 12 kDa, 105 amino acids | control of redox | oxidative stress |

[58,59]. Furthermore, since chemokine pretreatment induces desensitization and thus inhibits chemotaxis, elevated levels of Trx have been shown to inhibit neutrophil and monocyte recruitment [58]. However, the mechanism of desensitization is unclear because Trx does not act through a 7TM-R and/or G proteins. Few data are available on the intracellular effectors and signaling pathways controlled by Trx. Apoptosis signaling-regulating kinase 1 (ASK1) is negatively regulated by Trx, thus preventing apoptosis [59].

Trx80, a truncated form of Trx produced by monocytes, has been purified in human peripheral blood mononuclear cell (PBMC) cultures. Trx80 lacks the C-terminal part of Trx, and consists of the 80 N-terminal residues of the protein. Trx80 (most likely identical to eosinophilic cytotoxicity enhancing factor, ECEF), active as a dimer, has specific characteristics [60]. For instance, Trx80 stimulates eosinophilic cell cytotoxicity, and proliferation and activation of monocytes. Trx80, through secretion of IL-12, is also indirectly mitogenic for T lymphocytes [60].

## 7. Conclusions

In this review, we propose to create a new class of chemokines, named CLF, which groups presently together eight proteins. This list will probably expand. This new classification, required by the need to give to proteins such as HMGB1 a proper and well-deserved status, is based only on functions. CLF chemokines do not share the traditional structures of classical CXC, CC, C or CX3C chemokines. Table 1 summarizes the characteristics of all CLF chemokines presented here, and compares them to a classical chemokine. It is puzzling that these unrelated proteins that have very different primary roles (Table 2) can exert similar chemokine-like functions, basically recruitment and activation of leukocytes. It appears that these basic roles are achieved through various receptor types and signal transduction mechanisms as shown by different sensitivity to PT. However, the generated signals converge inside the cell and result in the activation of the MAP kinase pathway. Yet, some molecules have not been thoroughly studied, and future research might reveal more shared characteristics. Nevertheless, it is already possible to distinguish a common pattern. CLF chemokines are multifunctional proteins that exert various well-defined primary roles, and under special circumstances can exhibit chemokine-like functions as secondary roles (Table 2). This idea may better be illustrated by the other proposed family name, HULCK. They target not only leukocytes but also other normal or neoplastic cell types. Since CLF chemokines are ubiquitous or widely expressed, they can serve in order to obtain optimally adapted cellular responses, as convenient signals to transmit to immune cells and to the surrounding tissue the occurrence of a particular event (Table 2), for instance, tissue injury or necrosis in the case of HMGB1.

CLF class represents an innovative concept that may change our traditional view of chemokines and inflammation, and may lead to the identification of new therapeutic targets.

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