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REVIEW

An introduction to chemokines and their roles in transfusion medicine

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Chemokines are a set of structurally related peptides that were first characterized as chemoattractants and have subsequently been shown to have many functions in homeostasis and pathophysiology. Diversity and redundancy of chemokine function is imparted by both selectivity and overlap in the specificity of chemokine receptors for their ligands. Chemokines have roles impacting transfusion medicine in haematopoiesis, haematologic malignancies, transfusion reactions, graft-versus-host disease, and viral infections. In haematopoietic cell transplantation, chemokines are active in mobilization and homing of progenitor cells, as well as mediating T-cell recruitment in graft-versus-host disease. Platelets are rich source of chemokines that recruit and activate leucocytes during thrombosis. Important transfusion-transmissible viruses such as cytomegalovirus and human immunodeficiency virus exploit chemokine receptors to evade host immunity. Chemokines may also have roles in the pathophysiology of haemolytic and non-haemolytic transfusion reactions.

Key words: Chemokines, chemokine receptors, haematopoietic stem cell transplantation, graft-vs-host disease, transfusion reactions.

General characteristics of chemokines

Chemokines are small, secreted proteins in the range of 8–10 kDa that have numerous functions in normal physiology and pathology. The term derives from the words chemotactic cytokines, reflecting their important role in leucocyte chemoattraction. However, it is clear from an accumulating body of evidence that chemokines have many other functions in intercellular communication, cellular activation, and cell cycle regulation. The transcription of most chemokine genes is inducible and occurs in response to specific cellular stimuli. These have been classified as pro-inflammatory chemokines, as they have major roles in regulating immune and inflammatory responses, although inflammation is certainly not the only setting in which these mediators are produced. A few chemokines are produced at tonic levels physiologically, particularly in maintenance for normal bone marrow and lymphoid tissue, and are classified as homeostatic chemokines. This classification is not completely definite, as under some conditions homeostatic chemokines are inducible.

Most chemokines were originally named for their first identified biological activity, such as monocyte chemoattractant protein. This led to many chemokines having several synonyms before their molecular identities were established. Once it became clear that there are marked structural similarities among chemokines, a rational systematic nomenclature was established by The Chemokine Nomenclature Subcommittee of the Nomenclature Committee of the International Union of Immunological Societies [1].

Chemokines have been grouped according to structural similarities and contain characteristic conserved cysteine residues. The largest classes are CC chemokines, in which the first two of four cysteines are adjoining and CXC chemokines that have one intervening amino acid between the first two of four cysteines (Tables 1 and 2). CXC chemokines are further subdivided based on the presence or absence of Glu-Leu-Arg (ELR) motif near the amino terminus, designated ELR^+ and ELR^- chemokines, respectively. Two minor classes are C chemokines that retain only one cysteine at the amino terminus, and CX3C chemokines with three intervening amino acids. At present, only two C and one CX3C chemokines have been identified. In systematic nomenclature, each chemokine is

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Table 1 Nomenclature of human CC chemokines

Systematic name	Common names	Inflammatory (induced)/homeostatic (constitutive)/mixed function	References
CCL1	I-309	I	88
	P-500		
	TCA-3		
CCL2	MCAF	I	89
	TDCF		
	MCP-1		
CCL3	MIP-1α	I	90,91
	LD78α		
CCL4	MIP-1β	I	91
CCL5	RANTES	I	92
CCL7	MCP-3	I	93
CCL8	MCP-2	I	94
CCL11	Eotaxin-1	I	95
CCL13	MCP-4	I	96
CCL14	CKβ1	H	97
	HCC-1		
	MCIF		
CCL15	HCC-2	M	98,99
	Lkn-1		
	MIP-5		
	MCP-1γ		
CCL16	HCC-4	M	100,101
	LEC		
	LCC-1		
CCL17	TARC	M	102
CCL18	DC-CK1	H	103
	PARC		
	AMAC-1		
CCL19	EBI-1-Ligand	H	104,105
	ELC		
	MIP-3β		
	ckβ11		
CCL20	LARC	M	104,106
	MIP-3α		
CCL21	6CKine	M	107
	SLC		
	TCA-4		
	ckβ9		
CCL22	MDC	M	108
	STCP-1		
	abck-1		
	dc/β-ck		
CCL23	MPIF-3	I	109
	CKβ8–1		
CCL24	MPIF-2	I	109
	Eotaxin-2		
CCL25	TECK	H	110
CCL26	MIP-4α	I	111
	PTEC		
	Eotaxin-3		
CCL27	CTAK	H	112
	ILC		
CCL28	MEC	I	113

Gaps between numbers occur because human analogues of some chemokines identified in the mouse have not been recognized.

Table 2 Nomenclature of CXC chemokines

Systematic name	Common names	Inflammatory (induced)/homeostatic (constitutive)/mixed function	References
CXCL1	GROα MGSA-α MIP-2	I	114,115
CXCL2	GROβ MGSA-β MIP-2α	I	115,116
CXCL3	GROγ MGSA-γ MIP-2β	I	115,117
CXCL4	PF4	H	118
CXCL5	ENA-78	M	119
CXCL6	GCP-2	I	120
CXCL7	PPBP (Protolytic cleavage yields CTAP-III, β-thromboglobulin, NAP-2)	H	121,122
CXCL8	IL-8 MDNCF	I	123
CXCL9	MIG	I	124
CXCL10	CRG-2 IP-10	I	125
CXCL11	I-TAC IP9	I	126
CXCL12	SDF-1α SDF-1β	H	127
CXCL13	BLC BCA-1	H	128
CXCL14	BRAK	I	129
CXCL16	SR-PSOX	I	130

designated by its class, followed by the letter L (for ligand) and a number based on the chronological order in which it was identified (Tables 1 and 2). Chemokine receptors are similarly indicated by the class to which it binds, the letter R, and a number (Table 3).

The sequence homologies among chemokines results in similarities in tertiary structure. Both CC and CXC chemokines have a basic structure with three anti-parallel β-sheets with the amino terminus held in relative orientation by disulphide bonds [2–7]. Characteristically, chemokines spontaneously associate into homodimers. The manner in which these dimers associate can be strikingly different between chemokines, despite the similarities in tertiary structure. CCL5 forms dimers in which the amino termini are closely associated and anti-parallel [8]. CXCL8, on the other hand, associates between the first β-sheet of the monomers, leaving the amino termini externally exposed [9]. Furthermore, heterodimers between chemokines of the same class and even between chemokines of different classes are possible. The combinations of CXCL1/CXCL7, CXCL4/CXCL8, CCL2/CCL5, CCL2/CCL8, CXCL4/CCL5, CXCL4/CCL2, and CXCL8/CCL2 have been demonstrated [10]. The biological significance of such mixed dimers has yet to be defined.

Chemokine receptors

Chemokine receptors belong to the G-protein coupled receptor superfamily of molecules containing seven transmembrane domains. Structural commonalities are an extracellular portion consisting of three peptide loops and an amino terminus, and an intracellular portion with three peptide loops and a serine/threonine-rich carboxy terminus. Chemokines receptors transduce signals through G-protein coupling. Chemokine receptors contain a conserved asp-arg-tyr (DRY) motif that is common to virtually all G-protein coupled receptors.

In the language of chemokine communication, the message, that is, the end result on cellular function, depends on the ligand, the receptor, and the target cell. Thus, there are synonyms, homonyms, antonyms and even nonsense words in this vocabulary. For example, CXCL6 and CXCL8 are synonymous in the sense that both induce chemotaxis of neutrophils through CXCR2, although they differ in potency,

Table 3 Ligands and cellular distribution of CXC and CC chemokine receptors

CXC ligands	Receptor	CC ligands	Principle leucocyte receptor distribution	References
CXCL6	CXCR1		N, Ba, Plt	131–135
CXCL8				
CXCL1	CXCR2		N, Ba	131,132,134,136
CXCL2				
CXCL3				
CXCL5				
CXCL6				
CXCL7				
CXCL8				
CXCL9	CXCR3	CCL5	PDC, BC, TH1, NK	137–144
CXCL10		CCL7		
CXCL11		CCL13		
		CCL19		
		CCL20		
CXCL12	CXCR4		N, BC, IDC, M, MDC, TH1, TH2, Ba, NK, PDC, HPC, PC, Plt	134,28,145–150
CXCL13	CXCR5		BC, NT	128,151
CXCL16	CXCR6		MT, NK, PC	139,150,152
	CCR1	CCL3	IDC, M, BC, Ba, NK, Plt	134,146,147,153–161
		CCL5		
		CCL7		
		CCL13		
		CCL14		
		CCL15		
		CCL16		
		CCL23		
	CCR2	CCL2	IDC, M, B, Ba, PDC, TH1, TH2	134,140,146,161–164
		CCL7		
		CCL8		
		CCL13		
		CCL16		
	CCR3	CCL5	Eo, TH2, Ba, IDC, PC, Plt	146,147,150,158, 163,165–168
		CCL7		
		CCL11		
		CCL15		
		CCL24		
		CCL26		
	CCR4	CCL17	Eo, TR, TH2, Ba, BC, Plt	143,146,147,161,169
		CCL22		
	CCR5	CCL3	IDC, M, Ba, TH1, NK	140,143,146,170–172
		CCL4		
		CCL5		
		CCL8		
	CCR6	CCL20	BC, IDC, M, N, NK	173–176
	CCR7	CCL19	MDC, TH1, NT	148,177–179
		CCL21		
	CCR8	CCL1	TR, TH2	148,180
	CCR9	CCL25	MT	181,182
	CCR10	CCL27	MT, PC	113,150,183–185
		CCL28		
Strong	DARC	Strong	RBC, Endo	21

Table 3 *Continued*

CXC ligands	Receptor	CC ligands	Principle leucocyte receptor distribution	References
CXCL5		CCL2		
CXCL6		CCL5		
CXCL8		CCL7		
CXCL11		CCL11		
Weak		CCL13		
CXCL9		CCL14		
CXCL10		CCL17		
CXCL13		Weak		
		CCL1		
		CCL8		
		CCL18		
		CCL16		
	D6	CCL2	Endo	25,186
		CCL3		
		CCL4		
		CCL5		
		CCL7		
		CCL8		
		CCL11		
		CCL13		
		CCl14		
	CCX-CKR	CCL19	Unknown	26
		CCL21		
		CCL25		

Ba, basophil; BC, B-cell; Endo, endothelial cell; Eo, eosinophil; HPC, haemaopoietic progenitor cell; IDC, immature dendritic cell; M, monocyte; MDC, mature dendiritic cell; MT, memory T-cell; N, neutrophil; NK, NK cell; NT, naïve T-cell; PC, plasma cell; PDC, plasmacytoid dendritic cell; Plt, platelet; TH1, T_H1/T_C1 T-cell; TH2, T_H2/T_C2 T-cell; RBC, red blood cell.

lending subtle nuance to the language. CXCL8 signalling through CXCR1 in neutrophils contributes to the inflammatory response, while in endothelial cells this receptor/ligand combination stimulates angiogenesis, and so can be thought of as homonyms. CCL7 acts as an antagonist of CCL4 binding and signalling through CCR5, so these two chemokines are in a sense antonyms. Non-signalling receptors, such as Duffy antigen receptor for chemokines (DARC), cause no cellular response, so binding of ligands to this receptor can be thought of as non-sense communication. The place of heterodimers in the chemokine vocabulary is uncertain, but raises the possibility of complex neologisms.

In addition to functional receptors, there are several silent, non-functional receptors that bind many chemokines. These silent receptors facilitate localization, transport, and metabolism of chemokines. Glycosaminoglycans (GAG) on the luminal surface of endothelial cells bind all classes of chemokines in an orientation that facilitates presentation to leucocyte receptors [11]. The common tertiary structure of chemokines allows for the binding of these molecules to GAGs in an orientation that presents the chemokine receptor binding site to circulating leucocytes. As leucocytes roll along the endothelial surface, they encounter GAG-bound chemokines. Leucocyte signalling through chemokine receptors then rapidly stimulates intergrin-mediated adhesion. Such activated leucocytes can then transmigrate into the extravascular space. GAGs in the intercellular matrix also bind chemokines, which allows for a concentration gradient to be established and maintained in tissue. Leucocytes can then travel up this stabilized gradient, a process that has been termed 'haptotaxis'. There are considerable differences between chemokines with respect to where the GAG binding domains reside. In CCL5, the binding motif is located within the 40s loop between the second and third β-sheets [12]. In CXCL8, the GAG binding domain is located at the carboxy terminus [13].

Duffy antigens and interceptors

Duffy (Fy) antigens bind both CC and CXC chemokines, a phenomenon termed the DARC. The Fy protein lacks the DRY motif necessary for G-protein signalling. While absence of Fy antigens on erythrocytes is common in some populations,

DARC expression on endothelial cells of postcapillary venules of skin, kidney, lung, spleen and high endothelial venules of lymph nodes is nearly universal [14–16]. DARC expression can be induced by inflammation in giant cell arteritis, rheumatoid arthritis, nephritis, and renal transplant rejection [17–20]. Proinflammatory chemokines bind preferentially to DARC, while this receptor has low affinity for homeostatic chemokines [21]. The angiogenic ELR$^+$ chemokines CXCL1, CXCL3, CXCL5, CXCL6 and CXCL8 bind to DARC while the angiostatic chemokines CXCL9 and CXCL10 do not. Although DARC does not appear to be a signalling receptor, ligation of DARC can result in pinocytosis and transport of the internalized vesicles across endothelial cells to the opposite membrane [22]. Because of this activity, DARC has been termed an 'interceptor', for internalizing receptor. Thus, DARC can present proinflammatory chemokines to circulating leucocytes at sites of inflammation and promote neoangiogenesis. An alternative explanation of DARC function has recently been proposed. DARC constitutively forms oligiomers on the cell surface and is capable of forming hetero-oligomers with CCR5 [23]. Such hybrid receptors have impaired signalling, but are internalized normally. While it has been suggested that lack of Fy antigens on red blood cells (RBCs) may be a contributing factor to poor renal allograft survival in Fy-negative individuals, more recent data call this into question [24]. At the present time, there is no definitive evidence that RBC Fy non-expression has any significant physiologic or pathologic effect.

The chemokine interceptor D6 binds at least nine proinflammatory chemokines, and like DARC in non-signalling. D6 mediates rapid internalization of chemokines, which are then degraded rather than transported across the cell [25]. The interceptor is recycled to the cell surface so that exposed membrane levels of D6 are not affected by internalization. A third chemokine interceptor is CCX-CKR. Like DARC and D6, CCX-CKR is non-signalling. It is more selective than the other silent receptors in that it binds CCL19, CCL21 and CCL25 [26]. CCX-CKR mediates rapid internalization and degradation of CCL19 [27]. While CCR7 becomes refractory to CCL19 uptake with continuous exposure to the chemokine, the sequestration activity of CCX-CKR actually increases.

Haematopoietic progenitor cells

Chemokines play a role in mobilization of haematopoietic progenitor cells (HPCs) for transplantation and the homing of transplanted HPCs. CXCR4 is expressed by CD34+ HPCs, and its ligand CXCL12 is constitutively expressed by osteoblasts and bone marrow endothelial cells [28,29]. Blockade of CXCR4 prevents human HPC engraftment and repopulation of the bone marrow of NOD/SCID mice [30]. GCSF mobilization of HPCs results in reduced surface expression of CXCR4, as well as other adhesion molecules such as VLA-4, most likely through enzymatic cleavage [31]. Similarly, GCSF stimulation results in enzymatic degradation of CXCL12. A competitive inhibitor of CXCR4, AMD3100, has been developed and tested in HPC mobilization. Early studies showed that a single dose of AMD3100 increased circulation CD34+ HPC more than 10-fold [32]. AMD3100 in combination with GCSF has been compared to GCSF alone in autologous transplantation of patients with multiple myeloma and non-Hodgkin's lymphoma [33]. Nine of 25 patients in this trial failed to achieve collection of 2×10^6 CD34+ cells/kg by GCSF alone, but were all successfully mobilized with the combination of GCSF and AMD3100. A median 21-fold increase in HPCs collected was observed with AMD3100 and GCSF, compared to GCSF alone. All patients transplanted with the AMD3100 and GCSF mobilized product engrafted (median day 10–11). No late graft failures were seen. There were no significant adverse effects attributed to the study drug in this trial.

Larger clinical trials with AMD3100 are in progress, including patients who have failed other mobilization regimens. This drug appears to have considerable potential for improving HPC collection, simplifying the collection of autologous donors, and avoiding cytotoxic agents. A potential concerned in the autologous transplantation setting is possible mobilization of malignant cells, but to date this does not appear to be a problem. A potential additional advantage to using ADM3100 with GCSF is the higher level of CXCR4 expression on collected CD34+ HPCs that may facilitate homing to bone marrow and earlier engraftment.

Graft-versus-host disease

There is emerging evidence that chemokines have fundamental roles in the pathophysiology of graft-versus-host disease (GvHD). To date, our knowledge of chemokines in GvHD comes from experimental models. Shortly after HPC transplantation donor T-cells traffic to host lymphoid tissue where they encounter host histocompatibility antigens [34]. After several days of maturation, the engrafted donor T-cells traffic to non-lymphoid organs, including the typical targets of GvHD, such as skin, gut and liver, and non-classical organs such as kidney and brain. This orderly sequence is orchestrated by chemokines. Soon after transplantation, CXCL9, CXCL10, and CXCL11 are expressed in lymphoid tissue followed shortly by CCL2, CCL3, CCL4 and CCL5 [35]. On the donor T-cells, CCR5 expression plays an essential role in localization to lymphoid tissue. In the liver, CCL2, CCL3, CCL4, CCL5, CXCL9, CXCL10 and CXCL11 are expressed during experimental GvHD [35–37]. Elimination of CCL3 results in reduced liver pathology. In skin, CCL2, CCL6, CCL7, CCL9, CCL11 and CXCL1 are expressed early after transplantation [38]. CCL17 and CCL27 have been shown to be involved in recruitment of memory T-cells to skin during GvHD, suggesting that these chemokines may participate in tissue-specific migration of alloreactive T-cells during GvHD.

Chemokines expressed in lung after allogeneic transplantation include CXCL9, CXCL10, CXCL11, CCL2, CCL3, CCL4, CCL5 and CCL11 [35,37,39]. CXCR3 on transplanted lymphocytes has been shown to be critical for T-cell recruitment to the lung. The function of these chemokines networks is dependent at least in part on pre-transplant conditioning. In the non-conditioned model, elimination of CCR5 from transplanted T-cells results in less accumulation in liver and lung, and less pathology. However, after myeloablative conditioning, CCR5 knock-out CD4+ and CD8+ T-cell are more abundant in liver and lung, and there is greater tissue injury. CXCL9 and CXCL10 mediate recruitment of donor T-cells to the lung in allogeneic transplantation. Blockade of CXCL9 and CXCL10, as well as elimination of CXCR3 on donor T-cells, significantly reduces cellular infiltration and pathology in idiopathic pulmonary syndrome [40]. Donor T-cells themselves participate in the recruitment of alloreactive T-cells to the lung. Elimination of CCL5 expression by donor T-cells significantly reduces pulmonary infiltration and pathology in idiopathic pulmonary syndrome [41].

Multiple myeloma

Chemokines have pathologic roles in multiple myeloma (MM) [42]. Similar to HPC, MM cells circulate in peripheral blood, home to marrow, and express CXCR4 [43]. Approximately one quarter of MM cells in bone marrow express surface CXCR4, while about 60% of peripheral blood MM cells express the receptor. MM cells migrate along a gradient of CXCL12, and the ligand induces CXCR4 internalization as well as cytoskelatal reorganization. Similar to CD34+ HPCs, in MM cells CXCL12/CXCR4 binding promotes localization on marrow endothelium with up-regulation of VLA-4/VCAM-1 mediated attachment allowing for trafficking into the bone marrow microenvironment. While it may seem paradoxical that MM cells in marrow have lower levels of CXCR4, this may be explained by receptor internalization or down-regulation in an environment where the ligand is abundant. Also similar to HPCs, CXCL12/CXCR4 facilitates binding to MM cells to osteoblasts and marrow stromal cells. Based on these data, it is not surprising that AMD3100 inhibits homing of MM cells to bone marrow niches. CCL2, which is also expressed by marrow stromal cells in myeloma, is similarly a chemotactic factor for MM cells through CCR2. CCL3 and CCL4 are constitutively secreted by MM cells and induce the development of osteolytic bone lesions through stimulation of oseoclasts. Systemic levels of CCL3 increase in most patients with MM, and correlate with worse prognosis [44].

Platelets

CXCL4 and CXCL7 were identified in platelets as PF4 and NAP-2, respectively, well before the first leucocyte derived chemokine, IL-8, was described. Subsequently, platelets were found to contain CCL3, CCL5, CCL7, CCL17, CXCL1, CXCL5 and CXCL8 [45]. These chemokines are contained within α granules and are secreted upon activation, making platelets a rich source of chemokines during response to injury or in thrombosis. CXCL4 is an ELR^- chemokine, and lacks neutrophil chemotactic activity. However, CXCL4 potentiates degranulation of neutrophils primed by tumour necrosis factor-α (TNF-α) and promotes their adhesion to endothelium. CXCL4 has better defined roles in coagulation. CXCL4 binding to heparin is immunogenic, and antibodies to the complex may cause heparin-induced thrombocytopenia. CXCL4 inhibits heparin-dependent acceleration of thrombin inactivation by antithrombin III and potentiates platelet aggregation in the presence of suboptimal concentrations of agonists. As is common with other chemokines, CXCL4 binds to endothelial GAGs. Under normal conditions a substantial amount of CXCL4 is associated with GAGs. Intravenous injection of heparin results in an immediate 15–30-fold increase in plasma concentrations of CXCL4 without affecting platelet-associated CXCL4 [46]. CXCL4 also promotes the uptake of oxidized low density lipoprotein by endothelial cells, which may play a role in atherosclerosis [47]. CXCL7 is derived from platelet basic protein by proteolytic cleavage of the 24 aminoterminal amino acids. CXCL7 induces neutrophil degranualtion and reactive oxygen products, though it is approximately 100-fold less potent a neutrophil chemoattractant than CXCL8.

Platelets have also been shown to possess the receptors CCR1, CCR3, CCR4, CXCR1 and CXCR4. In general, it appears that chemokines that signal through these receptors are weak platelet agonists. However, in the presence of adenosine diphosphate at low levels, CXC12, CCL17 and CCL22 have been shown to induce near maximal platelet aggregation [48]. There is some question as to whether sufficient concentrations of chemokines occur *in vivo* to activate platelets, but it is likely that such conditions can exist locally at sites of inflammation or thrombosis. Because CXCR4 is a cofactor for human immunodeficiency virus (HIV) entry into cells, this receptor may also contribute to HIV-associated thrombocytopenia by facilitating infection of megakaryocytes.

Transfusion-transmissible diseases

A number of pathogens have evolved mechanisms of exploiting chemokine receptors to attack host cells or to evade the immune response. The use of Fy antigens by Plasmodium to enter RBCs was discovered before the identity of Fy and DARC was known. HIV exploits chemokine receptors to infect T-cells. After binding of viral gp120 to CD4, CCR5 or CXCR4 is engaged [49]. This allows the gp41 subunit to become firmly attached to the cell and fusion between the viral capsule and cell membrane to take place. Individuals who are homozygous for a 32 basepair deletion within the

coding region of the CCR5 gene have a high degree of protection from HIV infection [50].

The human cytomegalovirus genome encodes for a chemokine decoy receptor, US28, with the characteristic seven transmembrane domain structure of native chemokine receptors [51]. US28 is expressed on infected cells and binds most CC chemokines. It prevents leucocyte recruitment by degrading chemokines through internalization and receptor recycling. Cytomegalovirus also encodes for a secreted chemokine receptor, pUL21·5. This glycoprotein shares no structural similarities with native chemokine receptors. It binds CCL5, but not other proinflammatory CC chemokines, such as CCL2 and CCL3. Thus, pUL21·5 is a high affinity and relatively specific chemokine decoy receptor [52].

Transfusion reactions

There is emerging evidence for a role of chemokines in the pathophysiology of transfusion reactions. Haemolytic transfusion reactions are analogous to the systemic inflammatory response syndrome. Red blood cells coated with immunoglobulin G (IgG), and/or complement, stimulate phagocytes to produce inflammatory mediators. In models of ABO incompatibility both CXC and CC chemokines are produced at high levels [53,54]. In haemolytic reactions, RBC membrane bound IgG and complement interact with receptors on mononuclear phagocytes stimulating the production of mediators including proinflammatory cytokines TNF-α, IL-1β, CCL2 and CXCL8. Temporally, proinflammatory is produced first. CCL2 and CXCL8 production is partially, but not completely, inhibited by neutralization of TNF-α. In models of IgG-mediated RBC incompatibility, CCL2 and CXCL8 are also produced, though at lower levels [53,55].

In the setting of a non-haemolytic transfusion reaction with fever, chills, pain and dyspnoea that was associated with transfusion of plasma containing human leucocyte antigen DR antibodies reactive with recipient specificities, CXCL1 and CXCL8 have been implicated [56]. *In vitro* incubation of antigen positive peripheral blood mononuclear cells resulted in chemokine expression, as well as production of TNF-α, IL-1β and IL-6. Chemokine production was substantially reduced by blockade of the IgG receptors CD16 (FcγRIII) and CD32 (FcγRII), although not CD64 (FcγRI). While the roles of chemokines in such reactions are incompletely understood, it is likely that CXC ligands participate in neutrophils activation, which in turn contributes to the capillary leakage phenomenon of transfusion-related acute lung injury.

Intravenous immunoglobulin and anti-D

Intravenous immunoglobulin (IVIG) is well-known to have complex immunomodulatory effects. Little is known about the impact of IVIG on chemokines that may mediate inflammatory or autoimmune diseases. Gene expression profiling of peripheral blood cells from healthy subjects given a single dose of IVIG showed up-regulation of all chemokine genes examined: CCL2, CCL3, CCL4, CCL7, CCL8, CXCL9, CXCL10, CXCL11, CXCL12 and CL1 [57]. In patients with chronic inflammatory demyelinating polyneuropathy treated with IVIG serum levels of CCL2 decreased, whereas in patients with Kowasaki disease serum levels of CXCL8 were unaffected [58,59]. In a study of patients with congestive heart failure randomized to receive IVIG or placebo monthly for 5 months, there was a decrease in serum levels of CCL3, CCL4 and CXCL8 [60]. Peripheral blood mononuclear cell mRNA levels decreased after IVIG treatment for CCL3, CCL4, CCR1, CCR5 and CXCR1, but not for CXCL8. IVIG has been shown to contain antibodies to CCR5 capable of blocking CCL5 binding and HIV infection of lymphocytes and monocytes *in vitro* [61]. In patients with common variable immunodeficiency, serum levels of CXCL8 have been shown to increase after a single infusion of IVIG [62,63]. In contrast, a study of patients with immune-mediated neuropathies receiving IVIG found no effect on T-cell or monocyte expression of CCR1, CCR2, CCR4, CCR5, CCR6 or CXCR3 [64].

Similarly, there are spare data on the effects of anti-D administration of chemokine expression. In children with chronic immune thromobocytopenic purpura, infusion of anti-D caused a rapid reduction in serum CXCL8, as well as several other inflammatory cytokines [65]. In two other studies, a transient increase in serum CCL2 and CCL3 levels was observed after anti-D administration [66,67]. It is not clear at this time whether these changes are directly causes by IVIG or anti-D, or are secondary to the underlying disease process.

Accumulation of chemokines during blood component storage

Chemokines may accumulate in the supernatant of blood components during storage, either from platelet degranulation or from activation of leucocytes. The platelet-derived chemokines found in blood components include CCL5, CXCL4 and CXCL7. CXCL8 is the principle leucocyte-derived chemokine that has been identified in the supernatant of blood components. CCL5 may also be leucocyte-derived, but in blood components, the contribution from platelets appears to greatly out weigh that on leucocytes.

Most work to date has focused on platelet concentrates. In non-leucocyte-reduced platelets, whether prepared from whole blood, by the buffy-coat method, or by apheresis, there is progressive accumulation of CXCL4, CXCL7, CCL5 and CXCL8 [68–73]. CXCL8 can reach particularly high levels in non-leucoreduced platelets by the end of the storage period. Pre-storage leucocyte reduction can prevent the accumulation of leucocyte-derived CXCL8, but not platelet-derived

chemokines [68,69,71,73–76]. Photochemical pathogen reduction treatment or ultraviolet B irradiation prevents accumulation of leucocyte-derived chemokines, but γ-irradiation does not [70,77,78]. γ-Irradiation does not prevent release of platelet-derived CCL5 [79]. Platelet additive solutions appear to be little effect on the accumulation of chemokines, although one solution containing magnesium and potassium reduces release of CCL5, CXCL4 and CXCL7 [76,80,81].

Both leucocyte-derived and platelet-derived chemokines progressively accumulate in RBC during storage [82–85]. Pre-storage leucocyte reduction eliminates chemokine accumulation in RBCs [82,83,85]. This is seen with both leucocyte- and platelet-derived chemokines, most likely because leucocyte reduction filters designed for RBC also remove platelets. Not surprisingly, peripheral blood HPC components can contain significant levels of CCL7 [86].

Some leucocyte reduction filters that have a net negative surface charge are capable of removing CXCL8 and CCL5 from blood components [87,70,71]. In contrast, positively charged filters have no effect, most likely because these chemokines have a net positive charge at physiologic pH [70].

Clinical implication for transfusion medicine

It is probable that chemokine agonists and antagonists will have a major impact on HPC transplantation, similar to the influence of recombinant haematopoietic growth factors over the past decade. The most immediate impact will likely be in improvement of HPC mobilization by AMD3100 or similar CXCR4 blockers. Alternatively, strategies to increase CXCR4 expression on marrow stromal cells may facilitate HPC engraftment. Antagonists of CCR5 may be good candidates for drugs to reduce GvHD in allogeneic transplantation. However, we still have much to learn about the complexities of chemokine networks in GvHD.

The recent discoveries of chemokine decoy receptors encoded by several diverse human viral pathogens open new opportunities for antiviral therapies. The development of drug that specifically targets viral chemokine receptors could enhance the immune response to viral infections. Alternatively, recombinant viral decoy receptors have the potential use as drug to modulate chemokines in other diseases, such as autoimmune diseases. Such antichemokines would have the potential advantage over humanized monoclonal antibodies of binding multiple related chemokines.

Efforts to improve blood component storage to reduce the accumulation of chemokines would likely have a beneficial impact on transfusion reactions and on transfusion-related immune modulation. Pre-storage leucocyte reduction has clearly been shown to virtually eliminate the generation of leucocyte-derived chemokines, as well as other cytokines, in stored platelets and RBCs. The next major challenge will be to find ways to prevent the degranulation of platelets during storage, without negatively affecting their post-transfusion function.

There are many opportunities for future research into the roles of chemokines in transfusion medicine. However, we need to be careful in interpreting studies of chemokines in transfusion medicine. As we have noted, there is considerable redundancy and overlap in the biological function of individual chemokines, as well as chemokine receptors. Chemokines are only a part of a much larger and even more complex network of cytokines and other effort molecules of the immune and inflammatory systems.

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