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Review

Chemokines, G Proteins and Natural Killer Cells

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Abstract. Natural killer (NK) cells are anti-tumor and anti-viral effector cells. Members of C, CC, CXC and CX_3C chemokines induce the chemotaxis and enhance the cytotoxicity of NK cells, suggesting that these cells express receptors for chemokines. The ability of members of chemokines to inhibit the replication of HIV-1 strains, combined with the ability of the same chemokines to activate the anti-viral NK cells, provide compelling evidence for the role of NK cells in eradicating HIV-1 infection. In addition, chemokines induce various intracellular signaling pathways in NK cells, which include activation of the heterotrimeric, and perhaps the small guanine nucleotide binding (G) proteins, as well as the mobilization of intracellular calcium, among other activities. Further, chemokines induce the phosphorylation of chemokine receptors through the recruitment of G protein-coupled receptor kinases (GRKs) resulting in the desensitization and turning off the signals. In this review, I will update the knowledge of the effect of chemokines on NK cell motility and the signal transduction pathways induced by chemokines in these cells.

Key words: chemokines; natural killer cells; G proteins; chemotaxis; cytolysis.

Background

Natural killer (NK) cells are blood-born leukocytes which represent the first line of defence against microbial infection, but also have a potent anti-tumor activity (reviewed in[53]). To perform these functions, NK cells must extravasate into the infected tissues, or tumor growth sites. The mechanisms by which NK cells are involved in controlling viral replication or tumor spread are now starting to be understood. NK cells respond by chemoattraction to a family of cytokines known as chemokines, which are pro-inflammatory mediators implicit for the recruitment of various cell types into the inflammatory sites (reviewed in[35]). Depending on the presence and the arrangement of the first cystein residues in the N-terminal region, chemokines are divided into 4 subfamilies: CXC (α), CC (β), C (γ), and CX_3C (δ) (Table 1).

Chemokines Induce the Motility and the Cytolysis of NK Cells

Examination of the role of chemokines revealed that the CC chemokines MIP-1α, MCP-1 and RANTES, but not MIP-1β, induce the chemotaxis of NK cells[36]. Similarly, ALLAVENA et al.[5] reported that MCP-1, -2 and -3 induce the chemotaxis of these cells. Later work by others[32, 51] supports these findings. Additionally, the C chemokine lymphotactin[9, 23, 38], the CXC chemokine IP-10[38, 51], or SDF-1α[33, 45], the CC chemokine MDC[22, 29], MIP-3α[3], MIP-3β[3, 30], or 6Ckine[30], and the CX_3C

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Table 1. Members of the chemokine family, their receptors and their effects on NK cell chemotaxis

Subfamilies	Receptor	Chemotactic for NK cells
CXC		
IL-8	CXCR1, CXCR2	Yes
NAP-2	CXCR2	ND
GRO-α	CXCR2	ND
GRO-β	CXCR2	ND
GRO-γ	CXCR2	ND
PF4	CXCR2	ND
ENA	CXCR2	ND
VCXC-1	CXCR2	ND
IP-10, MIG	CXCR3	Yes
SDF-1α	CXCR4	Yes
BCA-1	CXCR5	ND
CC		
MIP-1α	CCR1, CCR5	Yes
MIP-1β	CCR5, CCR8	Yes and No
MCP	CCR2	Yes
Eotaxin	CCR3	ND
RANTES	CCR1, CCR3, CCR5	Yes
MDC	CCR4	Yes
MIP-3α (exodus, LARC)	CCR6	Yes
MIP-3β (ELC, CKβ-11)	CCR7	Yes
I-309	CCR8	Yes
VMIP-I	CCR8	Yes
TARC	CCR4, CCR8	Yes
TECK	CCR9	ND
6Ckine (exodus 2, SLC)	CCR7	Yes
PARC (DC-ck1)	ND	ND
leutactin	ND	ND
C		
lymphotactin	XCR1	Yes
CX_3C		
fractalkine	CX_3CR1	Yes

CXC chemokines: BCA-1 – B cell-attracting chemokine 1, ENA – epithelial-derived neutrophil chemoattractant; GCP-2 – granulocyte chemotactic protein-2; GRO – growth related oncogene; IL-8 – interleukin-8; IP-10 – interferon-inducible protein-10; MIG – monokine induced by interferon; NAP-2 – neutrophil activating protein-2; PF4 – platelet activating protein; SDF-1 – stromal-derived factor-1.

CC chemokines: ELC – EBV ligand chemokine; LARC – liver and activation-regulated chemokine; MCP – macrophage chemotactic protein; MDC – macrophage-derived chemokine; MIP – macrophage inflammatory protein; PARC – pulmonary and activation regulated chemokine; RANTES – regulated upon activation normal T cell expressed and secreted; SLC – secondary lymphoid chemokine; TARC – thymus and activation-regulated chemokine; TCA – T cell activation gene; TECK – thymus expressed chemokine. V – viral product. ND – not determined.

chemokine fractalkine[3, 28], have been shown to induce the chemotaxis of NK cells. Recently, the CC chemokines TARC, I-309 and vMIP-I were found to be chemotactic for NK cells[29]. Table 1 shows an up-to--date knowledge of the effect of chemokines on NK cell motility.

Chemokines not only induce the chemotaxis of NK cells, but also induce calcium mobilization in these cells[33, 38, 45], and activate them to kill tumor target cells[37, 39]. These killer cells are designated as CHAK (chemokine activated killer) cells. They may play a significant role in eradicating tumor metastases and in destroying virally-infected cells. An interesting work by MAHALINGAM et al.[39] showed that administration of recombinant vaccinia virus into athymic mice resulted in the death of these mice. However, administration of recombinant vaccinia virus encoding the CXC chemokine Mig resulted in a significant recruitment of NK cells into the liver and in their enhanced cytolytic activity. Consequently, these mice successfully resolved the vaccinia virus infection. In addition, it was observed that infection with murine cytomegalovirus resulted in increased expression of MIP-1α[47]. As a result, NK cells were recruited toward the infected tissues, which led to reduced infection with this virus. These results implicate a primary role for chemokines in the recruitment of NK cells toward the sites of viral infection, resulting in the eradication of these infections.

The importance of chemokines in human diseases has generated great interest. It has been known for several years that HIV-1 infects $CD4^+$ cells, but that the CD4 molecule is not sufficient for the entry of this virus into peripheral blood cells. HIV-1 exists in at least two forms. One predominates in the early stages of the disease and does not induce the fusion of the virally-infected cells with peripheral blood lymphocytes (non--syncytium inducing). This strain infects $CD4^+$ macrophages, hence it is called M-tropic. The second form predominates when the patients progress toward AIDS. This form induces the fusion of infected cells with neighboring $CD4^+$ T cells, hence the name T-tropic (reviewed in[7]). In late 1995, COCCHI et al.[15] observed that the CC chemokines MIP-1α, RANTES and MIP--1β inhibit the replication of the M-tropic HIV-1. Also, the CXC chemokine stromal derived factor-1α (SDF--1α) inhibits the replication of the T-tropic HIV-1[11, 40]. Later it was observed that the M-tropic strain of HIV-1 binds the chemokine receptor CCR5[4, 16, 18]. Some M-tropic isolates also bind CCR3 and CCR2b[14, 17]. These receptors act as this strain's coreceptors. In addition, the coreceptor for the T-tropic HIV-1 strain was identified and was designated as fusin[20], and later as

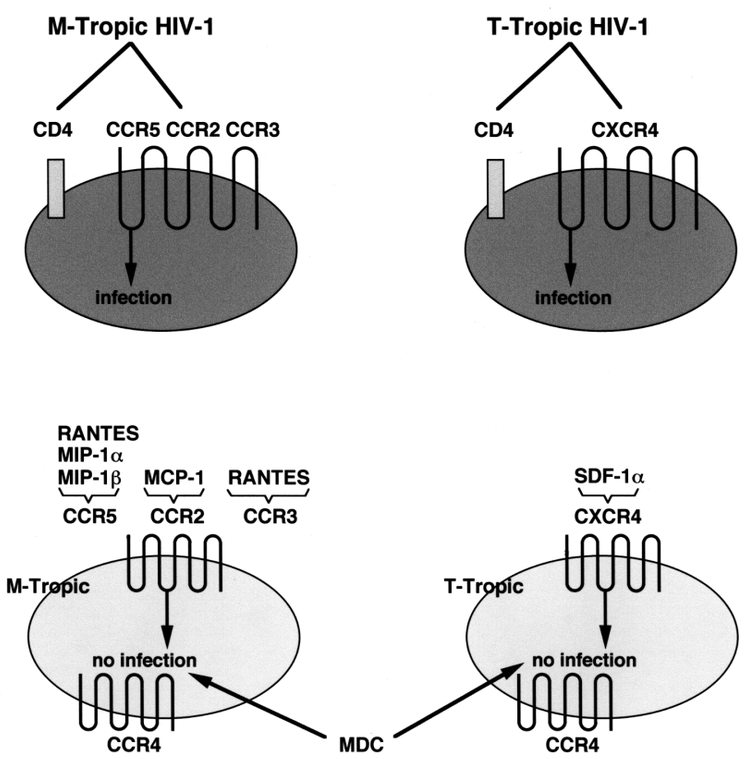

Fig. 1. Expression of HIV-1 co-receptors in $CD4^+$ cells. CCR5, CCR2 and CCR3 are coreceptors for M-tropic HIV-1 (upper left panel), whereas CXCR4 is the coreceptor for T-tropic HIV-1 (upper right panel). Only upon binding to CD4 molecule plus the HIV-1 coreceptors (chemokine receptors), the virus infects $CD4^+$ cells. Chemokines such as RANTES, MIP-1α and MIP-1β, and possibly MCP-1 (also MDC and I-309) inhibit the infection by M-tropic HIV-1. On the other hand, SDF-1α (also MDC and I-309) inhibits infection by T-tropic HIV-1. MIP-1α, MIP-1β and RANTES bind CCR5. RANTES also binds CCR3, and SDF-1α binds CXCR4. MDC binds CCR4 expressed in $CD4^+$ macrophages, or $CD4^+$ T cells

CXCR4[11, 40]. Additional work showed that the truncated form of the CC chemokine monocyte-derived chemokine (MDC) inhibits the replication of both the M-tropic and the T-tropic HIV-1 strains[43]. This chemokine binds to the CC chemokine receptor CCR4, which is not a co-receptor for HIV-1[27]. Another CC chemokine known as I-309 was found to inhibit the replication of both the syncytium forming (T-tropic) and the non-syncytium forming (M-tropic) HIV-1 strains[25]. The receptor for I-309 has been recently cloned and is designated as CCR8[46, 52].

Taken together, the anti-HIV-1 chemokines include MIP-1α, MIP-1β, RANTES, MDC, I-309 and SDF-1α. Except for MDC, all other chemokines bind HIV-1 coreceptors, which include CCR5, CCR8, CXCR4, and possibly CCR2 and CCR3. The presumed mechanisms by which chemokines inhibit the replication of HIV-1 include, among others, the following: 1) down regulation of chemokine receptors which act as HIV-1 coreceptors, 2) blocking chemokine receptors, hence competing out the virus, and 3) activation of immune cells such as NK cells. Figure 1 shows the effect of chemokines on the replication of HIV-1 strains.

There is overwhelming evidence showing that NK cell activity and NK cell numbers are reduced in HIV--1-infected individuals[24, 26, 31]. This is evident at the later stages of the disease, and in particular during the progression toward AIDS. Upon treatment of these patients with drugs such as AZT, a rebound of NK cell activity and number, associated with improved prognosis, has been observed. Several conclusions can be drawn from these observations: 1) NK cells may be infected with the HIV-1 virus, especially during the late stages of AIDS. If this is true, then it indicates that there are receptors on NK cells that mediate the entry of HIV-1 into these cells. These receptors include the already known, but may also include new receptors not yet cloned or characterized; 2) there is a cause-effect relationship between the recovery of NK cell activity and improved prognosis, strongly suggesting that NK cells are important in killing the HIV-infected cells and for inhibiting the replication of this virus. Recently, two groups observed that NK cells recovered from HIV-1-infected individuals secrete significant amounts of the CC chemokines MIP-1α, MIP-1β and RANTES[19, 41]. This coincided with suppression of HIV-1 viral replication, implicating NK cells as important effector cells responsible for the chemokine anti-HIV-1 activity.

Although MIP-1α and RANTES can induce the chemotaxis of IL-2-activated NK cells, in our study, MIP-1β did not[36]. In the studies of other investigators,

MIP-1β induced the chemotaxis of non-activated NK cells[32, 51]. Furthermore, we observed that MIP-1β is a weak stimulator of NK cell cytolysis as compared to MIP-1α, MCP-1 and RANTES[37]. These findings suggest that, although NK cells respond to MIP-1β, it is a weak response and disappears upon activation with IL-2. Corroborating these results, our recent finding showed that IL-2-activated NK cells lack the expression of CCR5[29], the major receptor for MIP-1β. Hence, it is reasonable to assume that freshly isolated NK cells respond to MIP-1β by expressing chemokine receptors other than CCR5. This may include CCR8, which has been recently shown to bind MIP-1β[8]. In support of this are our recent results demonstrating that IL-2-activated NK cells express CCR8[29]. Alternatively, it can be speculated that non-activated NK cells may express CCR5 and that IL-2 activation may down-regulate such expression. If this is true, then this may have important applications for the treatment of AIDS patients, since treatment with IL-2 could down-regulate the expression of HIV-1 coreceptors such as CCR5 resulting in reduced infection with the M-tropic HIV-1 strains. This may provide an impetus for the utilization of IL-2 in the treatment of AIDS patients.

The expression of chemokine receptors in immune cells is highly important, since this may provide tools for dissecting the ontogeny and the functional activities of these cells. For example, it has been reported by several groups that CCR3, CCR4 and CCR8 are preferentially expressed in T helper type 2 (Th2), whereas CCR5 and CXCR3 are preferentially expressed in T helper type 1 (Th1) cells[12, 55]. This differentiation is important since the only distinction between these cell types is by secretion of cytokines; the classical Th1 cytokines are IL-2 and IFN-γ, whereas IL-4 and IL-10 are secreted by Th2 cells[1]. Recently, an attempt was made to classify NK cells into NK1 and NK2 based on the nature of cytokines secreted by these cells[44]. This classification is based on the same cytokine profile secreted by Th1 and Th2 cells, and no other difference was observed between NK1 and NK2 except that NK1 cells produce IL-10 and IFN-γ, whereas NK2 cells produce IL-5 and IL-13. Hence, such classification may not reflect the actual nature of NK cell subsets. Our hypothesis is that chemokine receptors may discriminate at the NK cell subsets, i. e. certain chemokine receptors may be expressed on some NK cell subsets but not on others. Such expression could be important for NK cell function. For example, engagement of certain chemokine receptors which are predominantly expressed on certain subsets of NK cells may induce cytolysis and/or cytokine production by these cells.

Signal Transduction Pathways Induced by Chemokines in NK Cells

Receptors for chemokines are coupled to the heterotrimeric guanine nucleotide binding (G) proteins, which are composed of 3 subunits α, ß and γ. The α subunits belong to 4 subfamilies: 1) α_s (α_s and α_{olf}), 2) α_q (α_q, α_{11}, α_{14}, α_{15}, and α_{16}), 3) α_i (α_{i1}, α_{i2}, α_{i3}, α_{o1}, α_{o2}, α_z, α_t, α_{gus}, α_{con} and α_{rod}), and 4) α_{12} (α_{12} and α_{13}). The presence and identity of G proteins present in human and rat NK cell membranes have been investigated. It was reported that these membranes express G_i, G_o, G_s, G_q, G_z and G_{13}. These G proteins play vital roles as early transducers of various biological functions in NK cells[34, 35]. In addition to G protein coupled receptors (GPCR), receptor tyrosine kinases (RTK) transmit intracellular signals resulting in the activation of common second messengers, such as: a) non-receptor tyrosine kinase, b) the phosphoinositide 3 kinase (PI3K) and c) the mitogen-activated protein kinase (MAP kinase, also known as extracellular regulated kinase "ERK").

The role of the heterotrimeric G proteins in chemokine-induced signaling in NK cells has been described elsewhere[34, 35]. Recently, we observed that chemokine receptors, particularly CCR4, are internalized upon pretreatment of NK cells with the corresponding ligands[29]. Also, we observed that activation of NK cells with chemokines resulted in the recruitment of G protein--coupled receptor kinases (GRKs) into the cell membranes. The serine/threonine kinases GRK2 and GRK3 are responsible for phosphorylating the receptors. Also, we observed that the tyrosine kinase inhibitor herbamycin A (HA) inhibits chemokine-induced NK cell chemotaxis and calcium mobilization, suggesting that tyrosine kinases are involved in these activities. The best candidates are the non-receptor tyrosine kinases such as c-Src, Lyn, Fyn, Syk or ZAP-70. The picture emerging is that GRKs phosphorylate chemokine receptors. Phosphorylated receptors then bind β-arrestin, which may recruit members of the non-receptor tyrosine kinases, important for the chemotaxis and the mobilization of intracellular calcium in NK cells (Fig. 2). This model is analogous to the system described by AHNE et al.[2], who observed that activation of β2-adrenergic receptors results in the recruitment of c-Src which phosphorylates dynamin and facilitates the endocytosis of these receptors. A recruitment of GRK2 and β-arrestin into the membranes of monocytes was also reported after stimulating CCR2b with MCP-1[6]. Also, CCR1 or CXCR4 are internalized into clathrin-coated pits after stimulation of CHO cells expressing CCR1 with RANTES[48], or various other cell lines expressing

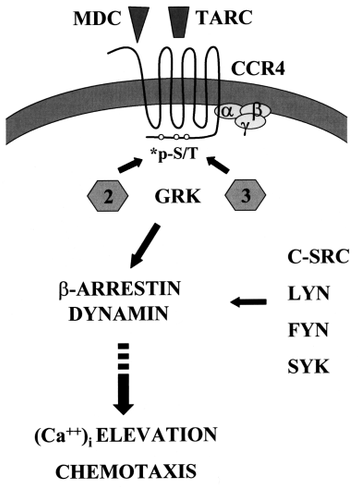

Fig. 2. A model of chemokine-induced internalization of chemokine receptors. Upon activation with chemokines such as MDC or TARC, which binds CCR4, the heterotrimeric G proteins coupled to this receptor dissociate into α and βγ subunits. The latter recruits GRKs which phosphorylate the receptors at the serine/threonine residues. Phosphorylated receptors bind β-arrestin, which recruits non-receptor tyrosine kinases such as c-Src, Lyn, Fyn or Syk. These in turn may phosphorylate dynamin, which binds clathrin-coated endocytic pits. This pathway results in the termination of the signals which include, but are not limited to, chemotaxis and calcium mobilization. *p-S/T – phosphoserine/phosphothreonine

CXCR4 with SDF-1α[50]. In addition, GRK2 and GRK3 phosphorylate CCR5 in various cell types upon activation with RANTES[42, 54].

Recent work showed that small G proteins, also known as GTPases because they hydrolyze GTP, play an important role in NK cell activity, such as NK-mediated cytolysis. Several GTPases are presently known. These exist in an inactive GDP-bound form. They exchange GDP for GTP very slowly due to the low dissociation rate of GDP. In order to activate these GTPases, GTP must bind these proteins. This is facilitated by guanine nucleotide exchange factors (GEFs), which are specific but may be shared among several members of the GTPase family of proteins (Table 2). The mechanism of this activation is beginning to be solved. All GTPases have two switch regions, known as switch I and switch II. These are so called because conformational changes occur upon GDP-GTP exchange and upon GTP hydrolysis[49]. Although each GEF is unique in its structure and function, GEFs apparently have a common activity, which is to insert themselves between these switch regions of the GTPases, exposing those areas in the GTPase that bind the GTP[49]. Binding of GTP will result in the activation of these proteins, which in turn bind several effectors

Table 2. List of the most important members of small G proteins (GTPases), their functions and relevant GTP exchange factors (GEFs)

GTPase	GEF	Function
RAS	SOS	MAPK activation
ARF	ARNO	PLD activation, vesicular trafficking
RAC	TIAM1, VAV, DBL	MAPK/JNK activation, actin polymerization, NF-κB activation
RHO	GEF p115, LBC, VAV, DBL	actin polymerization, filopodia, lamellopodia
CDC42	CDC25, VAV, DBL	actin polymerization, MAPK/JNK activation
RAB	RABEX	endocytic formation
RAN	RCC1	nuclear transport
EF-Tu	EF-Ts	aminoacyl-tRNA transport

and induce multiple biological activities as shown in Table 2. Recently, two groups reported that activation of NK cells through the engagement of CD16 molecule resulted in the phosphorylation of the GEF Vav[10, 21]. Abrogation of Vav expression[21], or inhibition of the GTPase Rac[10], leads to inhibition of NK cell mediated killing, or to reduced polarization and binding to target cells. These findings show novel effects for GTPases/GEFs in NK cell function. It remains to be seen whether GTPases are invloved in chemokine-induced activation of NK cells. This seems to be logical considering that some of these GTPases are invloved in the formation of lamellopodia and filopodia, which are important for the polarization of the cells and their extravasation into various tissues.

Concluding Remarks

NK cells are anti-tumor/anti-viral effectors, and may be important for the immunotherapeutic treatment of cancer and AIDS patients. These cells are recruited toward the sites of inflammation, virus infection and tumor metastases. Chemokines are the major chemoattractants for these cells, suggesting that NK cells express receptors for these chemokines. Examination of this expression will provide a scheme by which the ontogeny and functional activities of NK cells can be investigated. In addition, the ability of chemokines to attract and activate NK cells at the sites of viral infection, combined with the ability of chemokines to inhibit the replication of HIV-1 in infected cells, suggests that NK cells play significant roles in the eradication of virally-infected cells. The ability of chemokines to activate various intracellular signaling pathways in NK cells may provide an understanding of the migratory

behavior of these cells and to their activation, and should facilitate in planning the strategies to utilize NK cells for therapeutic purposes. Also, examination of various intracellular signaling pathways is highly important and may lead to therapeutic and preventive interventions which would benefit AIDS and cancer patients.

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