The Role of Chemokines in Melanoma Tumor Growth and Metastasis

Aimee S. Payne and Lynn A. Cornelius

Department of Dermatology, Washington University School of Medicine, St Louis, Missouri, U.S.A.

Chemokines represent a large family of polypeptide signaling molecules that are notable for their role in chemotaxis, leukocyte homing, directional migration, and G protein coupled receptor activation. Chemokines have recently been implicated in tumor progression and metastasis. The demonstration of chemokine expression and receptor activation in melanoma tumor cells themselves, and the tumor infiltrating leukocytes, may have important implications in terms of tumor progression and tumor cell homing to metastatic sites. In addition to their chemotactic and cell homing properties, chemokines and their receptors also play a part in other biologic functions relevant to oncogenesis, including cell proliferation, protease induction, tumor growth, and angiogenesis. Melanomas, and the cells derived from them, have been found to express a number of chemokines, including CXCL8 (interleukin-8), CXCL1–3 (MGSA-GROα–γ), CCL5 (RANTES), and CCL2 (monocyte chemotactic protein-1), which have been implicated in tumor growth and progression. Furthermore, recent studies have demonstrated organ-specific patterns of melanoma metastasis that correlate with their expression of specific chemokine receptors, including CXCR4, CCR7, and CCR10. This review will focus on the current biology of chemokines and chemokine receptors in the context of understanding their potential roles in melanoma progression and metastasis, and is not meant to be a comprehensive review of chemokine biology. Continued understanding and progress in the determination of the role of chemokines and their receptors in tumorigenesis and metastasis, including melanoma, may lead to novel approaches in the treatment and management of this disease. ***Key words: chemokine receptor/chemokine/G protein-coupled receptor/melanocyte/melanoma. J Invest Dermatol 118:915–922, 2002***

Cellular transformation, tumor growth, and metastasis are complex biochemical processes that involve, among other events, autonomous cell growth and host–tumor interactions. The molecular basis for many of these events is increasingly becoming understood, particularly in understanding deregulation of the cell cycle, programmed cell death, angiogenesis, extracellular matrix remodeling, and evasion of host immune surveillance. In melanoma, some of the mechanisms of cellular transformation have been identified and a portion of the genetics of familial melanoma have been determined (Greene *et al*, 1983; Halpern *et al*, 1991; Gruis *et al*, 1993; Hussussian *et al*, 1994; Newton-Bishop *et al*, 1994, 2000; Platz *et al*, 1998, 2000). Similarly, the mechanisms of melanoma metastasis are under investigation. The metastatic potential of melanoma contributes to the poor rate of survival following tumor invasion, together with the lack of effective systemic therapies. As in other cancers, matrix-degrading matrix metalloproteinases have been implicated in facilitating melanoma invasion and dissemination (MacDougall *et al*, 1999; Hofman *et al*, 2000). Additionally, an increasing body of literature is accumulating that identifies immune evasion as a critical step in melanoma disease progression (Bröcker *et al*, 1988; Giavazzi *et al*, 1990; Bottazzi *et al*, 1992; Kirkwood *et al*, 1996; Brinckerhoff *et al*, 2000; Torisu *et al*, 2000; Fishman *et al*, 2001). Downregulation of major histocompatibility complex class I expression (Fishman *et al*, 2001) and modulation of the inflammatory response via cytokines (Balkwill and Mantovani, 2001) have been described. Other recent reports suggest that expression of chemokines and chemokine receptors by melanoma may contribute to the ability to escape tumor surveillance and may partially explain preferential patterns of melanoma metastasis to sites such as lymph nodes, skin, and lungs (Muller *et al*, 2001).

Chemokines are structurally related, small (8–14 kDa) polypeptide signaling molecules (Zlotnik and Yoshie, 2000) that bind to and activate a family of seven transmembrane G protein-coupled receptors, more specifically, the chemokine receptors (Murphy, 1996). Chemokines were originally characterized by their ability to induce chemotaxis of leukocytes. They have since been shown to act on multiple cell types, including endothelial cells and tumor cells, where they elicit a broad range of cellular signals that may affect cell proliferation and the promotion of angiogenesis. The chemokines and their respective receptors are divided into the CXC, CC, C, and CX_3C families, based upon the positions of their conserved two N-terminal cys residues. Their genes are clustered on genomic loci, including chromosome 4q12–q13 (CXC acting mainly on neutrophils), 4q21, and 17q11.2 (CC chemokines acting mainly on monocytes) (Zlotnik and Yoshie, 2000). Although a detailed review of chemokine biology is beyond the scope of this manuscript, comprehensive overviews may be found in recent

Manuscript received August 1, 2001; revised December 16, 2001; accepted for publication December 18, 2001.

Reprint requests to: Dr. Lynn A. Cornelius, Division of Dermatology, 660 S. Euclid Ave., Washington University School of Medicine, St Louis, MO 63110, U.S.A. Email: cornelil@msnotes.wustl.edu

Abbreviations: GRO, growth-related oncogene; MGSA, melanoma growth stimulatory activity.

Table I. CXC, C, and, CX_3C chemokine/receptor families[a]

Systematic name	Human chromosome	Human ligand	Mouse ligand	Chemokine receptor(s)
CSC chemokine/receptor family				
CXCL1	4q12–q13	GROα/MGSA-α	GRO/KC?	CXCR2 > CXCR1
CXCL2	4q12–q13	GROβ/MGSA-β	GRO/KC?	CXCR2
CXCL3	4q12–q13	GROγ/MGSA-γ	GRO/KC?	CXCR2
CXCL4	4q12–q13	PF4	PF4	Unknown
CXCL5	4q12–q13	ENA-78	LIX?	CXCR2
CXCL6	4q12–q13	GCP-2	CKα-3	CXCR1, CXCR2
CXCL7	4q12–q13	NAP-2	Unknown	CXCR2
CXCL8	4q12–q13	IL-8	Unknown	CXCR1, CXCR2
CXCL9	4q21.21	Mig	Mig	CXCR3
CXCL10	4q21.21	IP-10	IP-10	CXCR3
CXCL11	4q21.21	I-TAC	Unknown	CXCR3
CXCL12	10q11.1	SDF-1α/β	SDF-1	CXCR4
CXCL13	4q21	BLC/BCA-1	BLC/BCA-1	CXCR5
CXCL14	Unknown	BRAK/bolekine	BRAK	Unknown
(CXCL15)	Unknown	Unknown	Lungkine	Unknown
C chemokine/receptor family				
XCL1	1q23	Lymphotactin/SCM-1α/ATAC	Lymphotactin	XCR1
XCL2	1q23	SCM-1β	Unknown	XCR1
CX_3C chemokine/receptor family				
CX3CL1	16q13	Fractalkine	Neurotactin	CX3CR1
CC chemokine/receptor family				
CCL1	17q11.2	I-309	TAC-3, P500	CCR8
CCL2	17q11.2	MCP-1/MCAF	JE?	CCR2
CCL3	17q11.2	MIP-1α/LD78α	MIP-1α	CCR1, CCR5
CCL4	17q11.2	MIP-1β	MIP-1β	CCR5
CCL5	17q11.2	RANTES	RANTES	CCR1, CCR3, CCR5
(CCL6)		Unknown	C10, MRP-1	Unknown
CCL7	17q11.2	MCP-3	MARC?	CCR1, CCR2, CCR3
CCL8	17q11.2	MCP-2	MCP-2?	CCR3
(CCL9/10)		Unknown	MRP-2, CCF18 MIP-1χ	Unknown
CCL11	17q11.2	Eotaxin	Eotaxin	CCR3
(CCL12)		Unknown	MCP-5	CCR2
CCL13	17q11.2	MCP-4	Unknown	CCR2, CCR3
CCL14	17q11.2	HCC-1	Unknown	CCR1
CCL15	17q11.2	HCC-2/Lkn-1/MIP-1	Unknown	CCR1, CCR3
CCL16	17q11.2	HCC-4/LEC	LCC-1	CCR1
CCL17	16q13	TARC	TARC	CCR4
CCL18	17q11.2	DC-CK1/PARC AMAC-1	Unknown	Unknown
CCL19	9p13	MIP-3β/ELC/exodus-3	MIP-3β/ELC/exodus-3	CCR7
CCL20	2q33–q37	MIP-3α/LARC/exodus-1	MIP-3α/LARC/exodus-1	CCR6
CCL21	9p13	6Ckine/SLC/exodus-2	6Ckine/SLC/exodus-2/TCA-4	CCR7
CCL22	16q13	MDC/STCP-1	ABCD-1	CCR4
CCL23	17q11.2	MPIF-1	Unknown	CCR1
CCL24	7q11.23	MPIF-2/Eotaxin-2	Unknown	CCR3
CCL25	19p13.2	TECK	TECK	CCR9
CLL26	7q11.23	Eotaxin-3	Unknown	CCR3
CCL27	9p13	CTACK/ILC	ALP/CTACK/ILC ESkine	CCR10*

Reprinted from: Zlnotnick A, Yoshie O: Chemokines: a new classification system and their role in immunity. *Immunity* 12: 121–127; 2000, with permission from Elsevier Science. Authors comments follow:

[a]We recently identified the receptor for CCL27, which has been named CCR10 (Homey *et al*, 2000). While we have tried to include most of the names with which a particular chemokine has been described, we may have missed some; for this we apologize in advance. We have also tried to list the main receptors for each chemokine, although some may bind other receptors but may not be their primary ligands. A question mark indicates that the listed mouse homolog may not correspond to the listed human ligand (see text). A systematic name in parenthesis indicates that the human homolog has not yet been identified. This provisional nomenclature proposal has been submitted to the International Union of Immunological Societies (IUIS) Subcommittee on Chemokine Nomenclature (Chairman R. Thorpe) for consideration as an internationally approved nomenclature.

articles (Belperio *et al*, 2000). The known human chemokines are listed in **Table I** with their systematic name, chromosome location and receptor(s).

Chemokine receptor gene clusters exist on chromosomes 2 and 3 (Zlotnik and Yoshie, 2000). There is significant redundancy and promiscuity in chemokine signaling as many chemokines share common receptors and, conversely, bind to multiple receptors. Chemokines and their receptors have been implicated in the regulation of various immune-mediated responses. For example, chemokines serve as attractant cytokines for T cells, and differential expression of chemokine receptors may contribute to the homing and activation of specific T cell subsets. Relevant to autoimmune, allergic, and dermatologic disease, it has been demonstrated that specific chemokine tissue expression profiles function to recruit differentially corresponding T cell subtypes (Sebastiani *et al*, 2001). Memory T cells differentially express chemokine receptors; T helper 1 lymphocytes expressing CCR5 and CXCR3, and T helper 2 lymphocytes CCR3, CCR4, and CCR8 (Gerber *et al*, 1997; Sallusto *et al*, 1997, 1998; Bonecchi *et al*, 1998; D'Ambosio *et al*, 1998; Zingoni *et al*, 1998). More recent investigations demonstrate a role for chemokines in T cell differentiation (Luther and Cyster, 2001). In addition, an entirely distinct role for

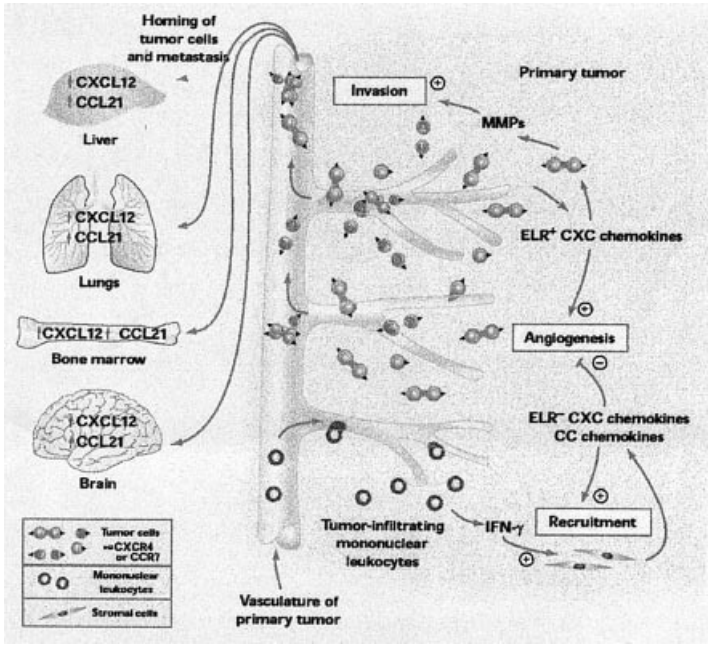

Figure 1. Chemokine role in cellular transformation, tumor growth, invasion and homing and metastasis to distant preferential organs. Reprinted with permission from: Strieter RM: Chemokines: Not just leukocyte chemoattractants in the promotion of cancer. *Nat Immunol* 2:285–286, 2001.

chemokine receptors has been identified with regard to infectious disease processes. An important example are the chemokine receptors CCR5 and CXCR4, present on monocytes and resting T cells, respectively, that bind to their ligands murine macrophage inflammatory protein (MIP) 1-α/β and RANTES (Regulated upon Activation Normal T cell Expressed and Secreted; CCR5) and SDF-1α (CXCR4). Interestingly, these chemokine receptors also act as coreceptors for human immunodeficiency virus, and this differential utilization partially explains the basis for human immunodeficiency virus tropism to specific cell types.

Such varied utilization of chemokine receptors, their activation and signaling are integrated into, and partially regulate, complex inflammatory and immune responses. Like other G-protein linked signal pathways, activation of chemokine receptors, characteristically by binding of their respective chemokine ligands, initiates a cascade of downstream biochemical events, including hydrolysis of phosphotidylinositol triphosphate, PI-3 kinase, protein kinase C activation, calcium influx, and activation of rac and Rho (reviewed in Mukaida, 2000). The latter pathway is involved in cell migration and has recently been implicated through large genomic screens to be a participant in melanoma metastasis (Clark *et al*, 2000).

Chemokines have also been implicated in the cellular transformation, tumor growth, invasion, and homing of metastasis to distant sites (**Fig 1**) (Strieter *et al*, 1995) and in the host–tumor response. The recruitment of leukocytes to the site of a tumor represents a delicate balance between the host–anti-tumor response and the elaboration of inflammatory mediators that may induce or facilitate invasion by the primary tumor. The role of inflammation in cancer has recently been reviewed (Balkwill and Mantovani, 2001), with much evidence supporting the theory that, for many cancers, the presence of leukocytes in a primary tumor negatively impacts prognosis. Relevant to inflammation and tumor biology, some chemokines have an effect on angiogenesis. More specifically, certain CXC chemokines, including CXCL8 [interleukin (IL)-8], contain a three amino acid ELR motif (glutamine–leucine–arginine) (ELR^+) between the N-terminus and the first cysteine, and function as potent promoters of angiogenesis (reviewed by Belperio *et al*, 2000). Other members of this family include Groα, -β, and -γ (see below), epithelial neutrophil activating protein-78 (ENA-78), granulocyte chemotactic protein-2 (GCP), and platelet basic protein-2 (PBP). CXC chemokines lacking this ELR motif (ELR^-) are angiostatic, and include platelet factor-4 (PF-4), interferon-γ inducible protein (IP-10), and monokine induced by interferon-γ (MIG). The relevance of the angiogenesis-promoting or angiogenesis-inhibiting properties of these chemokines to melanoma genesis and tumor progression is as yet not established. In fact, the prognostic significance of angiogenesis in melanoma, unlike certain other tumors, is unclear. In one study, macrophage infiltration has been found to correlate with melanoma tumor stage and angiogenesis, with the inflammatory mediators IL-1α and tumor necrosis factor (TNF)-α implicated in the pathogenesis of this effect (Torisu *et al*, 2000). Although the role of chemokines was not specifically addressed in this study, the leukocyte-derived inflammatory cytokine TNF-α is angiogenic and is also capable of inducing CXC ELR^+ chemokines (Schröder *et al*, 1990). Their role on angiogenesis aside, given the role of chemokines as potent chemoattractants for inflammatory cells, as well as effectors in other cell types, recent attention has focused upon the expression of chemokines and their receptors in tumorigenesis and metastasis.

CXCL8/IL-8

The first described chemokine, CXCL8, was originally identified as a neutrophil chemotactic and activating peptide isolated from mononuclear cells, a cytokine involved in the acute inflammatory response. It was purified from supernatants of lipopolysaccharide-stimulated human monocyte cultures (Schröder *et al*, 1987; Walz *et al*, 1987; Yoshimura *et al*, 1987; Mrowietz *et al*, 1999). CXCL8 mRNA is expressed by monocytes, natural killer cells, T lymphocytes, neutrophils, endothelial cells, keratinocytes, fibroblasts, and smooth muscle cells (Baggiolini *et al*, 1989; Schröder *et al*, 1987, 1990; Walz *et al*, 1987; Matsushima *et al*, 1988; Kulke *et al*, 1998). There is no known mouse homolog. CXCL8 has been shown to induce lysosomal degranulation, generation of the free radical burst, and upregulation of certain adhesion molecules (reviewed in Mukaida, 2000); thus acting both as a chemoattractant and upon binding to its receptor, a neutrophil activator. CXCL8 binds with high affinity to two distinct receptors, CXCR1 and CXCR2, primarily expressed on neutrophils (Holmes *et al*, 1991; Murphy and Tiffany, 1991), but also on other cell types, including keratinocytes (Kulke *et al*, 1998). CXCR1 (IL8RA) binds CXCL8 (IL-8) and another CXC chemokine NAP-2 (Petersen *et al*, 1994; Zlotnik and Yoshie, 2000); CXCR2 is a more promiscuous receptor, binding multiple CXC ELR^+ chemokines, including CXCL1, and CXCL8 (Zlotnik and Yoshie, 2000). It has

been postulated that CXCR2 may, in fact, mediate the angiogenic activity of ELR^+ chemokines (Belperio *et al*, 2000). Like many of the chemokine receptors, binding of ligand to CXCR2 not only leads to receptor activation but may also regulate receptor availability through receptor desensitization (Mueller *et al*, 1997), clathrin-mediated receptor endocytosis (Yang *et al*, 1999), and subsequent receptor degradation (Mueller *et al*, 1995).

Melanoma cells express CXCL8 mRNA (Colombo *et al*, 1992) and secrete the protein (Förster *et al*, 1991). In contrast to neutrophils, CXCR2 is the major CXCL8 receptor in melanoma. CXCL8 has been implicated in melanoma progression through several mechanisms, including the promotion of tumor cell growth and migration (Wang *et al*, 1990; Norgauer *et al*, 1996). *In vitro*, CXCL8 has been described as a melanoma cell mitogen (Schadendorf *et al*, 1993). In one study, inhibition of CXCL8 via anti-sense oligonucleotides or neutralizing CXCL8 monoclonal antibodies decreased melanoma cell proliferation in culture suggesting a role for CXCL8 as a melanoma growth factor (Schadendorf *et al*, 1993). Separate studies utilizing different melanoma cell lines, however, showed that neutralizing antibodies to another chemokine, CXCL1 (see below), but not CXCL8, similarly inhibited cell proliferation (Fujisawa *et al*, 1999); this suggested that melanoma may utilize different chemokine ligands, among other proteins, to support growth. CXCL8 mediates the haptotactic migration of melanoma cells (Wang *et al*, 1990) and induces matrix metalloproteinase-2, facilitating extracellular matrix degradation and migration (Luca *et al*, 1997).

Chemokine studies in mice may be difficult to translate to the human system. In the murine system, IL-8 does not exist and the most likely functional murine equivalent to human IL-8 is MIP-2 (Hogaboam *et al*, 1999). Interestingly, however, murine MIP-2 is structurally similar to another chemokine, Gro-α (see below) and murine Gro is known as KC (**Table I**). Nonetheless, CXCL8 expression also correlates with metastatic potential in murine melanoma tumor models using human cell lines (Singh *et al*, 1994). In a nude mouse model, induction of ultraviolet-induced melanoma cell tumorigenesis and metastasis correlate with CXCL8 mRNA and protein expression (Singh *et al*, 1995). Using the same model, it was demonstrated that expression of CXCL8 was regulated by the tissue microenvironment (Gutman *et al*, 1995). CXCL8-expressing human melanoma cells injected into nude mice and harvested after metastasis to the subcutis, spleen, and liver differed in their chemokine expression levels. Melanoma cells metastatic to the skin consistently expressed higher levels of CXCL8 than cells that engrafted in the liver, and cross-over experiments demonstrated that highly expressing metastatic cells isolated from the skin expressed decreased amounts of CXCL8 after reinjection and isolation from liver metastases. *In vitro* experiments with these cells suggested that these findings were the result of induction of CXCL8 expression by keratinocyte-derived IL-1 and conversely, inhibition of CXCL8 by hepatic-derived transforming growth factor-β. These studies suggest a role for paracrine regulation of chemokines in the clinical behavior of melanoma *in vivo*. In fact, recent related work (Muller *et al*, 2001) demonstrates that chemokine receptor expression by tumor cells may direct their homing to metastatic sites (**Fig 1**).

Understanding chemokine and chemokine receptor regulation may contribute to our understanding of the metastatic patterns of melanoma. As indicated above, CXCL8 is constitutively expressed by some, but not all, melanoma cells *in vitro* (Schadendorf *et al*, 1993). CXCL8 expression is upregulated by the inflammatory cytokines IL-1 and TNF-α via AP-1 and NF-κB response elements in its promoter (Singh *et al*, 1995; Mohler *et al*, 1996). Conversely, interferons α and β inhibit this response (Singh and Varney, 1998). Potentially relevant to tumorigenesis, the CXCL8 promoter also contains an Oct1 repressor element (Wu *et al*, 1997) that is deactivated through retinoblastoma protein expression (Zhang *et al*, 1999). Parallel mechanisms of chemokine receptor expression regulation are similarly beginning to be understood (Mueller *et al*, 1997; Nieto *et al*, 1997; Sica *et al*, 1997; Sozzani *et al*, 1998; Romagnani *et al*, 2001; Zella *et al*, 1991)

CXCL1–3/MGSA-α–γ/GRO-α–γ

CXCL1 protein was originally purified as an autocrine growth factor MGSA (melanoma growth stimulatory activity protein) from supernatants of Hs29T melanoma cell cultures (Richmond *et al*, 1985; Richmond and Thomas, 1986; Bordoni *et al*, 1990) and described as the product of the growth-related oncogene (gro) locus (Anisowicz *et al*, 1987; Richmond *et al*, 1988), which is identical to the CXCL1–3 gene cluster on chromosome 4q12–q13. Later, it was purified from lipopolysaccharide-activated monocytes as a neutrophil chemoattractant (Schröder and Christophers, 1989) and from similarly stimulated human umbilical vein endothelial cells (Schröder *et al*, 1990). CXCL1 mRNA expression has also been demonstrated in keratinocytes of psoriatic skin (Kulke *et al*, 1998). CXCL1 is clustered on three homologous genes (α, β, and γ; CXCL1, 2, and 3, respectively) on chromosome 4 (Haskill *et al*, 1990). Some background on nomenclature regarding this group of related chemokines is necessary to understand the literature. CXCL1, 2, and 3 are also known as MGSA-α/Gro-α, MGSA-β/Groβ and MGSA-γ/Gro-γ, respectively. As previously described, the murine homolog is Gro/KC (Zlotnik and Yoshie, 2000). Suggesting a role in melanocyte transformation, overexpression of CXCL1, CXCL2, or CXCL3 in immortalized melanocytes results in their ability to form tumors (Balentien *et al*, 1991; Owen *et al*, 1997).

Whether there are independent biologic roles for the three ligands (CXCL1–3) is uncertain. In addition to its role as a purported autocrine growth factor for melanoma (Richmond *et al*, 1988), CXCL1 promotes angiogenesis in the rat cornea model, with a less robust angiogenic response generated by CXCL2 and 3 (Strieter *et al*, 1995). CXCL1–3 promote neutrophil chemotaxis with similar efficacy (Baggiolini *et al*, 1994), but demonstrate differing levels of potency with respect to calcium mobilization (an important indicator of receptor activation), which is dependent upon cell type (Geiser *et al*, 1993). CXCL1–3 bind with high affinity to a common receptor, CXCR2 (Haskill *et al*, 1990; Mueller *et al*, 1994), with CXCL1 having the highest affinity (Hammond *et al*, 1996). Conversely, CXCL1 binds with a lower affinity to CXCR1 (CXCL8 receptor, see above) (Lee *et al*, 1992). The biologic significance of multiple receptors for CXCL1 is unclear, as blocking antibodies to either CXCL1 or CXCR2 inhibit melanoma cell growth *in vitro* (Lawson *et al*, 1987; Norgauer *et al*, 1996), indicating that CXCR2 may be necessary and sufficient for melanoma growth signaling by CXCL1 in these cells. Mice lack expression of CXCR1, but do express CXCR2 that bind CXC chemokines (Lee *et al*, 1995). As previously discussed, human CXCR1 transcripts are primarily expressed in neutrophils, melanoma cells express transcripts for CXCR2 (Muller *et al*, 2001).

CXCL1 regulation occurs at both the transcriptional and translational level, although again, in a cell-type-specific manner. Relevant to cells of melanocyte lineage, CXCL1 mRNA is constitutively expressed in cultured nevocytes from benign and ''dysplastic'' nevi as well as melanoma cells, but is not detectable in cultured primary melanocytes (Bordoni *et al*, 1990). This tight regulation of CXCL1 mRNA expression in ''normal'' melanocytes, along with deregulated expression in transformed cells (melanoma) *in vitro* is analogous to the expression profile of cellular oncogenes (Campisi *et al*, 1984). In cells of melanocyte lineage, CXCL1 protein, unlike CXCL1 mRNA, is constitutively expressed only by melanoma cells (through activation of NF-κB; Shattuck-Brandt and Richmond, 1997) and protein release can be induced in nevocytes as well as melanocytes by exogenous growth factors (Bordoni *et al*, 1990). One proposed explanation for these findings is that CXCL1 mRNA contains 3′ regulatory sequences that, in a nontransformed cell, normally signal for rapid turnover, making steady-state detection in normal melanocytes difficult (Bordoni *et al*, 1990). It has also been proposed that the increased

level of mRNA observed in melanoma cells is due not only to this increased mRNA stability but also to transcriptional regulation in response to exogenous cytokines and growth factors, including CXCL1 itself (Anisowicz *et al*, 1987; Richmond *et al*, 1988). Finally, translational regulation of CXCL1 has also been demonstrated, where mRNA–polyribosome association is dependent upon specific growth factors, exogenously derived for melanocytes and endogenous in melanoma cells (Bordoni *et al*, 1990). When multiple melanoma cell lines were examined, it was similarly found that regulation of chemokine protein expression involves both transcription and mRNA stability, as well as mechanisms affecting translation and secretion (Yang and Richmond, 2001). In the context of these findings, the pattern of expression of the chemokine CXCL1, in the cellular transformation of the melanocyte presents a multitiered process that appears to involve a stepwise regulation of transcriptional and translational control. Stimulation by exogenous factors such as ultraviolet irradiation, as well as those yet to be determined, may serve as initiating events in the circumvention of normal growth control regulatory mechanisms in the melanocyte (Singh *et al*, 1995). If one proposes that melanocyte transformation progresses from either the melanocyte directly to melanoma or, in certain instances, the melanocyte to nevus cell to melanoma, determining such cellular and molecular characteristics that accompany this transformation may aid our understanding of the process. In fact, G protein-coupled receptor overexpression has previously been linked to cellular transformation in another cell type. Overexpression of the Kaposi's sarcoma virus chemokine-like G protein-coupled receptor (KSHV-G protein-coupled receptor), having similarities to CXCR2, in hematopoietic cells in mice leads to the spontaneous development of angioproliferative lesions resembling Kaposi's sarcoma (Yang *et al*, 2000).

CCL5/RANTES

Human and murine CCL5, or RANTES, share sequence homology and chromosomal location (17q11.2) (Zlotnik and Yoshie, 2000). RANTES, similar to the other chemokines, was originally identified as a leukocyte chemoattractant protein (Schall *et al*, 1990; Schall, 1999). More recently, however, its role in binding to one of its receptors, CCR5, one of the human immunodeficiency virus cell entry receptors on $CD4^+$ T cells (Ward and Westwick, 1998) has received much attention. RANTES is produced by $CD8^+$ T cells, platelets, epithelial cells, and fibroblasts, typically in response to inflammatory mediators (Appay and Rowland-Jones, 2001). Its receptors, CCR1, 3, 4, and 5 may be found on a variety of cell types, including T cells, monocytes, dendritic cells, and mast cells (Schall, 1999), and upon binding its ligand, mediate the effects of this chemokine at nanomolar concentrations (Nieto *et al*, 1997). The expression of multiple receptors on one cell type (i.e., the macrophage) may potentially explain the rapid receptor activation, that is, in fact, a characteristic of this chemokine. Interestingly, at micromolar concentrations, RANTES acts as a T cell mitogen that is independent of chemokine binding to its G protein-coupled receptor (Appay *et al*, 2000). Also unique to RANTES is its ability to self-aggregate on the cell surface, having potential implications in cell–cell cross-linking (Appay and Rowland-Jones, 2001).

With respect to melanoma, a subset of melanoma cells has been shown to express CCL5/RANTES (Mrowietz *et al*, 1999). In these cells, expression of CCL5 was 5–50-fold higher than CXCL8 and was upregulated by TNF-α. CCL5 expressing melanoma cells formed increasingly aggressive tumors in nude mice in a concentration-dependent fashion. CCL5-expressing tumors were highly chemotactic for leukocytes, and transplantation experiments demonstrated that CCL5 expression favored tumor progression.

CCL2/MCP-1

Monocyte chemotactic protein-1 (MCP-1, CCL2) is another highly conserved CC chemokine (Yoshimura *et al*, 1989). CCL2 is a potent monocyte chemoattractant protein, isolated from cultured smooth muscle vascular cells (Valente *et al*, 1988) that also recruits natural killer cells (Allavena *et al*, 1994) and certain T lymphocyte populations (Carr *et al*, 1994). CCL-2 is expressed in a variety of ''inflammatory'' conditions (Kuziel *et al*, 1997), including atherosclerosis (Yla-Herttuala *et al*, 1991), and its role in tumorigenesis and progression, including melanoma, has been widely investigated (Salcedo *et al*, 2000; Sica *et al*, 2000; Ueno *et al*, 2000; Nesbit *et al*, 2001). The predominant chemokine receptor is CCR2 (Kurihara *et al*, 1997). Mice deficient in this receptor demonstrate a severe reduction in leukocyte adhesion, monocyte extravasation (Kuziel *et al*, 1997) and recruitment (Boring *et al*, 1997). In ovarian carcinoma, tumor-associated macrophages isolated from ascites or solid tumors demonstrated defective CCR2 expression and the investigators propose that this may represent one mechanism of the defective immune response seen in advanced cancers (Sica *et al*, 2000).

Early studies demonstrated the expression of monocyte chemotactic protein (CCL2/MCP-1) by melanoma *in vivo* (Graves *et al*, 1992), a finding of some interest as macrophages are commonly found in melanoma as the quantitatively dominant leukocyte type (Bröcker *et al*, 1988; Van Ravenswaay-Claasen *et al*, 1992). Tumor-associated macrophages elaborate cytokines and growth factors, some of which are angiogenic and angiostatic, which can either promote or inhibit tumor progression (Mantovani *et al*, 1992) Along these lines, previous studies on gene transfer of human CCL2 into murine melanoma cells demonstrated that CCL2-producing melanoma clones showed a 2-fold increase in the percentage of tumor-associated macrophages *in vivo* as well as a 2-fold decrease in the rate of tumor growth, associated with increased survival (Bottazzi *et al*, 1992). This relationship is not straightforward, however, as inoculation of smaller numbers of CCL2-expressing cells resulted in increased tumorigenicity. As previously noted, one group has found that macrophage infiltration in human melanoma correlates with tumor stage and angiogenesis (Torisu *et al*, 2000). Interestingly, we have found that CCL2, as well as certain other chemokines, induce matrix-degrading matrix metalloproteinase secretion in macrophages, potentially relevant to tumors having macrophage infiltration (LAC, unpublished observations). Clearly, these relationships are complex, and further studies are needed to determine the role of these factors in the host immune response that is specific to melanoma.

CHEMOKINE RECEPTORS AND MELANOMA

The coordinated secretion of chemokines, and their binding to receptors on the cell surface, directs leukocyte cell homing to specific tissue sites. The secretion of chemokines and their receptor expression by dendritic cells during maturation, migration, and antigen presentation in the lymph node provides an elegant example of this homing process (Dieu *et al*, 1999; Förster *et al*, 1999; Zlotnik and Yoshie, 2000) as reviewed by Zlotnik and Yoshie (2000). Similar processes have been suggested in the homing of transformed cells to specific sites (**Fig 1**). In fact, a role for chemokine receptors has recently been demonstrated in the homing of metastatic tumor cells in breast cancer and melanoma (Muller *et al*, 2001). Investigators demonstrated that malignant cells express distinct and nonrandom patterns of chemokine receptors that guide their metastatic destination determined by the expression levels of chemokines by target organs. Specifically, melanoma cells were found to express high levels of CXCR4, CCR7, and CCR10 mRNA as compared with normal primary melanocytes. The respective ligands for these receptors, CXCL12/SDF-1α, CCL21/6Ckine, and CCL27/CTACK, exhibit peak levels of expression in lymph node, lung, liver, bone marrow, and skin, in accordance with the primary metastatic destinations of melanoma. CCL27/CTACK is a novel skin-specific chemokine (Morales *et al*, 1999) whose expression can be upregulated by the inflammatory cytokines IL-1 and TNF-α (Homey *et al*, 2000). The receptor for CCL27, CCR10, is expressed by melanocytes, melanoma

tumor cells, dermal fibroblasts, endothelial cells, T cells, and Langerhans cells (Homey *et al*, 2000), supporting the role for CCL27/CCR10 interaction in this, as well as other, immune-mediated processes of the skin (Rottman *et al*, 2001).

In the above breast cancer model of metastasis, signaling through CXCR4 or CCR7 induces actin polymerization, pseudopod formation, and chemotactic migration in tumor cells. Treatment of SCID mice with anti-CXCR4 neutralizing monoclonal antibody following intravenous or orthotopic injection of human breast cancer tumor cells significantly inhibited lymph node and lung metastases (Muller *et al*, 2001). In these investigations, however, it was unclear whether this effect was achieved by disruption of metastatic homing and/or primary inhibition of tumor cell growth. Prior studies using human melanoma cells in nude mice have demonstrated that treatment with a synthetic peptide that competitively inhibits CXC chemokine binding can inhibit pulmonary metastases *in vivo* (Fujisawa *et al*, 1999). Similarly, preliminary studies in a murine melanoma model using CCR-7-transduced B16 melanoma cells demonstrated a 200-fold increase in draining lymph node tyrosinase-related protein-1 mRNA (indicative of B16 tumor cell homing) than their vector-transduced littermates (Gonzolez *et al*, 2001). Given the multiple steps in the pathway toward metastasis, including tumor growth, cell migration, and engraftment, the actual mechanism for chemokine receptor dependent homing is undoubtedly more complex than mere ligand recognition and most likely includes the local induction of proteases and adhesion molecules, as well as other factors.

CHEMOKINES AND MELANOMA THERAPY

In addition to melanoma chemokine receptor expression and potential homing to preferential metastatic sites, the increasing data on the association of chemokines with dendritic cell maturation and lymph node homing (Zlotnik and Yoshie, 2000) is in itself also relevant to melanoma therapy. As previously cited, dendritic cell expression of chemokine receptors such as CCR7 has been shown to mediate dendritic cell migration to lymph nodes, an essential event for antigen-specific T cell activation (Förster *et al*, 1999; Sallusto *et al*, 2000) and an important mechanism for mounting an immune response to certain tumors. Dendritic cells also express specific chemokines such as CCL17 whose receptor CCR4 has been associated with the T helper 2 phenotype, suggesting that the particular chemokine secreted determines the subset of T cells targeted for recruitment and expansion (O'Garra *et al*, 1998; Imai *et al*, 1999). Separate investigations have determined that this recruitment of dendritic cells to tumor tissue involves chemokines and receptors that are distinct from those employed in leukocyte recruitment (tumor-associated macrophages) (Hillibrand *et al*, 1999). Further research into mechanisms of dendritic chemokine expression and receptor regulation as it relates to tumor cell antigen presentation may have significant clinical benefit relevant to melanoma vaccine adjuvant therapy (Brinckerhoff *et al*, 2000).

In the area of infectious disease, chemokine receptor blockade strategies are currently under investigation in human immunodeficiency virus chemokine receptor recognition and cell entry (Moore and Trkola, 1997; Hadida *et al*, 1998; Berger *et al*, 1999). Although this does not represent an entirely analogous biologic system, further investigations linking tissue and tumor cell chemokine and receptor expression to cellular transformation and tumor metastasis may provide a scientific basis for similar blocking strategies to be investigated in the area of cancer biology and metastasis.

REFERENCES

Allavena P, Bianchi D, Zhou D, van Damme J, Jilek P, Sozzani S, Mantovani A: Induction of natural killer cell migration by monocyte chemoattractant protein-1, -2 and -3. *Eur J Immunol* 24:3233–3236, 1994

Anisowicz A, Bardwell L, Sager R: Constitutive overexpression of a growth-regulated gene in transformed Chinese hamster and human cells. *Proc Natl Acad Sci USA* 84:7188–7192, 1987

Appay V, Dunbar PR, Cerundolo V, McMichael A, Czaplewski L, Rowland-Jones SL: RANTES activates antigen-specific cytotoxic T lymphocytes in a mitogen-like manner through cell surface aggregation. *Int Immunol* 8:1173–1182, 2000

Appay V, Rowland-Jones SL: RANTES. a versatile and controversial chemokine. *Trends Immunol* 22:83–87, 2001

Baggiolini M, Walz A, Kunkel SL: Neutrophil-activating peptide-1/interleukin-8, a novel cytokine that activates neutrophils. *J Clin Invest* 84:1045–1049, 1989

Baggiolini M, Dewald B, Moser B: Interleukin-8 and related chemotactic cytokines—CXC and CC chemokines. *Adv Immunol* 55:97–179, 1994

Balentien E, Mufson BE, Shattuck RL, Derynck R, Richmond A: Effects of MGSA/GRO alpha on melanocyte transformation. *Oncogene* 6:1115–1124, 1991

Balkwill F, Mantovani A: Inflammation and cancer: back to Virchow? *Lancet* 357:539–544, 2001

Belperio JA, Keane MP, Arenberg DA, Addison CL, Ehlert JE, Burdick MD, Strieter RM: CXC chemokines in angiogenesis. *J Leukoc Biol* 68:1–8, 2000

Berger EA, Murphy PM, Farber JM: Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism and disease. *Annu Rev Immunol* 17:657–670, 1999

Bonecchi R, Bianchi G, Bordignon PP, *et al*: Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells. *J Exp Med* 187:129–134, 1998

Bordoni R, Fine R, Murray D, Richmond A: Characterization of the role of melanoma growth stimulating activity MGSA in the growth of normal melanocytes, nevocytes and malignant melanocytes. *J Cell Biochem* 44:207–219, 1990

Boring L, Gosling J, Chensue SW, Kunkel SL, Farese RV: Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2. *Proc Natl Acad Sci USA* 94:12053–12058, 1997

Boring L, Gosling J, Cleary M, Charo IF: Decreased lesion formation in $CCR2^{-1-}$ mice reveals a role for chemokines in the initiation of atherosclerosis. *Nature* 394:894–897, 1998

Bottazzi B, Walter S, Govoni D, Colotta F, Mantovani A: Monocyte chemotactic cytokine gene transfer modulates macrophage infiltration, growth, and susceptibility to IL-2 therapy of a murine melanoma 148:1280–1285, 1992

Brinckerhoff LH, Thompson LW, Slingluff CLJ: Melanoma vaccines. *Curr Opin Oncol* 12:163–173, 2000

Bröcker EB, Zwadlo G, Holzmann B, Macher E, Sorg C: Inflammatory cell infiltrates in human melanoma at different stages of tumor progression. *Int J Cancer* 41:562–567, 1988

Campisi J, Gray HE, Pardee AB, Dean M, Sonenshein GE: Cell-cycle control of c-myc but not c-ras expression is lost following chemical transformation. *Cell* 36:241–247, 1984

Carr MW, Rioth SJ, Luther E, Rose SS, Springer A: Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. *Proc Natl Acad Sci USA* 91:3652–3656, 1994

Clark EA, Golub TR, Lander ES, Hynes RO: Genomic analysis of metastasis reveals an essential role for Rho C. *Nature* 406:532–535, 2000

Colombo MP, Maccalli C, Mattei S, Melani C, Radrizzani M, Parmiani G: Expression of cytokine genes, including IL-6, in human malignant melanoma cell lines. *Melanoma Res* 2:181–189, 1992

D'Ambosio D, Iellem A, Bonecchi R, Mazzeo D, Sozzani S, Montovani A, Sinigaglia F: Selective up-regulation of chemokine receptors CCR4 and CCR8 upon activation of polarized human type 2 Th cells. *J Immunol* 161:5111–5115, 1998

Dieu MC, Vanbervliet B, Vicari A, *et al*: Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J Exp Med* 188:373–386, 1999

Fishman D, Irena B, Kellman-Pressman S, Karas M, Segal S: The role of MHC class I glycoproteins in the regulation of induction of cell death in immunocytes by malignant melanoma cells. *Proc Natl Acad Sci USA* 98:1740–1744, 2001

Förster E, Kirnbauer R, Urbanski A, Keck A, Luger TA, Schwartz T: Human melanoma cells produce interleukin-8 which functions as an autocrine growth factor. *Arch Dermatol Res* 283:26, 1991

Förster R, Schubel A, Breitfield D, Kremmer E, Renner-Muller I, Wolf E, Lipp M: CCR7 coordinates the primary immature response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 99:23–33, 1999

Fujisawa N, Hayashi S, Miller EJ: A synthetic peptide inhibitor for α-chemokines inhibits the tumor growth and pulmonary metastasis of human melanoma cells in nude mice. *Melanoma Res* 9:105–114, 1999

Geiser T, Dewald B, Ehrengruber MU, Clark-Lewis I, Baggiolini M: The interleukin-8-related chemotactic cytokines GRO alpha, GRO beta, and GRO gamma activate human neutrophil and basophil leukocytes. *J Biol Chem* 268:15419–15424, 1993

Gerber BO, Zanni MP, Uguccioni M, *et al*: Functional expression of the eotaxin receptor CCR3 in T lymphocytes co-localizing with eosinophils. *Curr Biol* 7:836–843, 1997

Giavazzi R, Garofalo A, Bani MR, *et al*: Interleukin 1-induced augmentation of experimental metastases from a human melanoma in nude mice. *Cancer Res* 50:4771–4775, 1990

Gonzolez EB, Wiley H, Hwang ST: CC chemokine receptor 7 (CCR-7) - transduced B16 melanoma cells show enhanced early metastasis to regional lymph nodes. *Meet Soc Invest Dermatol* 35:200, 2001

Graves DT, Barnhill R, Galanopoulos T, Antoniades HN: Expression of monocyte chemotactic protein-1 in human melanoma in vivo. *Am J Pathol* 140:9–14, 1992

Greene MH, Sanders RJ, Chu FC, Clark WH, Elder DE, Cogan DG: The familial occurrence of cutaneous melanoma, intraocular melanoma, and the dysplastic nevus syndrome. *Am J Opthalmol* 96:238–245, 1983

Gruis N, Sandkuijl L, Weber J, Zee A, Borgstein A, Bergman W, Frants R: Linkage analysis in Dutch familial atypical multiple mole–melanoma syndrome families. Effect of naevus count. *Melanoma Res* 3:271–277, 1993

Gutman M, Singh RK, Xie K, Bucana CD, Fidler IJ: Regulation of interleukin-8 expression in human melanoma cells by the organ environment. *Cancer Res* 55:2470–2475, 1995

Hadida F, Vieillard V, Autran B, Clark-Lewis I, Baggiolini M, Debre P: HIV-specific T cell cytotoxicity mediated by RANTES via the chemokine receptor CCR3. *J Exp Med* 188:609–614, 1998

Halpern AC, Guery D 4th, Elder DE, Clark WH Jr, Synnestvedt M, Norman S, Ayerle R: Dysplastic nevi as risk markers of sporadic (nonfamilial) melanoma. *Arch Dermatol* 127:995–999, 1991

Hammond MEW, Shyamala V, Siani MA, *et al*: Receptor recognition and specificity of interleukin-8 is determined by residues that cluster near a surface-accessible hydrophobic pocket. *J Biol Chem* 271:8228–8235, 1996

Haskill S, Peace A, Morris J, *et al*: Identification of three related human GRO genes encoding cytokine functions. *Proc Natl Acad Sci USA* 87:7732–7736, 1990

Hillibrand EF, Neville AM, Coventry BJ: Immunohistochemical localization of CD1a-positive putative tumor infiltrating dendritic cells in human breast tumors. *Br J Cancer* 79:940–944, 1999

Hofman UB, Westphal JR, Waas ET, Becker JC, Ruiter DJ, van Muijen GNP: Coexpression of integrin avb3 and matrix metalloproteinase-2 (MMP-2) coincides with MMP-2 activation: Correlation with melanoma progression. *J Invest Deratol* 115:625–632, 2000

Hogaboam C, Bone-Larson C, Steinhauser M, *et al*: Novel CXCR2-dependent liver regenerative qualities of ELR-containing CXC chemokines. *FASEB J* 13:1565–1574, 1999

Holmes WE, Lee J, Kuang WJ, Rice GC, Wood WI: Structure and functional expression of a human interleukin-8 receptor. *Science* 253:1278–1280, 1991

Homey B, Wang W, Soto H, *et al*: Cutting edge: the orphan chemokine receptor G protein-coupled receptor-2 (GPR-2, CCR10) binds the skin-associated chemokine CCL27 (CTAK/ALP/ILC). *J Immunol* 164:3465–3470, 2000

Hussussian C, Struewing JP, Goldstein AM, *et al*: Germline p16 mutations in familial melanoma. *Nat Genet* 8:15–21, 1994

Imai T, Nagira M, Takagi S, *et al*: Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int Immunol* 11:81–88, 1999

Kirkwood J, Strawderman M, Ernstoff M, Smith T, Borden E, Blum R: Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. *J Clin Oncol* 14:7–17, 1996

Kulke R, Bornscheuer E, Schluter C, Bartels J, Rowert J, Sticherling M, Christophers E: The CXC receptor 2 is overexpressed in psoriatic epidermis. *J Invest Dermatol* 110:90–94, 1998

Kurihara T, Warr G, Loy J, Bravo R: Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. *J Exp Med* 186:1757–1762, 1997

Kuziel WA, Morgan SJ, Dawson TC, Griffin S, Smithies O, Ley K, Maeda N: Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2. *Proc Natl Acad Sci USA* 94:12053–12058, 1997

Lawson DH, Thomas HG, Roy RG, Gordon DS, Chawla RK, Nixon DW, Richmond A: Preparation of a monoclonal antibody to a melanoma growth-stimulatory activity released into serum-free culture medium by Hs0294 malignant melanoma cells. *J Cell Biochem* 34:169–185, 1987

Lee J, Horuk R, Rice GC, Bennett GL, Camerato T, Wood WI: Characterization of two high affinity human interleukin-8 receptors. *J Biol Chem* 267:16283–16287, 1992

Lee J, Cacalano G, Camerato T, Toy K, Moore M, Wood W: Chemokine binding and activities mediated by the mouse IL-8 receptor. *J Immunol* 155:2158–2164, 1995

Luca M, Huang S, Gershenwald JE, Singh RK, Reich R, Bar-Eli M: Expression of interleukin-8 by human melanoma cells up-regulates MMP-2 activity and increases tumor growth and metastasis. *Am J Pathol* 151:1105–1113, 1997

Luther SA, Cyster JG: Chemokines as regulators of T cell differentiation. *Nature Immunol* 2:102–107, 2001

MacDougall JR, Bani MR, Muschel RJ, Kebel RS: "Proteolytic switching" opposite patterns of regulation of gelatinase B and its inhibitor TIMP-1 during human melanoma progression and consequences of gelatinase B overexpression. *Br J Cancer* 80:504–512, 1999

Mantovani A, Bottazzi B, Colotta F, Sozzani S, Ruco L: The origin and function of tumor-associated macrophages. *Immunol Today* 13:265–270, 1992

Matsushima K, Morishita K, Yoshimura T, *et al*: Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. *J Exp Med* 167:1883–1893, 1988

Mohler T, Scheibenbogen C, Hafele J, Willhauck M, Keilholz U: Regulation of interleukin-8 mRNA expression and protein secretion in a melanoma cell line by tumor necrosis factor-alpha and interferon-gamma. *Melanoma Res* 6:307–311, 1996

Moore JP, Trkola A: HIV type 1 coreceptors, neutralization serotypes, and vaccine development. *AIDS Res Hum Retroviruses* 13:733–736, 1997

Morales J, Homey B, Vicari AP, *et al*: CTAK, a skin-associated chemokine that preferentially attracts skin-homing memory T cells. *Proc Natl Acad Sci USA* 96:14470–14475, 1999

Mrowietz U, Schwenk U, Maune S, *et al*: The chemokine RANTES is secreted by human melanoma cells and is associated with enhanced tumour formation in nude mice. *Br J Cancer* 79:1025–1031, 1999

Mueller SG, Schraw WP, Richmond A: Melanoma growth stimulatory activity enhances the phosphorylation of the class II interleukin-8 receptor in non-hematopoietic cells. *J Biol Chem* 269:1973–80, 1994

Mueller SG, Schraw WP, Richmond A: Activation of protein kinase C enhances the phosphorylation of the type B interleukin-8 receptor and stimulates its degradation in non-hematopoietic cells. *J Biol Chem* 270:10439–10448, 1995

Mueller SG, White JR, Schraw WP, Lam C, Richmond A: Ligand-induced desensitization of the human CXC chemokine receptor-2 is modulated by multiple serine residues in the carboxyl-terminal domain of the receptor. *J Biol Chem* 272:8207–8214, 1997

Mukaida N: Interleukin 8. An expanding universe beyond neutrophil chemotaxis and activation. *Int J Hematol* 72:391–398, 2000

Muller A, Homey B, Soto H, *et al*: Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410:50–56, 2001

Murphy PM: Chemokine receptors: structure, function and role in microbial pathogenesis. *Cytokine Growth Factor Rev* 7:47–64, 1996

Murphy PM, Tiffany HL: Cloning of complementary DNA encoding a functional human interleukin-8 receptor. *Science* 253:1280–1283, 1991

Nesbit M, Schaider H, Miller TH, Herlyn M: Low-level monocyte chemoattractant protein-1 stimulation of monocytes leads to tumor formation in nontumorigenic melanoma cells. *J Immunol* 166:6483–6490, 2001

Newton-Bishop JA, Bataille V, Pinney E, Bishop DT: Family studies in melanoma: identification of atypical mole syndrome. *Melanoma Res* 4:199–206, 1994

Newton-Bishop JA, Wachsmuth RC, Harland M, *et al*: Genotype/phenotype and penetrance studies in melanoma families with germline CDKN2A mutations. *J Invest Dermatol* 114:28–33, 2000

Nieto M, Frade JMR, Sancho D, Mellado M, Martinez AC, Sanchez-Madrid F: Polarization of chemokine receptors to the leading edge during lymphocyte chemotaxis. *J Exp Med* 186:153–158, 1997

Norgauer J, Metzner B, Schraufstutter I: Expression and growth-promoting function of the IL-8 receptor beta in human melanoma cells. *J Immunol* 156:1132–1137, 1996

O'Garra A, McEvoy LM, Zlotnik A: T-cell subsets: chemokine receptors guide the way. *Curr Biol* 8:R646–R649, 1998

Owen JD, Strieter R, Burdick M, Haghnegahdar H, Nanney L, Shattuck-Brandt R, Richmond A: Enhanced tumor-forming capacity for immortalized melanocytes expressing melanoma growth stimulatory activity/growth-regulated cytokine β and γ proteins. *Int J Cancer* 73:94–103, 1997

Petersen F, Flad H-D, Brandt E: Neutrophil-activating peptides NAP-2 and IL-8 bind to the same sites on neutrophils but interact in different ways: Discrepancies in binding affinities, receptor densities, and biologic effects. *J Immunol* 152:2467–2478, 1994

Platz A, Hansson J, Ringborg U: Screening of germline mutations in the CDK4, CDKN2C and TP53 genes in familial melanoma: a clinic-based population study. *Int J Cancer* 78:13–15, 1998

Platz A, Ringborg U, Hansson J: Hereditary cutaneous melanoma. *Cancer Biol* 10:319–326, 2000

Richmond A, Lawson DH, Nixon DW, Stevens JS, Chawla RK: Characterization of autostimulatory and transforming growth factors from human melanoma cells. *Cancer Res* 45:6390–6394, 1985

Richmond A, Thomas HG: Purification of melanoma growth stimulatory activity. *J Cell Physiol* 129:375–384, 1986

Richmond A, Balentien E, Thomas HG, *et al*: Molecular characterization of melanoma growth stimulatory activity, a growth factor structurally related to β-thromboglobulin. *EMBO J* 7:2025–2033, 1988

Romagnani P, Annunziato F, Lasagni L, *et al*: Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial mediates angiostatic activity. *J Clin Invest* 107:53–63, 2001

Rottman JB, Smith TL, Ganley KG, Kikuchi T, Krueger JG: Potential role of the chemokine receptors CXCR3, CCR4 and integrin alphaEbeta7 in the pathogenesis of psoriasis vulgaris. *Lab Invest* 81:335–347, 2001

Salcedo R, Resau JH, Halverson D, *et al*: Differential expression and responsiveness of chemokine receptors (CXCR1-3) by human microvascular endothelial cells and umbilical vein cells. *FASEB J* 14:2055–2064, 2000

Sallusto F, Mackay CR, Lanzavecchia A: Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. *Science* 277:2005, 1997

Sallusto F, Lenig D, Mackay CR, Lanzavecchia A: Flexible programs of chemokine receptor expression in human polarized T helper 1 and 2 lymphocytes. *J Exp Med* 187:875–883, 1998

Sallusto F, Mackay CR, Lanzavecchia A: The role of chemokine receptors in primary effector and memory immune responses. *Annu Rev Immunol* 18:593–620, 2000

Schadendorf D, Mueller A, Alermissen B, Worm M, Sticherling M, Czarnetzki BM: IL-8 produced by human malignant melanoma cells in vitro is an essential autocrine growth factor. *J Immunol* 151:2667–2675, 1993

Schall TJ, Bacon K, Toy KJ, Goeddel DV: Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. *Nature Immunol* 347:669–671, 1990

Schall TJ: Biology of the RANTES/SIS cytokine family. *Cytokine* 3:165–183, 1999

Schröder J, Christophers E: Secretion of novel and homologous neutrophil-activating peptides by LPS-stimulated human endothelial cells. *J Immunol* 142:244–251, 1989

Schröder J, Mrowietz U, Morita E, Christophers E: Purification and partial biochemical characterization of a human monocyte-derived, neutrophil-activating peptide that lacks interleukin 1 activity. *J Immunol* 139:3474–3483, 1987

Schröder JM, Sticherling M, Henneicke HH, Preissner WC, Christophers E: IL-1α or tumor necrosis factor-α stimulate release of three NAP-1/IL-8-related

neutrophil chemotactic proteins in human dermal fibroblasts. *J Immunol* 144:2223–2232, 1990

Sebastiani S, Allavena P, Albanesi C, *et al*: Chemokine receptor expression and function in CD4+ T lymphocytes with regulatory activity. *J Immunol* 166:996–1002, 2001

Shattuck-Brandt RL, Richmond A: Enhanced degradation of IkB contributes to endogenous activaiton of NFkB. *Cancer Res* 57:3032–3039, 1997

Sica A, Saccani A, Borsatti CA, *et al*: Bacterial lipopolysaccharide rapidly inhibits expression of C-C chemokine receptors in human monocytes. *J Expm Med* 185:969–974, 1997

Sica A, Saccani A, Bottazzi B, *et al*: Defective expression of the monocyte chemotactic protein-1 receptor CCR2 in macrophages associates with human ovarian carcinoma. *J Immunol* 164:733–738, 2000

Singh RK, Gutman M, Radinsky R, Bucana CD, Fidler IJ: Expression of interleukin 8 correlates with the metastatic potential of human melanoma cells in nude mice. *Cancer Res* 54:3242–3247, 1994

Singh RK, Gutman M, Reich R, Bar-Eli M: Ultraviolet B irradiation promotes tumorigenic and metastatic properties in primary cutaneous melanoma via induction of interleukin 8. *Cancer Res* 55:3669–3674, 1995

Singh RK, Varney ML: Regulation of interleukin 8 expression in human malignant melanoma cells. *Cancer Res* 58:1532–15367, 1998

Sozzani S, Allavena P, D'Amico G, *et al*: Cutting edge: Differential regulation of chemokine receptors during dendritic maturation: a model for their trafficking properties. *J Immunol* 161:1083–1086, 1998

Strieter RM, Polverini PJ, Kunkel SL, *et al*: The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. *J Biol Chem* 270:27348–27357, 1995

Torisu H, Ono MH, Kiryu H, *et al*: Macrophage infiltration correlates with tumor stage and angiogenesis in human melanoma. Possible involvement of TNF-α and IL-1α. *Int J Cancer* 85:182–188, 2000

Ueno T, Toi M, Saji H, *et al*: Significance of macrophage chemoattractant protein-1 in macrophage recruitment, angiogenesis and survival in human breast cancer. *Clin Cancer Res* 6:3282–3289, 2000

Valente AJ, Graves CE, Vialle-Valentin R, Delgado R, Schwartz CJ: Purification of a monocyte chemotactic factor secreted by nonhuman primate vascular cells in culture. *Biochemistry* 27:4162–4168, 1988

Van Ravenswaay-Claasen HH, Kluin PM, Fleuren GJ: Tumor infiltrating cells in human cancer. On the possible role of CD16+ macrophages in antitumor cytotoxicity. *Lab Invest* 67:166–174, 1992

Walz A, Peverni P, Aschauer H, Bagglioni M: Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. *Biochem Biophys Res Commun* 149:755–761, 1987

Wang JM, Taraboretti G, Matsushima K, Van Damme J, Mantovani A: IL-8 induces haptotaxis and chemotaxis of melanoma cells. *Biochem Biophys Res Commun* 169:165–169, 1990

Ward SG, Westwick J: Chemokines: understanding their role in T-lymphocyte biology. *Biochem* 333:457–470, 1998

Wu GD, Lai EJ, Huang N, Wen X: Oct-1 and CCAAT/enhancer-binding protein (C/EBP) bind to overlapping elements within the interleukin-8 promoter: the role of Oct-1 as a transcriptional repressor. *J Biol Chem* 272:2396–2403, 1997

Yang J, Richmond A: Constitutive Ikb kinase activity correlates with nuclear factor-kB activation in human melanoma cells. *Cancer Res* 61:4901–4909, 2001

Yang T-Y, Chen S-C, Leach MW, *et al*: Transgenic expression of the chemokine receptor encoded by human herpesvirus 8 induces an angioproliferative disease resembling Kaposi's sarcoma. *J Exp Med* 191:445–454, 2000

Yang W, Wang D, Richmond A: Role of clathrin-mediated endocytosis in CXCR2 sequestration, resensitization and signal transduction. *J Biol Chem* 274:11328–11333, 1999

Yla-Herttuala S, Lipton B, Rosenfeld M, *et al*: Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. *Proc Natl Acad Sci USA* 88:5252–5256, 1991

Yoshimura T, Matsushima K, Tanaka S, Robinson EA, Appella E, Oppenheim JJ, Leonard EJ: Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. *Proc Natl Acad Sci USA* 84:9233–9237, 1987

Yoshimura T, Yuhki N, Moore SK, Appella E, Lerman MI: Human monocyte chemoattractant protein-1. Full length DNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE. *FEBS Lett* 244:487–493, 1989

Zella D, Barabitskaja O, Burns JM, *et al*: Interferon-gamma increases expression of chemokine receptors CCR1, CCR3 and CCR5, but no CXCR4 in monocytoid U937 cells. *Blood* 91:444–450, 1991

Zhang H, Shepherd AT, Eason DD, *et al*: Retinoblastoma protein expression leads to reduced Oct-1 DNA binding activity and enhances interleukin-8 expression. *Cell Growth Differ* 10:457–465, 1999

Zingoni A, Soto H, Hedrick JA, *et al*: The chemokine receptor CCR8 is preferentially expressed in Th2 but not in Th1 cells. *J Immunol* 161:547–551, 1998

Zlotnik A, Yoshie O: Chemokines: a new classification system and their role in immunity. *Immunity* 12:121–127, 2000