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The countercurrent principle in invasion and metastasis of cancer cells. Recent insights on the roles of chemokines

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ABSTRACT Chemokine production by cancer cells constitutes a duality. Leukocyte recruitment under the pressure of chemokines may be beneficial for the host or for the tumor. Here, the emphasis will be on the detrimental effects of chemokines in tumor biology. A decade ago, the countercurrent principle of tumor-derived chemokine and peritumoral protease production was formulated to explain chemokine expression as a selective advantage for specific tumors and as a phenotype of invasive and metastasizing cancer cells. Chemoattracted leukocytes may provide trophic factors and produce invasion and metastasis-promoting proteinases. On the basis of the consensus sequence glutamic acid-leucine-arginine (ELR) preceding the canonical cysteine-any amino acid-cysteine (CXC), ELR-positive CXC chemokines, such as interleukin-8 and granulocyte chemotactic protein-2, are angiogenic and thus instruct the host to feed the tumor and bring the vessels into closer contact with the tumor cells. These mechanisms may enhance lymphogenic and hematogenic metastasis. Recent research and proofs of this countercurrent concept are here reviewed and compared. In addition, we discuss how alterations in chemokine ligand and receptor expression profiles may contribute to tumor growth, invasion, metastasis and immune evasion. These comparisons imply practical consequences for future cancer diagnosis and therapy. The implications include methods to diminish metastasis by inhibiting angiogenic CXC chemokine ligands and receptors, therapeutic combinations of chemokine overexpression with antigenic stimuli and co-treatment with angiostatic chemokines and tumor antigens.

KEY WORDS: *chemokine, gene transfer, chemokine receptor*

Introduction

The understanding of the underlying biochemical events is gradually growing in the case of several classical observations in tumor biology. This statement certainly is applicable for the observations of paraneoplastic effects, tumor-associated macrophages and the countercurrent model of invasion and metastasis. It is well established that many tumors, in comparison with normal cells, overproduce specific proteins by coincidence. At the molecular level, this phenomenon is explained by accumulation of (random) genetic changes that deregulate gene promoter activities and lead to overproduction of specific mRNAs and proteins. If such proteins possess pharmacological activities, the clinical cancer phenotype is extended by *paraneoplastic effects*. The production of peptide hormones by oat cell tumors of the lungs forms a classical example of this situation. Chemokine expression may also lead to paraneoplastic effects. Disseminated pustulosis by tumoral expression of interleukin-8 forms an example (Poszepczynska *et al.*, 2001). Expression of macrophage inflammatory protein-1α (MIP-1α/CCL3) by myeloma cells can have bone destruction as a pharmacological effect, which may be mediated by activation of osteoclasts (Choi *et al.*, 2001).

At the microscopic level, the infiltration of leukocytes into tumors is also a classical observation. Although the underlying mechanism may be random and as such the phenomenon may be classified as paraneoplastic, in specific settings, selection of tumor cell clones during cancer progression may occur. Clones with such selective advantage may persist longer in the host. For instance, a tumor cell clone which produces an autocrine growth factor may become independent from serum platelet-derived growth factor. Similarly, clones that produce net proteolytic activity with the capacity to degrade extracellular matrix components may leave efficiently the primary tumor site and invade the surrounding tissues or cross the basement membrane of endothelia and metastasize to distant organs. Invasion and metastasis

Abbreviations used in this paper: CXC, cysteine-any amino acid-cysteine; ELR, glutamic acid-leucine-arginine.

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of such clones will more readily occur than of clones which overproduce net inhibitory activity against proteases. Cancer cell phenotypes, such as uncontrolled growth and invasive behavior have been extensively reviewed (Coussens and Werb, 2002; Mareel and Leroy, 2003).

Tumor-associated leukocytes were originally conceived as a beneficial host response to tumors. This is certainly the case for immunogenic tumors or when additional immune signaling or insults occur. For instance, a tumor may become necrotic or infected. Furthermore, many cancer treatments, in particular chemotherapy, radiotherapy and immunotherapies induce necrosis and inflammatory signals. In other words, many circumstances exist in which chemokines may be induced in the tumor surroundings and this may account for tumor-associated leukocytes and, indeed, these recruited cells may be detrimental for the tumor and beneficial for the host. A number of experimental conceptual proofs will be given from recent literature (*vide infra*). In addition, we will review here the current knowledge on another key question. What happens or what is the effect of autonomous chemokine expression by the tumor when no immunological danger signaling occurs? Obviously, leukocytes will be recruited to the tumor and maybe the tumor will strangle itself by the workings of the immune system. In principle, if it were so simple, by such negative selection one would not observe so often tumor-associated leukocytes in diagnostic tumor biopsies. While we were studying chemokine production by cancer cells, we observed that most invasive cancers produced the broadest spectrum and the highest levels of chemokines. For instance, we purified from the human osteosarcoma cell line MG-63 (Fig. 1) the angiogenic and granulocyte chemotactic proteins interleukin-8 (IL-8/CXCL8)(Van Damme *et al.*, 1988), GROα/CXCL1, granulocyte chemotactic protein-2 (GCP-2/CXCL6) (Proost *et al.*, 1993), the angiostatic CXC chemokine IP-10/CXCL10 (Proost *et al.,* 1993) and monocyte chemotactic protein -1 (MCP-1/CCL2), MCP-2/CCL8 and MCP-3/CCL7 (Van Damme *et al.*, 1992).

The early observations that cancer cells produce chemokines, the *countercurrent principle of invasion* (Opdenakker and Van Damme, 1992), the gene structures and biological functions of chemokines and the consequences for cancer biology were reviewed in 1999 (Opdenakker and Van Damme, 1999) and this theme was discussed in other review articles (Balkwill and Mantovani, 2001; Mareel and Leroy, 2003). Also, the role of proteinases from leukocytes in the invasive process was highlighted in the original countercurrent concept and has meanwhile been documented *in vivo* (Coussens *et al.*, 2000; Van Coillie *et al.*, 2001). The roles played by gelatinase B/matrix metalloproteinase-9 in pathology were extensively addressed in a recent extensive review (Van den Steen *et al.*, 2002). We originally coined the name "countercurrent model" for the involvement of chemokines in cancer, because the fluxes of tumor and host cells are opposite: chemoattracted leukocytes and growing vessels (angiogenesis) are towards the tumor, while the invasion of cancer cells is away from the primary tumor site and is facilitated by chemokine-induced proteolysis. By now, almost every type of tumor has been shown to produce chemokines or to be responsive to one or another chemokine. A literature search in the PubMed data library (www.ncbi.nlm.nih.gov) lists about 2000 entries on "chemokines and cancer" and the majority of manuscripts deals with the expression of chemokine ligands or receptors (Tables 1 and 2) in various types of tumors. This observation not only directly explains the so frequent occurrence of tumor-associated leukocytes, but it also suggests that this phenomenon is more than just random coincidence. Therefore, we will focus below on the functional aspects how chemokines may assist tumors and what may be the practical consequences of the countercurrent principle for the oncologist.

How may chemokines help tumors?

Chemokines may be direct autocrine growth factors for cancer cells

Particular chemokines have been found to stimulate in an autocrine way the growth of cancer cells. This implies that the tumor cells carry chemokine receptors that transduce a mitogenic signal. In this way IL-8 has been found to be mitogenic for Kaposi sarcoma cells (Masood *et al.*, 2001), ovarian cancer (Xu and Fidler, 2000), colon carcinoma (Brew *et al.*, 2000) and malignant mesothelioma (Galffy *et al.*, 1999). Alternatively, the chemokine Groα/CXCL1 was originally discovered as a growth factor for melanoma cells (Richmond *et al.*, 1988; Haghnegahdar *et al.*, 2000).

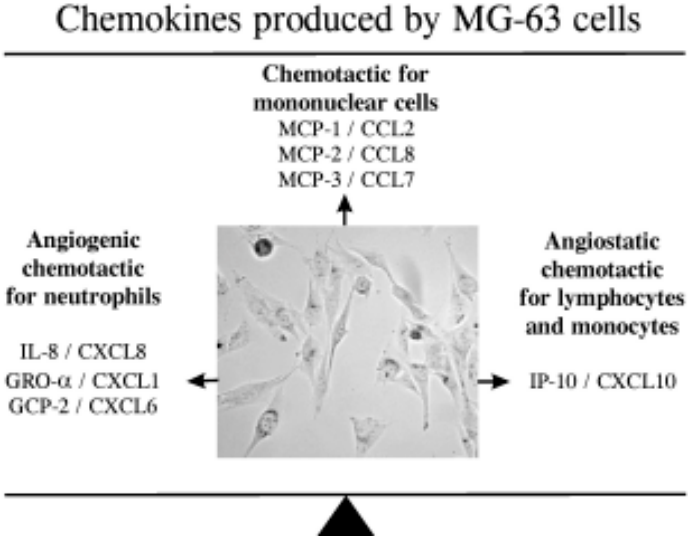

Fig. 1. Invasive tumor cells produce simultaneously various chemokines. *Around 1970, invasive primary tumors were systematically cultured to establish new tumor cell lines at the Rega Institute for Medical Research. The corresponding cell lines were named "MG" because "menselijk gezwel" means human tumor. One of these, MG-63 (Billiau* et al.*, 1977) is pictured here and has been widely used for cytokine (fibroblast interferon, interleukin-6) and chemokine production. A number of chemokines that were produced and characterized from MG-63 cells are here classified into the CXC chemokines and the mononuclear cell attractants of the CC family. In terms of angiogenesis a counterbalance exists between the angiogenic and angiostatic CXC chemokines, whereas the effect of CCLs is less documented. This historical example illustrates that tumor cells may produce simultaneously a variety of chemokines and that the biological effects in vivo depend on functional balances between e.g. angiogenesis and angiostasis and of the numbers, types and activation pathways of the chemokine-recruited leukocytes. For chemokine abbreviations see Tables 1 and 2. Photograph and montage by the courtesies of Chris Dillen and Pierre Fiten.*

SDF-1/CXCL12 has been found to stimulate glioblastoma cell proliferation (Barbero *et al.*, 2003). I-309/CCL1 possesses anti-apoptotic effects and thus sustains the growth of T cell lymphomas (Van Snick *et al.*, 1996) and adult T cell leukemia cells (Ruckles *et al.*, 2001). Finally, IL-8/CXCL8 has been found to inhibit TNF-related apoptosis in ovarium carcinoma (Abdollahi *et al.*, 2003).

Chemokines provide paracrine growth advantages via angiogenesis

Angiogenesis has been well studied in the biology of organ development, tissue repair and cancer growth and invasion. Vessel growth, relevant for cancer, varies widely (e.g blood and lymph vessels) and is governed by many factors that stimulate (e.g. angiogenesis by vascular endothelial growth factors) or dampen (e.g. angiostatin) proliferation of the various types of endothelial cells (Folkman, 1995). Chemokines belong to these factors and their introduction in the angiogenesis field coincided with the early studies of platelet factor-4/CXCL4, a heparin binding molecule with the CXC signature that possesses strong anti-angiogenetic activity (Maione *et al.*, 1990). Shortly thereafter, IL-8/CXCL8 was discovered as an angiogenic chemokine (Koch *et al.*, 1992; Strieter *et al.*, 1992). The critical difference between the angiogenic and angiostatic CXC chemokines is dependent on a tripeptide ELR motif in front of the CXC (Strieter *et al.*, 1995). According to this rule, the CXC chemokines (without ELR), such as PF-4/CXCL4 (Maione *et al.*, 1990), IP-10/CXCL10 (Arenberg *et al.*, 1996) and Mig/CXCL9 are angiostatic, whereas the ELR$^+$CXC chemokines are angiogenic. Prototypes for the latter subgroup are IL-8/CXCL8 in humans and GCP-2 in the mouse (Strieter *et al.*, 1995b; Belperio *et al.*, 2000). Although it is known that angiostatic chemokines bind to CXCR3 (Proost *et al.*, 2001; Romagnani *et al.*, 2001), it remains not fully understood how these inhibit the angiogenic activity of ELR$^+$ CXC chemokines.

TABLE 1

HUMAN CC CHEMOKINES:
CHROMOSOMAL LOCALIZATION AND RECEPTOR USAGE

Systemic Name	Synonym [a]	Chromosome	Receptor(s)[b]
CCL1	I-309	17q11.2	CCR8
CCL2	MCP-1/MCAF	17q11.2	CCR2
CCL3	MIP-1α/LD78α	17q11.2	CCR1,CCR5
CCL3L1	MIP-1α/DL78β	17q11.2	CCR1, CCR3, CCR5
CCL4	MIP-1β	17q11.2	CCR1, CCR2, CCR5
CCL5	RANTES	17q11.2	CCR1, CCR3, CCR5
CCL7	MCP-3	17q11.2	CCR1, CCR2, CCR3, CCR5
CCL8	MCP-2	17q11.2	CCR1, CCR2, CCR3, CCR5
CCL11	Eotaxin	17q11.2	CCR2, CCR3
CCL13	MCP-4	17q11.2	CCR1, CCR2, CCR3
CCL14	HCC-1	17q11.2	CCR1, CCR3, CCR5
CCL15	HCC-2/Lkn-1/MIP-1δ	17q11.2	CCR1, CCR3
CCL16	HCC-4/LEC	17q11.2	CCR1
CCL17	TARC	16q13	CCR4
CCL18	DC-CK1/PARC/AMAC-1	17q11.2	Unknown
CCL19	MIP-3β/ELC/exodus-3	9p13	CCR7
CCL20	MIP-3α/LARC/exodus-1	2q33-q37	CCR6
CCL21	6Ckine/SLC/exodus-2	9p13	CCR7
CCL22	MDC/STCP-1	16q13	CCR4
CCL23	MPIF-1/MIP-3	17q11.2	CCR1
CCL24	MPIF-2/eotaxin-2	7q11.23	CCR3
CCL25	TECK	19p13.2	CCR9
CCL26	Eotaxin-3	7q11.23	CCR3, CCR10
CCL27	CTACK/Eskine	9p13	CCR10
CCL28	MEC		CCR10

[a] The CCL nomenclature is restricted for human ligands.
[b] receptor binding by intact or posttranslationally modified chemokine

Chemokines enhance invasion

Invasion defined as the process of cell movement through basement membrane barriers and the dense network of extracellular matrix molecules is in fact a physiological function that leukocytes constantly execute in inflammation. The direction of leukocyte migration is towards increasing chemokine concentrations. Some molecular mechanisms to perform chemotaxis functions rely on the production and function of cytoskeletal motor proteins, secreted proteinases and glycanases and the expression of adhesion molecules, including mucins, selectins, integrins and immunoglobulin family receptors. Chemokines, while attracting leukocytes, often activate the production of such enzymes and cell surface markers. Whereas chemokine actions have been mainly studied in immune responses, these activities have also been observed in specific tumor models. For instance, prostate tumor cells, responding to IL-8/CXCL8 through CXCR2 are more invasive *in vitro* (Reiland *et al.*, 1999) and chemokines induce migrational responses (chemotaxis and cytoskeletal changes) in various human breast carcinoma cell lines (Youngs *et al.*, 1997). *In vivo*, the invasive phenotype may be assisted by tumor-derived chemokines. Indeed, expression of IL -8/CXCL8 mRNA in ovarium carcinoma is correlated with histological grading (Davidson *et al.*, 2002). Chemoattracted leukocytes may enhance the local proteinase load and thus assist in the invasive process. Thus, overexpression of mouse granulocyte chemotactic protein-2 (which is one of the most potent neutrophil attractants in the mouse) in human melanoma cells, assisted tumor growth by angiogenesis and induced *in vivo* gelatinase B/MMP-9 as a matrix enzyme (Van Coillie *et al.*, 2001). In many models, xenografting with chemokine-expressing cancer cells has been used to show chemokine action (i.e. tumor infiltration of leukocytes and proteinase induction) (Melani *et al.*, 1995; see also Tables 3 and 4). It needs to be noticed that in most of these settings a strong immune response is elicited and thus the tumor may eventually grow, but often is rejected by the workings of the hosts immune response (*vide infra*).

TABLE 2

HUMAN CXC CHEMOKINES:
CHROMOSOMAL LOCATION AND RECEPTOR RECOGNITION

Systemic Name	Synonym	Chromosome	Receptor(s)
CXCL1	GROα/MGSA-α	4q12-q13	CXCR2
CXCL2	GROβ/MGSA-β	4q12-q13	CXCR2
CXCL3	GROγ/MGSA-γ	4q12-q13	CXCR2
CXCL4	PF4	4q12-q13	CXCR3B
CXCL5	ENA-78	4q12-q13	CXCR2
CXCL6	GCP-2	4q12-q13	CXCR1, CXCR2
CXCL7	NAP-2	4q12-q13	CXCR2
CXCL8	IL-8	4q12-q13	CXCR1, CXCR2
CXCL9	Mig	4q21.21	CXCR3
CXCL10	IP-10	4q21.21	CXCR3
CXCL11	I-TAC	4q21.21	CXCR3
CXCL12	SDF-1α/β	10q11.1	CXCR4
CXCL13	BLC/BCA-1	4q21	CXCR5
CXCL14	BRAK/bolekine	Unknown	Unknown

Chemokines enhance metastasis by vessel entry

After local growth and crossing tissue barriers, the tumor cells may enter blood or lymph vessels. This forms the first phase of tumor cell entry during the process of metastasis. In many *ex vivo* studies, the expression of chemokines has been associated with higher clinical tumor staging (Table 3). Along this line, studies are available on IL-8/CXCL8 in cutaneous melanoma (Nurnberg *et al.*, 1999), malignant melanoma (Singh *et al.*, 1999), colorectal carcinoma (Haraguchi *et al.*, 2002), ovarian carcinoma (Davidson *et al.*, 2002), non-Hodgkin lymphoma (Retzlaff *et al.*, 2002), breast cancinoma (Bendre *et al.*, 2002) and lung cancer (Chen *et al.*, 2003). RANTES/CCL5 was studied in non small cell lung cancer (Moran *et al.*, 2002), whereas MCP-1/CCL2 expression was correlated with glioblastoma (Huang *et al.*, 2002). Whether the process of vessel entry is determined by enzyme induction, by the process of angiogenesis, by both or by still other mechanisms must await further experimentation, e.g. by using specific knockout models or by specifically blocking proteinases or chemokines with monoclonal antibodies.

Chemokines determine the location of secondary tumors

Once tumor cells have entered the blood or lymph vessels they circulate passively. Studies on the roles played by chemokines in metastasis have been skewed more towards chemokine receptor expression than to chemokine ligand production. One reason for this may be the early finding that CXCR4-positive breast cancer cells are responsive to SDF-1 (Muller *et al.*, 2001). At the time of this discovery, SDF-1 was well known for its homing effect on immature (CD34-positive) progenitor cells in the process of bone marrow repopulation (Aiutti *et al.*, 1997). Meanwhile, VEGF was shown to upregulate CXCR4 in breast carcinoma, possibly in hypoxic zones, making these cells responsive to SDF-1 (Bachelder *et al.*, 2002). SDF-1 also enhances motility and adhesion of lung cancer cells expressing CXCR4 (Kijima *et al.*, 2002) and breast cancer cells (Helbig *et al.*, 2003). Furthermore, the outgrowth of micrometastases in colon carcinoma cells was found to be enhanced by SDF-1 (Zeelenberg *et al.*, 2003).

The expression of chemokine receptors, easily experimentally accessible with the use of fluorescent-activated cell sorting-(FACS)-analysis and immunohistopathology, may thus determine the type of tumor that metastasizes, whereas the site of dissemination is dictated by expression of the chemokine ligand. For this process to occur, not only chemokines but also adhesion events need to take place. For instance, it has been found that mouse B16 melanomas, transfected with CXCR4, become more metastatic to the lungs (Murakami *et al.*, 2002) via interaction with endothelial beta1 integrins (Cardones *et al.*, 2003). Another place of SDF-1 expression is the lymph node. This may explain why CXCR4-expressing tumors metastasize to lymph nodes (Kato *et al.*, 2003). SDF-1 expression in the eye has been invoked for cancer spreading to this organ (Chan *et al.*, 2003). Along a similar line, LARC/CCL20 is constitutively expressed in the liver and this ligand interacts with CCR6. The latter receptor is commonly overexpressed in colon, thyroid and ovarian carcinoma. These observations may explain the commonly observed metastases of these tumors to the liver (Dellacassagrande *et al.*, 2003). In the future, it will become clearer whether chemokine ligand and receptor expression determine the metastasis target organ. Such studies can now be done by gene profiling of primary tumors and metastases and by investigating organ specific chemokine ligand and receptor expression profiles (Ohshima *et al.*, 2003).

Chemokine expression may induce immune evasion for specific cancers

Within the concept that chemo-attracted leukocytes may damage the tumor by increasing the immunogenicity through the action of antigen-presenting cells or by stimulating the adaptive immune response through T-helper or cytotoxic T cells and NK cells, the reduction of chemokine action may enhance immune evasion. This may be caused by diminished chemokine ligand or receptor production or activity. The chemotactic response may be decreased in specific tumor settings. For instance, in patients with primary and metastatic melanoma, circulating monocytes have been found to be less responsive to MCP-1/CCL2 in chemotactic migration than those of controls. Possibly, this is due to deactivation or modulation of the MCP-1-receptor expression on these cells (Muller *et al.*, 1997). In another study on the etiology of cervical carcinoma caused by human papilloma virus oncogenes E6 and E7, it was observed that these oncogenes, individually or acting together, suppressed the production of MCP-1/CCL2 in primary epithelial cells from the female genital tract. Other chemokines, such as IP-10/CXCL10, IL-8/CXCL8 and RANTES/CCL5, were less affected. Furthermore, 4 of 6 cervical carcinoma cell lines that scored positive for human papilloma virus transformation, did not express MCP-1/CCL2. Suppression of MCP-1/CCL2 expression

TABLE 3

CHEMOKINE EXPRESSION *EX VIVO* IN HUMANS

Chemokine	Tumor	Association	Reference
RANTES/CCL5	Non small cell lung cancer	predictor of survival in stage I NCCLC	Moran *et al.,* 2002
IL-8/CXCL8	T cell lymphoma	association with disseminated pustulosis	Poszepczynska *et al.,* 2001
IL-8/CXCL8	Primary cutaneous melanoma	correlation with worse prognosis	Nurnberg *et al.,* 1999
IL-8/CXCL8	Malignant melanoma	correlation with metastatic phenotype	Singh *et al.,* 1999
IL-8/CXCL8	Colorectal carcinoma	expression in tumor and serum correlates with liver metastasis, microvessel density	Haraguchi *et al.,* 2002
MCP-1/CCL2	Glioblastoma	MCP-1 as autocrine growth factor	Huang *et al.,* 2002
IL-8/CXCL8	Ovarian carcinoma	IL-8 mRNA expression in effusions associated with higher tumor grade	Davidson *et al.,* 2002
IL-8/CXCL8	Primary gastrointestinal non Hodgkin lymphoma	higher pretreatment IL-8 serum levels associated with higher stage	Retzlaff *et al.*, 2002
IL-8/CXCL8	Non small cell lung cancer	higher vessel density, higher metastatic potential *in vitro*	Chen *et al.,* 2003
IL-8/CXCL8	Gastric carcinoma	association with vascularity	Kitadai *et al.*, 1998
IL-8/CXCL8	Malignant melanoma	association with aggressiveness	Kunz *et al.*, 1999

seems to coincide with the program of E6/E7-induced transformation of primary epithelial cells (Kleine-Lowinski *et al.*, 2003).

Therapeutic application of chemokines in cancer

Factors influencing the effect of intratumoral overexpression of chemokines on antitumoral activity

Five years ago, it was already clear that the effect of chemokine expression in a tumor was different for immunogenic tumors *versus* nonimmunogenic tumors. In fact, we questioned whether chemokines are tumor suppressors, since in all the available examples of chemokine gene transfer that demonstrated tumor suppression, secondary immunostimulating signals were provided, e.g. by activation with LPS or by using immunogenic tumors (Opdenakker and Van Damme, 1999; Paul *et al.*, 2002). This statement has only been reinforced by more recent data (Table 4). For instance, whereas IP-10/CXCL10 showed little effect as antitumoral agent by adenoviral expression, in synergy with IL-12, the effects were profound in a murine model of colorectal adenocarcinoma (Narvaiza *et al.*, 2000).

A number of influencing factors need to be stressed here. In many experimental models strong *immunogenic signals* are provided along with the chemokine signals. In particular, the use of xenografted tumor cells and of viral gene transfer vectors (that confer expression of "non self" viral proteins) provide such signals (Table 4). Immunogenic stimuli are absent in most human tumors. This implies that the positive effects of chemokine gene transfection in experimental animal models (including immunogenic signals) may not necessarily be observed in humans.

In addition, the *level of chemokine expression* determines the outcome. For example low levels of adenoviral expression of MCP-1/CCL2 in xenogeneic tumors lead to tumor growth, whereas high expression leads to massive infiltration and tumor rejection (Nesbit *et al.*, 2001). This gene-dose effect seems to be difficult to control in human schemes for gene therapy. Also*, the balance of tumoral chemokine production* and chemokine production by the surrounding tissue will determine whether a tumor cell (having the corresponding chemokine receptor) will migrate to form secondary tumor sites (Menten *et al.*, 2002).

Therefore, at present one may better advocate to study first the outcome of chemokine gene transfection in syngeneic tumor host models and with nonimmunogenic transfer systems (e.g. by not using viral vector systems). As an alternative, the effect of chemokine function in experimental tumor models can be analysed with the use of inhibitory monoclonal antibodies against chemokine ligands or receptors or by other types of interference with chemokine mRNA or protein. In the situation that the chemokine action is blocked by immune evasion mechanisms (rather than the chemokine expression is enhanced) in the tumor, also secondary immunogenic signals, dose effects and the contributions of the surrounding tissues have to be taken into account. Information in the recent literature is in agreement with this thesis and provides a number of examples of this phenomenon (Table 5). The observation that, in a number of examples, the strategies of chemokine engineering or interference work *in vivo*, gives hope that biotechnological development is possible. However, the fact that so many different chemokines exist makes the choice more difficult.

TABLE 4

ANTITUMORAL EFFECTS BY FORCED CHEMOKINE EXPRESSION IN TUMORS *IN VIVO*

Chemokine DNA	Vector or antibody	Tumor	Effect on tumor	Immune mediator/response	Reference
Human MDC/CCL22	Adenovirus	murine syngeneic tumors	growth ↓	$CD8^+$ cytolytic effectMHC I	Lee *et al.*, 2003
Mouse fractalkine	pCDNA3.1/Myc-HisA tag	murine syngeneic Lewis lung	growth ↓	$CD8^+$ cytolytic effect $CD4^+$ helper effect	Guo *et al.*, 2003
Mouse MCP-3	pCMV MCP-3	mouse colon rectal cancer cells (CMT93) in C57Bl6 mice	growth ↓ metastasis ↓	increased tumoral leukocytes	Hu *et al.*, 2002
Human MCP-3/CCL7	parvovirus vector	human cervical carcinoma in mouse xenograft	growth ↓	activated macrophages and dendritic cells	Wetsel *et al.*, 2001
Human MCP-1/CCL2	adenovirus	human melanoma xenograft	growth ↓ (high dose)	massive monocyte infiltration at high dosis	Nesbit *et al.*, 2001
Human MCP-1/CCL2	bovine papillomavirus	human malignant glioma xenograft	no effect	massive numbers of monocytes and natural killer cells	Nagai *et al.*, 2001
Mouse SLC Mouse ELC Mouse SDF-1α	PCXN2 pThioHisA	fibrosarcoma or ovarian carcinoma (immunogenic)	growth ↓	T lymphocytes and adjuvant effect of cytokines	Nomura *et al.*, 2001
Human Mig/CXCL9	adenovirus	human non small cell lung carcinoma	growth ↓ metastasis ↓	angiogenesis ↓	Addison *et al.*, 2000
IP-10	neutralizing antibody	neuroblastoma syngeneic	growth ↓	$CD8^+$ induction and vaccination effect	Pertl *et al.*, 2001
LEC/HCC-4/CCL16	vector transfection	mouse adenocarcinoma syngeneic in Balb c mice	growth ↓	$CD8^+$ and neutrophils	Giovarelli *et al.*, 2000
IP-10 in synergy with IL-12	adenovirus vector no effect of IP-10 alone	syngeneic CT26 mouse colorectal adenoca rumonia	growth ↓	$CD4^+$ cells increased $CD8^+$ cells increased	Narvaiza *et al.*, 2000
Mouse CTACK	adenovirus	syngeneic mouse ovarium carcinoma	growth ↓	$CD4^+$and $CD8^+$ NK cells $CD3^+$ lymphocytes	Gao *et al.*, 2003
Mouse SLC	plasmid vector	syngeneic colon carcinoma	growth ↓	$CD8^+$ cells	Vicari *et al.*, 2000

TABLE 5

INHIBITION OF CHEMOKINES *IN VIVO* HAS PROTECTIVE EFFECT AGAINST CANCER

Inhibition	Tumor	System	Effect	Reference
Antisense human IL-8 via adenovirus vector	Transitional cell carcinoma of bladder	Athymic nude mice xenograft	↓ growth ↓ angiogenesis	Inoue *et al.*, 2001
Sense and antisense vectors	Human pancreas carcinoma	Balb c nude mice xenograft	↓ growth ↓ metastasis	Shi *et al.*, 1999
Inhibitors of CXCR4	Human ALL	*in vitro*	↓ growth ↓ migration	Juarez *et al.*, 2003

Pharmacological interference on the basis of the countercurrent model

Several experimental procedures have been worked out and illustrate that the knowledge of the countercurrent principle can be successfully used for cancer therapy. Most of the chemokine literature on invasion and metastasis promotion is about IL-8/CXCL8 as an example, and some *in vivo* successes were obtained by inhibition of chemotaxis or angiogenesis mediated by IL-8/CXCL8. Enprostil is a prostaglandin E2 analogue that blocks IL-8/CXCL8 production (Toshina *et al.*, 2000). Fujisawa and colleagues produced a hexapeptide inhibitor of Gro-α/CXCL4 and IL-8/CXCL8, called antileukinate (Fujisawa *et al.*, 1999 and 2000). Monoclonal antibodies against IL-8/CXCL8 and antileukinate inhibited tumor cell growth and pulmonary metastasis *in vivo*. Monoclonal antibody against IL-8/CXCL8 was also efficient in inhibiting the progression of malignant pleural mesothelioma in nude mice (Galffy *et al.*, 1999). Rebamipide, an antiulcer agent, reduces the inflammatory potential of *Helicobacter pylori* by reducing IL-8/CXCL8 production by gastric cancer cells (Masamune *et al.*, 2001). The fusion of a chemokine with a tumor antigen induced potent antitumoral response for IP-10/CXCL10 and MCP-3/CCL7, presumably by recruitment and activation of antigen presenting cells, since T cell responses were detected (Biragyn *et al.*, 1999). The latter study constitutes another example that chemokine plus a second signal (antigen) will provoke an antitumoral response. Some antitumor agents induce antiangiogenic chemokines (e.g. IP-10/CXCL10) and may be useful for inducing tumor necrosis (Cao *et al.*, 2001). Another mechanism by which chemokine-producing cancer cells may have an advantage is by chemokine-receptor desensitization and reduced chemotaxis (Kurt *et al.*, 2001).

Conclusions

We here addressed the various possibilities how chemokine expression by cancer cell clones may assist these clones for growth, invasion and metastasis. This knowledge is superimposed on the original findings that tumor-associated leukocytes may dampen tumor growth and invasion. Further conclusions can be drawn by an analysis and comparison of the literature on *in vivo* overexpression of chemokines in tumor models. First, beneficial effects of tumor-specific chemokine overexpression predominate for the host, if the immune system is activated by a second signal. In other words, a therapeutic effect with reduced tumor growth, invasion and metastasis is observed if, in addition to the chemokine signal, the tumor itself or the used vector signal are immunogenic. The effect may be spectacular with xenografted human tumors in mouse systems. We caution that this is an artificial situation, since most human cancers, even when producing chemokines, do not necessarily provide this additional signal, because they often are poorly immunogenic. The use of immune stimulation may help the host to kill the tumor. Second, angiogenic chemokine expression by tumors needs to be blocked, whereas angiostatic chemokines are best enhanced. Various pharmacological ways to achieve these goals are presently being investigated. Finally, chemokine-induced proteases may be other targets for therapy. Since the countercurrent model is mainly documented with neutrophil chemokines (IL-8/CXCL8 in humans and GCP-2 in mice) and these chemokines induce the release of mainly neutrophil proteases (MMP-8, MMP-9 and neutrophil elastase), these enzymes may in the future become good targets for pharmacological inhibitors in invasive and metastatic cancers.

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