Prospects for Cytokine and Chemokine Biotherapy

Joost J. Oppenheim, William J. Murphy, Oleg Chertov, Volker Schirrmacher, and Ji Ming Wang
Laboratory of Molecular Immunoregulation, Division of Basic Sciences, National Cancer Institute [J. J. O.] and Intramural Research Support Program, Science Applications International Corporation-Frederick [W. J. M., O. C., J. M. W.], Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201, and German Cancer Institute, D69120 Heidelberg, Germany [V. S.]

Abstract

Cytokines with immunostimulating effects have the capacity to induce tumor immunity in animal models, whereas some cytokines interfere with tumor growth based on their angiostatic effects. Despite these capabilities, cytokines, such as IFN-α, IFN-γ, tumor necrosis factor, interleukin (IL)-1, and IL-2, have had limited clinical efficacy and many undesirable side effects. In preclinical models, cytokines can even promote tumor growth and increase metastatic spread. Although chemokines have had limited clinical evaluation, studies of animal models show that they can also have tumor-suppressive or tumor-enhancing effects. In mice, chemokines, such as IP-10, RANTES, and TCA3, have resulted in tumor regression and immunity to subsequent tumor challenge. Those chemokines that are angiostatic (e.g., PF4, IP-10, and MIG) can also induce tumor regression by reducing the tumor blood supply. Conversely, IL-8, which is angiogenic, can promote tumor growth. Our studies show that nasopharyngeal cell line cells (FADU) show a chemotactic as well as a proliferative response to MIP-1. In addition, a variant murine T cell lymphoma cell line Esb-MP, unlike the parental variant Esb, was selectively chemoattracted by murine MCP-1/JE. When injected s.c. into mice, the Esb-MP variant metastasized to the kidney with much higher frequency than the Esb variant. Both cultured kidneys from normal mice and a mesangial cell line constitutively produced chemoattractants that acted on Esb-MP but not Esb parental cells. Purification to homogeneity of these chemoattractants led to the identification of RANTES and JE. These results demonstrate that some chemokines may promote tumor growth and organ-specific metastatic spread of those tumors that have adapted and become responsive to chemokines. Finally, tumors appear to use numerous adaptative mechanisms to subvert and suppress the immune system. More effective therapy with cytokines and chemokines will require better characterization of the means by which tumors develop resistance to cytokines and overcome the immune system. Only then can we develop appropriate therapeutic approaches to antagonize cancer-induced immunosuppression.

Introduction

Although I (J. J. O.) did not realize it at the time, my 35-year career in research was launched, as well as shaped, by my initial introduction to clinical oncology research under the leadership of Drs. Emil Frei and Emil J Freireich at the National Cancer Institute (Bethesda, MD). This challenging and frustrating experience convinced me that more fundamental research was needed to learn how to harness the host’s own natural defense mechanisms to cope with cancer. It is to Dr. Freireich’s credit that Dr. Evan Hersh and I were not only permitted but encouraged to develop in vitro methods for studying human immune responses, which taught us that the immunological defense mechanisms of cancer patients were frequently defective (1). This led me to pursue studies aimed at characterizing the regulatory cytokines responsible for orchestrating inflammatory and immunological reactions. My initial experiences on the chemotherapy service under the guidance of Drs. Frei and Freireich motivated my subsequent clinical studies of IL-1 and our current evaluation of the potential role of chemokines in cancer. In this report, my collaborators and I briefly review the current status and future prospects for cytokines in the treatment of cancer patients and summarize our preclinical findings concerning the in vitro effects of chemokines on tumor cells, in vivo tumor growth, and metastatic spread.

Cytokine Cancer Therapy

There is abundant evidence that in patients with growing tumors, the levels of immunosuppressive cytokines, such as TGF-β and IL-6, increase at the expense of immunostimulating cytokines, such as IL-2, TNF-α, and IFN-γ (2). Direct attempts to correct this imbalance by treatment of cancer patients with recombinant cytokines, such as IFN-α and IL-2, only rarely have curative effects (3, 4). Cytokine therapy with IL-2 is complicated by the observation that a variety of tumors express receptors for IL-2 and upon ligand binding can be induced to proceed into the cell cycle, potentially resulting in tumor cell growth (5). Nevertheless, a small proportion of about 15% of the patients with melanoma or renal cell cancers in response to cytokine therapy do experience partial or complete responses.

The experience gained with IL-1 over a period of 5 years in


2 To whom requests for reprints should be addressed, at Laboratory of Molecular Immunoregulation, Building 560, Room 21-89A, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD 21702-1201. Phone: (301) 846-1551; Fax: (301) 846-7042.

3 The abbreviations used are: IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor; CSF, colony-stimulating factor; EC, endothelial cell; KCM, kidney conditioned medium; RP-HPLC, reverse phase high-performance liquid chromatography; PT, pertussis toxin.
the treatment of patients with a variety of solid tumors, including melanomas, renal cancers, and colon cancers, illustrates our experience with the limited rewards of cytokine biotherapy (6). The possibility that IL-1 has antitumor effects was based on the demonstrated in vitro cytostatic and cytotoxic effects of IL-1 on some tumor cells (7). IL-1 also was reported to augment a variety of cell-mediated immune responses in vivo that could contribute to tumor rejection. The best evidence for the antitumor effects of IL-1 was the demonstration that murine fibrosarcoma cells transfected to overexpress IL-1α, instead of exhibiting progressive growth, regress with the development of subsequent tumor-specific immunity to nontransfected fibrosarcoma cells (8). However, other experiments showed that i.v. administration of IL-1 to tumor-bearing mice can promote metastatic spread (9). This illustrates the potentially hazardous consequences of treatment with cytokines that have wide-ranging biological effects, including increased adhesiveness, mobility, and invasiveness of tumor cells. Unexpectedly, IL-1 treatment also induced the production of high levels of IL-1 receptor antagonists, which presumably down-regulates its desirable as well as undesirable effects (10).

As would be predicted from the radioprotective effect of IL-1 in lethally irradiated mice (11), IL-1 does have beneficial hematopoietic effects. IL-1 mobilizes immature and mature bone marrow cells and causes a peripheral neutrophilia (6, 12). IL-1 induces the production of numerous cytokines in patients, including IL-6, granulocyte CSF, and granulocyte-macrophage CSF, and this produces an increase in the bone marrow content of megakaryocytes with a consequent increase in the number of circulating platelets after a 1–2-week delay. These effects encouraged the evaluation of the utility of IL-1 as an adjunct to bone marrow-suppressive chemotherapy and led to the clinical finding that IL-1 administration following bone marrow suppressive carboplatin therapy reduced the degree of thrombocytopenia (13). The multiplicity of IL-1 activities also accounted for the many undesirable clinical side effects seen in cancer patients. Although IL-1 did not produce the vascular leak syndrome seen in patients treated with IL-2, vasodilatation and hypotension were the dose-limiting toxicities of IL-1 (6). In addition, IL-1 frequently induced a flu-like syndrome with fever, chills, rigors, headache, myalgias, gastrointestinal distress, and somnolence. Despite these problems, IL-1 did induce complete or partial remissions in about 10% of patients with nonvisceral melanoma (6).

Nevertheless, cytokine therapy has also had a number of more clear-cut successes. IFN-α therapy can maintain almost all patients with hairy cell leukemia in long-term remission based on its antiproliferative effects (14). Similarly, IFN-α can suppress chronic myelogenous leukemia in a considerable number of patients without engaging the immune response (15). Cytokines with specialized functions that have a limited number of target cells and fewer undesirable side effects are very useful therapeutics. For example, erythropoietin and granulocyte CSF have been administered with great success and restore the RBC and neutrophil counts of patients with iatrogenically suppressed bone marrow functions. IL-12 has been the most effective of all the cytokines in curing murine tumors (16). On the basis of its great promise in preclinical studies, IL-12, despite its broad spectrum of effects, is at present being evaluated for its antitumor activities in man. Cytokines, such as granulocyte-macrophage CSF and Flt3 ligand, which promote the development of antigen-presenting dendritic cells, have been potent inducers of antitumor immunity in animals and may prove to be of therapeutic benefit in man (17).

The Role of Chemokines in Tumor Biology

Chemokines consist of a family of M, 8,000–16,000 proteins that use homologous members of the seven transmembrane G-protein-coupled family of receptors (18). Chemokines induce directional migration and adhesion of various types of leukocytes. In addition, some of the chemokines also mobilize and activate other cell types, such as ECs, epithelial cells, and fibroblasts. In fact, the chemokine IL-8 has been shown to also induce the proliferation of ECs and to promote tissue vascularization (19). This angiogenic activity is seen with all C-X-C chemokines, including IL-8, that have a glutamic acid, leucine, and arginine sequence at the NH₂ terminus of the molecule. This has been reported to be the basis for the tumor growth-promoting effect of IL-8 (20). In contrast, PF4, IP-10, and MIG, three other C-X-C chemokines lacking the glutamic acid, leucine, and arginine sequence, have angiostatic effects and can suppress tumor growth (21). These observations show that some chemokines can induce tumor progression, whereas others contribute to tumor regression.

Transfection of genes encoding chemokines, such as IP-10, RANTES, and TCA3, into murine tumor cells promotes the development of tumor immunity in mice to tumors that overexpress these chemokines (22–24). Mice injected with tumors expressing these cytokine genes reject the tumors and develop specific resistance to subsequent challenge with nontransfected parental tumor cells. In contrast, transfection of tumor cells with murine MCP-1/JE results in the development of macrophage infiltrates and temporary tumor regression, but without the development of antitumor immunity (25). Thus, some of the C-C chemokines enhance immunogenic rejection of tumors, whereas others only induce nonspecific transient regression.

Because chemokines have been reported to have comitogenic effects on lymphocytes, ECs, and keratinocytes, we decided to evaluate whether the capacity of chemokines to promote cell proliferation might result in promoting the growth of some tumors. These possibilities were not unprecedented because IL-8, which can promote tumor growth, has been shown to chemotactract some melanoma tumor cells (26). We investigated chemokines for their effects on transformed human epithelial tumor cell lines. Pilot studies revealed that several human tumor cells of epithelial origin are able to migrate in response to MCP-1 across a filter precoated with the extracellular matrix proteins collagen type 4 or fibronectin. We chose to study a human nasopharyngeal squamous carcinoma cell line, FADU, in which chemokines enhance immunogenic rejection of tumors, whereas others only induce nonspecific transient regression.

Because chemokines have been reported to have comitogenic effects on lymphocytes, ECs, and keratinocytes, we decided to evaluate whether the capacity of chemokines to promote cell proliferation might result in promoting the growth of some tumors. These possibilities were not unprecedented because IL-8, which can promote tumor growth, has been shown to chemotactract some melanoma tumor cells (26). We investigated chemokines for their effects on transformed human epithelial tumor cell lines. Pilot studies revealed that several human tumor cells of epithelial origin are able to migrate in response to MCP-1 across a filter precoated with the extracellular matrix proteins collagen type 4 or fibronectin. We chose to study a human nasopharyngeal squamous carcinoma cell line, FADU, in which chemokines enhance immunogenic rejection of tumors, whereas others only induce nonspecific transient regression.
Table 1  Antibody inhibition of Esb-MP cell chemotactic activity*

<table>
<thead>
<tr>
<th></th>
<th>Migration to fraction treated with antibody</th>
<th>Migration to fraction treated with medium alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody</td>
<td>1</td>
<td>X 100%</td>
</tr>
<tr>
<td>Anti-JE</td>
<td>30%</td>
<td>80%</td>
</tr>
<tr>
<td>Anti-MIP1α</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Anti-RANTES</td>
<td>11%</td>
<td>0%</td>
</tr>
<tr>
<td>Anti-IL-8</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>MES supernatant</td>
<td>30,000</td>
<td>10,000</td>
</tr>
<tr>
<td>Fraction I</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Fraction II</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Fraction III</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

The human pharyngeal squamous cell carcinoma cell line FADU and the murine mesangial cell line were purchased from American Type Culture Collection (Rockville, MD). The chemokines MCP-1/JE, RANTES, and MIP-1α were purchased from PeproTech Inc. (Rocky Hill, NJ). Antisera to murine JE, RANTES, and MIP-1α were obtained from R&D Systems (Minneapolis, MN). PT was purchased from Sigma (St. Louis, MO).

Approximate molecular weight

<table>
<thead>
<tr>
<th>MES supernatant</th>
<th>Fraction I</th>
<th>Fraction II</th>
<th>Fraction III</th>
</tr>
</thead>
<tbody>
<tr>
<td>30,000</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>10,000</td>
<td>80%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>7,000</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

However, in contrast with leukocytes in which MCP-1-induced cell migration is abolished by PT, the migratory response of FADU cells to MCP-1 was not PT sensitive. Thus, MCP-1 does not use a Gi-protein-coupled receptor on FADU cells, but the nature of the receptor remains to be established.

To our surprise, MCP-1 also induced FADU cell proliferation as assessed by a 3-[3H]-thymidine incorporation assay. This effect of MCP-1 was also inhibited by anti-MCP-1 antibody. Furthermore, low levels of MCP-1 (10–50 pg/ml) were detectable by ELISA in FADU-conditioned media. Upon stimulation with TNF-α or IL-1, the secretion of MCP-1 by FADU cells increased to 5–10 ng/ml. This raises the possibility that under certain conditions, FADU cells may produce MCP-1 as an autocrine growth factor.

Because chemokines can promote leukocyte adhesion to ECs, their diapedesis, penetration of basement membranes, and migration to inflammatory sites, we wondered whether chemokines participate in promoting the tumor cell invasion and migration that are necessary for metastatic spread. We also were interested in determining the basis for the apparent tissue preference of metastatic tumor cells. Our collaborator, Dr. Volker Schirmacher, previously established a highly malignant variant (Esb) of the methylcholanganhene-induced murine T cell lymphoma (27). The Esb-MP cell line was a selected, plastic-adherent, more rapidly proliferating variant of Esb. When administered in vitro to normal mice by either s.c. or i.v. injection, both variants metastasized equally to the liver and lung. However, Esb-MP cells metastasized to the kidney with high frequency, whereas the parental Esb cells only rarely infiltrated the kidney. This preferential metastatic spread of Esb-MP cells to the kidneys raised the possibility of a specific interaction between Esb-MP cells and a kidney-derived chemoattractant(s).

We confirmed the previous observation that normal mouse kidneys incubated in serum-free media yielded KC, which consistently showed in vitro chemotactic activity for Esb-MP cells but not for the parental Esb variant cells (27). These observations suggested that a kidney-derived factor was selectively chemotactic for certain tumor cells. The correlation between migration in response to KCM in vitro and metastasis in vivo suggested that migration in response to a selective tissue-derived signal may direct the organ-specific colonization of these metastatic tumor cells.

We next proceeded to determine whether the tumor cells could be chemotactically directed to any of the known chemokines. Indeed, murine JE (MCP-1) has been implicated in the induction of in vitro migration of metastatic variant cells of a large cell lymphoma (28). Because several chemokines have been implicated in the induction of T lymphocyte migration, we examined the possibility that a known chemokine(s) might be responsible for the migration of Esb-MP T lymphoma cells. Binding studies showed that both metastatic (Esb-MP) and Esb tumor variants expressed high affinity binding sites for radiolabeled C-C chemokines, including radiolabeled murine MCP-1/JE. However, only metastatic Esb-MP cells were able to migrate in vitro in response to any of the C-C chemokines, including murine JE; these tumor cell variants presumably differ in their signal transduction capabilities. These results indicate that chemokines could potentially account for the chemotactic activity contained in the KCM supernatant.

We then proceeded to purify and identify the molecular nature of the kidney-derived tumor cell chemoattractant(s). To establish a relatively unlimited source of the KCM, we tested different cell types derived from various murine kidney-derived cell lines for their ability to produce tumor cell chemoattractant activity. One of the cell lines tested, a mesangial cell line (MES 13), was able to constitutively produce such activity in the supernatants. These mesangial cells could provide us with active supernatant in larger quantities, which greatly facilitated the
purification of the activity. This activity eluted from RP-HPLC in similar positions to chemoattractants present in the KCM, suggesting that the activities were identical. The chemotactic activity present in the MES 13 supernatant was resolved into three major fractions (I, II, and III) by RP-HPLC of the active fractions eluted from DEAE and CM ion-exchange chromatography. Further purification using microbore RP-HPLC and SDS-PAGE followed by immunoblotting revealed that fractions I, II, and III contained active proteins of $M_r$ 30,000, 10,000 and 7,000, respectively. The NH$_2$-terminal sequence of the $M_r$ 7,000 protein was identified as that of murine RANTES. The active $M_r$ 30,000 protein in fraction I appears to be murine JE because a rabbit anti-JE antiserum inhibited 85% of the chemotactic activity of this fraction. This was confirmed by NH$_2$-terminal sequencing of this fraction. However, the activity of fraction II (10 kDa) was not inhibited by anti-JE, anti-RANTES, or anti-MIP-1a (Table 1). Consequently, the activity in fraction II represents an as yet undefined chemoattractant. The majority of the chemotactic activity for Esb-MP cells present in KCM was also neutralized by antiserum to JE and RANTES.

Discussion

Although both of the murine Esb T cell lymphoma variants express high-affinity binding sites for C-C chemokines, only the Esb-MP variant cells migrated in response to these chemokines in vitro and metastasized to the kidney. The divergence in the responsiveness of these tumor cell variants merits further investigation to establish possible differences in their signal transduction events, receptor phosphorylation, expression of adhesion molecules, and/or pattern of gene activation. Overall, these studies suggest that some chemokines may promote the growth and spread of certain tumors to "favored" sites. Rather than considering all chemokines as potential antitumor therapeutics, the effects of these cytokines on tumors must be carefully characterized, and we must develop antagonists to those chemokines that prove to have tumor-promoting effects.

Tumor Subversion of Host Defenses. At present, gene therapy is being evaluated as a better means of delivering cytokines to tumor sites; alas, without any miraculous cures as yet (29). Furthermore, based on the premise that most tumor cells express immunogenic antigens, patients are being treated with a variety of tumor vaccines. Once again, the preliminary results of this approach are proving to be disappointing. The hypothesis that immunological host defenses can be simply marshaled to eliminate tumors has been difficult to substantiate and is again being seriously questioned. Of course, based on our experience in infectious diseases, the expectation that vaccines can eliminate preexisting well-established tumors may be expecting too much. After all, well-established tumors have had the opportunity to adapt to the immune system, and the large tumor burden produces a massive antigen overload.

The best evidence, however, that the immune system provides a serious threat to the development of tumors is supported by considerable evidence that tumors use numerous mechanisms to avoid, circumvent, obstruct or suppress immune defenses. For example:

(a) Tumors avoid detection by defects in antigen processing or failure to present MHC antigens that are necessary to present tumor antigens to initiate effective T cell-dependent antitumor responses (30).

(b) Tumors produce a variety of cytokines, such as IL-10, IL-4, and IL-13, that divert immune responses from effective cell-mediated antitumor reactions to more permissive humoral antibody responses that may even promote tumor growth (2).

(c) Tumors produce cytokines that down-regulate all types of immune responses, such as TGF-β (2).

(d) Alternatively, TGF-β functions as a potent inhibitor of epithelial cell growth by interacting with the human type II receptors for TGF-β to suppress the growth of colon cancer cells. However, colon cancers can become resistant to TGF-β based on mutational inactivation of the TGF-β receptor (31, 32).

(e) Tumors produce "factors" that decrease expression of adhesion proteins by the tumor vasculature and are associated with reduced leukocyte infiltration and greater tumor progression (33, 34).

(f) Tumors may produce autostimulating or use endogenous proinflammatory cytokines, such as IL-1, IL-2, and TNF, or chemokines, such as IL-8 and MCP-1, to promote their growth and metastatic spread.

(g) Tumors may induce the expression of CTLA-4, which serves to down-regulate T cell-mediated immune responses. This is based on observations that blockade by anti-CTLA-4 augments immunological rejection of tumors (35).

(h) Tumors may express FAS-ligand and can use it as a "rapier" to kill infiltrating T cells that express the Fas receptor (36).

In conclusion, tumors use multiple means to blunt and circumvent our best immunotherapeutic thrusts. These observations all point to the need to thwart the fundamental processes used by tumors to subvert and suppress the immune response. The anticipated beneficial effects of immunostimulants, such as treatment with cytokines and tumor vaccines, have been largely blocked by the ability of tumors to neutralize host defense mechanisms. Biotherapy with single modalities has therefore had limited success. We must learn to identify and counteract down-regulatory costimulants, such as CTLA-4 and FAS-ligand, to achieve effective immunotherapeutic measures. We must identify immunostimulants that overcome these mechanisms. We must consider using combinations or, if necessary, a battery of immunostimulants capable of overcoming the immunosuppressive signals emanating from well-established tumors.

Acknowledgments

We are grateful for the critical reading of the paper by Drs. Ruth Neta, Ken Wasserman, Robert Fenton, and Robert Wiltrout. We are also grateful for the invaluable editorial assistance of Cheryl Fogle.

References


Clinical Cancer Research

Prospects for cytokine and chemokine biotherapy.
J J Oppenheim, W J Murphy, O Chertox, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/3/12/2682

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.