

Chemokines in Tumor Immunotherapy

Kenneth Flanagan¹ and Howard L. Kaufman^{1,2}

Department of¹ Pathology and ² Surgery, Columbia University, New York, NY

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Structure and Function of Chemokines and Chemokine Receptors
 - 3.1. Chemokines affect the magnitude of the immune responses
 - 3.2. Local chemokine expression affects tumor growth
4. Inflammatory chemokines for the therapy of neoplasia
5. Lymphoid chemokines for the therapy of neoplasia
6. Conclusion
7. References

1. ABSTRACT

The chemokines are a family of small molecules that mediate cell migration, activation, differentiation, angiogenesis, and perhaps other functions. The chemokines have been classified by their amino acid composition, functional activity, and receptor binding properties. The chemokines receptors are 7 transmembrane G proteins and there is considerable redundancy in ligand specificity. The role of chemokines in cancer is not well understood, but there is accumulating evidence that they play a major role in both tumorigenesis and the host immune response to tumors. Thus, chemokines and their receptors represent potential therapeutic targets for drug development. This article will briefly review the current understanding of chemokines biology of defined chemokines that are thought to be involved in tumor growth, metastasis, and the host immune response against cancer.

2. INTRODUCTION

Initiation and amplification of antigen-specific responses to tumors, requires the coordinated migration of the diverse cells of the innate and acquired immune systems. A tumor-specific immune response occurs when antigen presenting cells (APCs), specifically dendritic cells (DCs), identify the presence of a tumor either because the tumor is considered dangerous, or expresses unique tissue or cell-specific antigens (1, 2). Upon infiltration of a tumor, DC will take up necrotic or apoptotic tumor cells, process them into antigenic peptides and migrate to regional lymph nodes. The antigen-loaded DC then encounter naïve T cells which have the potential to recognize MHC-presented tumor antigens through cognate T cell receptors (TCRs). In the context of appropriate costimulation, DC will activate T cells into effector populations followed by emigration from the lymph node

and migration to the tumor where presumably anti-tumor effector functions are fulfilled (3). The mediators of this migration, from the tumor to the lymph node and back again, are a family of molecules called chemokines (4). In addition to migration, there is accumulating evidence that chemokines are also responsible for numerous regulatory functions by immune cells. This review will focus on the role of chemokines in tumor progression, and will emphasize the important therapeutic application that manipulation of the chemokine system can have for tumor immunotherapy, with a particular focus on DC and T cells given their central role in tumor immunology.

3. STRUCTURE AND FUNCTION OF CHEMOKINES AND CHEMOKINE RECEPTORS

The chemokines are a family of small, structurally related, highly basic molecules, with a molecular weight between 8 and 12 kDa (5). Though there are more than 50 known human chemokines, only 20 chemokine receptors have been identified to date highlighting the considerable redundancy in the chemokine system (6); many chemokines bind to multiple receptors, and most receptors are capable of binding multiple chemokines. This redundancy suggests that the chemokine system is vital to a functioning immune system, and may allow the system to function in the event of disruption by either mutation or infection (7, 8). Furthermore, it allows for subtle control and fine tuning of cell migration and other processes.

The chemokine family is distinguished by a motif of cysteine residues near the N-terminus of the molecule. The position of the cysteines within the primary structure further differentiates the chemokines family into four groups. Members of the CC chemokine family have two cysteines directly adjacent to each other, while the two cysteines are interrupted by one other amino acid in the CXC family of chemokines. The C chemokine family contains just one cysteine residue, and the CX₃C family, has three intervening amino acids.

The chemokine receptors belong to a family of 7 transmembrane G protein coupled receptors; the peptide chain comprising the chemokine receptor has three loops within the cell, and three extracellular loops (9). Similar to other G protein coupled receptors, when the extracellular N-terminus of the receptor binds to a particular chemokine ligand, heterotrimeric G proteins within the cytosol bind to one of the intracellular loops of the receptor. This initiates a signal transduction pathway culminating in activation of cell surface integrin molecules to a high affinity state as well as polarization of integrins resulting in increased integrin avidity (10, 11). In their high affinity/avidity state, integrins bind tightly to adhesion molecules expressed on the walls of the vasculature, allowing the cell to roll along the surface of blood vessels and eventually to arrest and diapedese into the tissues (12). Thus, though the chemokine receptor system is highly redundant, it is also highly specific; migration of a specific cell into a specific tissue depends upon the regulated expression pattern of

chemokine receptors, the induction of integrins, as well as the expression of vascular adhesion molecules.

The chemotactic properties of the chemokine family can be divided into two groups based upon function (13). The inflammatory chemokines are secreted from peripheral cells at sites of tissue damage and function to initially attract naive DCs and neutrophils, and later function to home activated T cells. In contrast, the lymphoid chemokines, which are constitutively expressed within lymphoid tissue, attract mature, antigen presenting DCs, as well as naive lymphocytes into lymph nodes and serve to co-localize DC and lymphocytes for communication. Thus, the priming of effector lymphocytes in secondary lymphoid tissue is mediated by lymphoid chemokines, while homing of activated cells to the site of antigen is controlled by inflammatory chemokines.

Though best known for their function in inducing chemotaxis, some members of the chemokine family are also involved in other processes which have a bearing on anti-tumor immune responses (14). Chemokines play a role in angiogenesis with some molecules inducing angiogenesis and others playing a role in angiostasis. Chemokines are also involved in the activation of T cells, as well as the polarization of CD4⁺ T cells into T_H1 or T_H2 immune responses (15). T_H1 responses are characterized by cytotoxic T lymphocyte (CTL) lysis and secretion of IFN γ , while T_H2 responses are characterized by antibody production and the secretion of such cytokines as IL-4 and IL-10. It is possible that chemokines are critical in determining which of these pathways is taken based upon the type and activation status of cells recruited to sites of inflammation, as well as direct effects of the chemokine upon those cells once they arrive.

3.1. Chemokines affect the magnitude of the immune responses

As described, the chemokine system is at the crux of priming antigen specific immune responses as the particular chemokines that are secreted will determine not only the types of cells that are attracted, but may also directly affect the direction of the immune response. In fact, disruption of chemokine secretion has been shown to lead to significant delays in T cell priming (16). Nowhere is this more evident than in the case of virally encoded proteins aimed at circumventing the chemokine system (17). There are numerous examples of viral proteins involved in immune evasion through disruption of local chemokines activity. Some members of the poxvirus family encode chemokine binding proteins capable of neutralizing secreted CC chemokines, while herpesviruses encode decoy chemokine receptors (18-21). Similarly, human herpes virus 8 expresses a chemokine receptor with homology to CXCR1 and CXCR2 that is constitutively active, and promotes tumor angiogenesis in HIV-related Kaposi's sarcoma (22). Chemokine homologs have also been detected in murine cytomegalovirus suggesting a role in controlling the host immune response (23). These examples illuminate that it is possible to markedly alter immune responses by manipulating the chemokine system,

and thus, manipulation of the chemokine system has become a topic of intense interest in tumor immunology.

3.2. Local chemokine expression affects tumor growth

The tumor-derived chemotactic factor (TDCF) was initially identified as playing a role in inhibiting tumor growth by stimulating macrophage extravasation (24). Consequently, TDCF was characterized as a member of the CC chemokine family (and renamed MCP-1, and later CCL2), and studies on the role of chemokines in tumor growth began (25). Because chemokines have a variety of functions that may affect the tumor microenvironment, it is often difficult to determine the effects that a chemokine may have in a given tumor (26). While some chemokines appear to orchestrate immune-mediated inhibition of tumor growth, others seem to promote tumor growth either by angiogenic or unknown mechanisms (24, 27).

The chemokine CXCL1, which was originally given the apt name of melanoma growth stimulatory activity (MGS), is an example of this complicated relationship between chemokines and tumors. CXCL1 is a growth factor and T cell chemotactic, but stimulates melanoma cell growth (28). Likewise, inhibition of CXCL1 results in delayed melanoma growth (29). This is not unique to CXCL1, as similar results have been demonstrated with CXCL8 (IL-8) (30). There are likely multiple mechanisms behind the role of chemokines in the transformation and growth of tumor cells, but it is likely due, at least in part to the dysregulation of NF- κ B, a downstream signaling molecule of all known chemokine receptors. NF- κ B is normally intricately regulated in the cytosol by its sequestration when complexed to its inhibitor I κ B (31). However, chemokine signaling invariably leads to dissociation of NF- κ B from I κ B, with subsequent transport of NF- κ B into the nucleus where it acts as a transcription factor for a variety of genes critical to cell cycle progression and survival (32). Thus, overexpression of CXCL1 or CXCL8 leads to autocrine effects upon tumor cells that encourage cell proliferation and escape from apoptotic signals, and allow the tumor to progress.

There is also evidence to suggest that chemokines can affect metastatic potential as well as tumor growth. Metastasis of tumor cells involves several steps, and culminates in migration of tumor cells to a distant site from the original tumor (33). Chemokine expression can affect the migration of tumor cells through the metastatic process, as many tumor cells migrate in response to end-organ chemokines expression (34). Over-expression of chemokine receptors on tumor cells can also affect the site of metastasis (35). For example, the chemokine receptor CXCR4 is highly expressed on some human breast cancer cells, which induces CXCL12 mediated chemoattraction and cell adhesion, hallmarks of metastasis (36, 37). Thus, it is clear that tumors exploit the pleiotropic functions of chemokines to enhance their survival and metastatic potential. These insights, however, also suggest that chemokines and their receptors may be powerful targets for therapeutic intervention.

4. INFLAMMATORY CHEMOKINES FOR THE THERAPY OF NEOPLASIA

Secretion of CXCL1 from tumor cells allows tumors to progress despite the fact that CXCL1 attracts leukocytes to the tumor (38). However, successful tumor immunotherapy is believed to hinge upon the priming of tumor specific T lymphocytes (39). As such, it is of interest to determine whether tumor-expressing chemokines that directly induce migration of T cells, or APCs, such as monocyte cells or DCs are capable of priming T cell responses against established tumors. To this end, a variety of chemokines have been employed in murine models to determine whether chemokines enhance infiltration of immune cells into the tumor, and whether increasing the number of immune cells encountering a tumor is adequate to elicit anti-tumor immunity.

The inflammatory CC chemokines, including CCL1 (TCA-3), CCL3 (MIP-1 α), and CCL5 (RANTES), have been transfected into tumor cells and demonstrated enhanced tumor rejection in murine models (40-42). In most cases it was shown that the anti-tumor effect depended upon the leukocyte subset for which the given chemokine is specific and was accompanied by potent tumor infiltration by that cell type. These therapeutic responses were highly dependent upon the function of T cells, and much investigation of chemokines strategies have focused on induction of T cell responses. However, it must be cautioned that the aforementioned pleiotropic effects attributed to chemokines must be considered before anti-tumor chemokine therapies are begun, as the effects of chemokine expression at a tumor site are unpredictable. This is best seen in the case of CCL5 where some studies have demonstrated an anti-tumor effect, while others have shown that CCL5 actually increases the rate of tumor growth (43). The pro-tumor effects of CCL5 have been attributed to increased levels of metalloproteinases as well as increased vascularity of the tumor, both of which have been shown to enhance tumor growth and metastasis.

Initial experiments in the field were aimed at enhancing the infiltration of monocytes using the chemokine CCL2 (44, 45). Expression of CCL2 by transfected adenocarcinoma cells reduced the ability of tumors to establish within the lungs of mice. The decreased tumorigenicity of CCL2 expressing tumor cells underscores that increasing the number of infiltrating APCs can result in therapeutic responses. Furthermore, *in vitro* analysis of these tumor cells revealed that such chemokine-transfected cell lines were particularly susceptible to monocyte lysis in conjunction with lipopolysaccharide (LPS), a potent bacterial endotoxin (46). Synergy with LPS, which is capable of stimulating the cytotoxic functions of macrophages, indicates a potential powerful combined therapy involving chemokines, capable of attracting immune cells, and cytokines, which are required for expanding and activating such cells.

The "attraction/expansion" of immune cells may be a powerful tumor therapy, as it not only results in an increase in the number of cells encountering the tumor, but

may also result in enhanced anti-tumor priming of these infiltrating cells. This hypothesis was examined using intratumoral injection of lymphotactin (XCL1/LPTN)-transduced fibroblasts (47). XCL1, one of two members of the C chemokine family, is an attractor of both natural killer (NK) cells and T cells (48). While transduction of XCL1 into tumors did not noticeably affect the growth of established tumors in mice despite significant T cell infiltration, addition of IL-2 to these same tumors resulted in markedly reduced rates of tumor growth which depended upon both CD4+ and CD8+ T cells. Thus, not only did the T cells need to be brought to the tumor, but these cells needed to be stimulated.

The power of attraction/expansion was shown in a vaccine approach in mouse models using a combination of the T cell chemokine CXCL10 and the T_H1 cytokine IL-12 (49). Using co-administration of recombinant adenoviruses expressing these molecules, it was possible to eradicate established tumors. Though it was not directly confirmed that the powerful anti-tumor CTL responses that were detected were primed at the tumor site, the therapeutic response was only seen when both vaccines were administered within the same tumor, which argues strongly that the tumor was the site of priming. Furthermore, once the CTL response was primed within the injected tumor, untreated tumors on the opposite flanks of the mice were similarly eradicated. This is of clear importance in treating neoplasia, as the chemokine/cytokine treatment may be capable of attracting and priming sufficient numbers of tumor specific T cells to eradicate metastatic disease once the original tumor mass is resected. Though chemokine/cytokine combination therapies are in their infancy, they clearly have potential as a powerful anti-tumor therapy. Further studies are required to determine which of the myriad of chemokines and cytokines will prove to have the greatest effect against tumors. Thus, current cancer vaccines and adoptive immunotherapy approaches aimed at activating tumor infiltrating lymphocytes may be augmented by providing chemokines to increase the number of cells at sites of established tumor growth (50, 51). Alternatively, local priming at the site of tumor growth may be possible through delivery of lymphoid chemokines to the tumor site and we will consider this possibility next.

5. LYMPHOID CHEMOKINES FOR THE THERAPY OF NEOPLASIA

An attempt to turn the tumor into a neo-lymphoid environment has been tested with the chemokines CCL19 (ELC) and CCL21 (SLC), two of the most potent attractors of naïve T cells and mature DCs (52). Secreted constitutively from the lymphoid tissue, CCL19 and CCL21 function to colocalize these cells and encourage contact. Thus CCL19 and CCL21 are vital in the priming, clonal expansion, and activation of antigen specific T cell responses within the lymph node (53). Because the lymph node is so critical in initiating T cell responses, it has been proposed that initiation of an anti-tumor T cell response requires that tumor cells reach the lymph nodes; conversely tumors that avoid entering the lymphoid tissue are largely

ignored by the immune system (54). However, it is possible that the chemokine system can be harnessed to make the lymph node dispensable in priming an anti-tumor response by providing these lymph node secreted chemokines at the tumor site, thus allowing the priming of an anti-tumor T cell response within the tumor. To this end, CCL19 or CCL21 treated tumors display a markedly reduced growth rate compared to controls, and are heavily infiltrated by both CD4+ and CD8+ T cells (55, 56). Importantly, SLC continues to be of therapeutic value in mice lacking peripheral lymph nodes. This strongly implies that the tumor is the site of priming anti-tumor immune response (57).

The ability to manipulate the immune system to prime T cells within the tumor, rather than relying on the tumor cells, or tumor cell containing DC, to migrate to the lymph nodes, has important consequences. If priming anti-tumor immune responses truly requires migration to the lymph node, it is likely that the migrating tumor cells may metastasize to other sites as well. It is possible that by the time a tumor is capable of metastasis, sending cells to the lymphoid tissue and initiating an anti-tumor immune response, that the tumor has reached sufficient size, and established ample immune escape mechanisms to render an immune response futile (58). The ability to circumvent the lymphoid organs, and prime an immune response within the tumor may allow initiation of immune responses while the tumor remains small and vulnerable. Recent evidence suggests that CCL19 and CCL21 have properties unrelated to chemotaxis that may give additional potency to these chemokines as therapeutic agents against cancer. In *in vitro* systems, CCL19 has demonstrated the ability to induce DC maturation and stimulates them towards induction of Th1 T cell responses (59). On the other side of the immunological synapse, CCL21 has costimulatory properties through the induction of TCR-dependent T cell stimulation (60). Thus, these chemokines act in both the colocalization of DC with T cells, and also in the direct stimulation of both cell types. Further studies are needed to better understand how early immune responses are generated against tumor cells and hence, how to optimize chemokines therapy.

Another interesting possibility that has arisen recently is the manipulation of regulatory T cells using chemokines. These cells function to inhibit T cell responses by directly inhibiting T cell function, as well as dendritic cell antigen presentation (61, 62). The most well studied population of such cells are the CD4⁺ T cells constitutively expressing CD25 (Tregs) (63, 64). Though the mechanisms behind Treg suppression of immune responses are not fully understood, it is clear that anti-tumor immune responses are strengthened in the absence of Tregs (65, 66). The intricately regulated system of chemokines and their receptors may allow the specific attraction of non-regulatory T cells to the tumor, to allow them access to tumor cells in the absence of Tregs. The lymphoid chemokines CCL19 and CCL21 attract non-regulatory T cells to a larger extent than their regulatory counterparts (60, 67), and thus represent a promising method for priming T cell responses extranodally with the added benefit of circumventing Treg mediated suppression.

Further analysis of the migration patterns and chemokine receptor usage of Tregs is necessary.

6. CONCLUSION

It is difficult to believe that the chemokine family was first described little more than fifteen years ago. Since that time they have been shown to be involved in nearly every aspect of immunology. From mediating the migration of cells throughout the periphery and within the lymphoid tissue, to affecting the activation and polarization of immune cells, chemokines likely have an extensive role in determining the magnitude and type of immune response elicited. By determining which cells are involved in response to a given antigen, the chemokines likely form the foundation of anti-tumor and pathogen-specific immunity. Thus, while many strategies attempt to enhance tumor-specific immune responses, modification of the earliest interactions of the innate and adaptive immune responses through chemokines may be a powerful method for improving the therapeutic potential of tumor immunotherapy.

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Send correspondence to: Dr. Howard L. Kaufman, Columbia University, 177 Fort Washington Avenue, MHB-7SK, New York, NY 10032, Tel: 212-342-6042, Fax: 212-342-0234, E-mail: hlk2003@columbia.edu

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