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COMMENTARY

MONOCYTE CHEMOTACTIC PROTEINS FROM HUMAN TUMOR CELLS

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Tumors are characterized by infiltration of macrophages which frequently constitute 20–30% of the cellular mass of the tumor [1]. Tumor-associated macrophages (TAM) are found around the periphery of and within human and animal tumors. It is now generally accepted that most TAM are derived from peripheral blood monocytes that are recruited into the tumor mass from the circulation [2–5]. However, the local proliferation of macrophages may also contribute to the large numbers of TAM frequently associated with solid tumors [3]. It is also apparent that TAM are a heterogenous population of cells demonstrating non-uniformity with respect to size, morphology, and biochemical and cytochemical characteristics [6, 7]. The presence of monocytes/macrophages at various stages of maturation or activation probably reflects the continuous nature of the monocyte recruitment process. The presence of macrophage sub-populations which are functionally distinct is another important source of TAM heterogeneity [6, 7].

Studies in mice with primary and early-passage chemically induced tumors indicate that the population of TAM, for the most part, is relatively stable in malignant tumors [4]. For example, tumors which have been irradiated tend to be re-populated with TAM to a constant macrophage/tumor cell ratio [5]. These studies suggest that the TAM population is in some way regulated by, and a characteristic of, the tumor itself.

The functional consequence of monocyte recruitment and the presence of TAM are controversial, due to the potential of activated macrophages to produce different factors which have the capacity to either inhibit or enhance tumor growth [8]. Whatever view is espoused concerning the function and role of TAM in the growth and proliferation of neoplastic tissue, the lack of consensus and understanding should not obscure the point that these macrophages very likely carry out some important function and are not just neutral bystanders. Therefore, the basic mechanisms which control monocyte recruitment from the circulation and their maturation into macrophages are likely to be significant.

The presence of macrophages is dependent upon several discrete steps [9]. Peripheral blood monocytes, macrophage precursors, are formed in the bone marrow from committed progenitor cells. At the appropriate stage of maturation they are released into the circulation. The migration of peripheral blood monocytes into different tissues is thought to be an infrequent and random event in the absence of inflammation. However, in the presence of locally generated monocyte chemoattractants, peripheral blood monocytes are recruited in large numbers as described below. *In situ*, monocytes undergo final maturation to macrophages. Macrophages can then be stimulated or activated to produce a slew of regulatory factors, enzymes, or oxygen metabolites [8, 9]. It is through the production of these biologically active factors that macrophages can influence the growth of tumor cells.

The recruitment of circulating monocytes into the tumor mass probably involves those mechanisms which regulate leukocyte infiltration in other forms of inflammation. These involve the adherence of marginating leukocytes to the adjacent vascular endothelium, increased leukocyte locomotion, extravasation into the tissue spaces and finally the homing of monocytes to the inflamed site along positive gradients of chemotactic substances (i.e. chemoattractants). The infiltration of leukocytes shows a high degree of specificity *in vivo* [10]. This selectivity can be controlled at several levels. One level of control is the adhesion of a given type of leukocyte to vascular endothelium. Adhesion glycoproteins specific for lymphocytes, referred to as "addressins" have been identified on endothelial cell surfaces that enhance the adherence of lymphocytes [10]. Likewise, endothelial-leukocyte adhesion molecule-1 (ELAM-1) enhances the adherence of neutrophils and, to a lesser extent, peripheral blood monocytes to vascular endothelium [11]. Perhaps additional, as yet unidentified adhesion molecules will be found on vascular endothelium, which specifically regulate monocyte adhesion to endothelium.

Other factors influence the adhesion of leukocytes to endothelium. These include glycoprotein complexes on the leukocytes themselves. Of particular interest is the CDw18 antigen complex, which is found on neutrophils and peripheral blood monocytes [12]. Studies both *in vitro* and *in vivo* suggest a role for the CDw18 antigen complex in monocyte–endothelial interactions [13, 14]. Chemotactic factors can also influence the adhesion of leukocytes to endothelial cells. The monocyte-specific chemoattractant, MCP-1, increases adhesion of monocytes to endothelium *in vitro*.*

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An essential component to monocyte recruitment is induction of diapedesis across the endothelial wall of blood vessels and migration into tissue. The process of directed cell movement in tissue is initiated by chemotactic factors. It was formerly thought that the primary signalling event initiating the recruitment of monocytes resulted from immune recognition of tumor cells [15]. However, this is unlikely since there is generally little correlation between immunogenicity and the level of tumor associated macrophages [16–18]. In addition, macrophage recruitment by tumors does not depend on the presence of an intact immune system [18, 19]. However, a functional bone marrow system capable of producing monocytes is needed for the accumulation of macrophages in tumors [19, 20]. This suggests that the presence of TAM is dependent upon a pool of circulating monocytes which are recruited through the generation of chemotactic factors. Such a mechanism is supported by findings that tumor infiltration by macrophages in animals is correlated with the production of monocyte chemoattractants by the tumor cells *in vitro* [21]. The chemotactic activity described in this report was partially purified and shown to be mediated by a cationic protein with $M_r = 12{,}000$ that stimulates chemotaxis of monocytes but not polymorphonuclear leukocytes [22].

Normal cells have also been shown to produce monocyte chemoattractants. Jauchem and co-workers [23] identified a monocyte chemoattractant produced by baboon smooth muscle cells, smooth muscle cell produced chemotactic factor, SMC-CF. Valente and colleagues have characterized and purified SMC-CF; it is a cationic, monomeric protein of $M_r = 14{,}000$ that stimulates migration of monocytes but not polymorphonuclear leukocytes or lymphocytes [24, 25]. The similarity in biologic specificity and physical properties of the monocyte chemoattractants produced by tumor cells and vascular smooth muscle cells, as reported in these studies, suggested that they might be related.

To investigate the potential relationship between the monocyte chemotactic factors produced by vascular smooth muscle and tumor cells, immunochemical studies were undertaken using an antisera generated against homogenous SMC-CF [26]. Immunoprecipitation experiments using SMC-CF antiserum demonstrated that the MG-63 human osteosarcoma cell line synthesized and secreted a protein that was antigenically related to SMC-CF. Serum free medium conditioned by MG-63 cells was tested for stimulation of monocyte migration and for the capacity of SMC-CF antiserum to block this activity. Antiserum to SMC-CF blocked all of the monocyte chemotactic activity secreted by the MG-63 osteosarcoma cell line, demonstrating that proteins related to SMC-CF are the predominant monocyte chemoattractants produced by these cells *in vitro*. The above observations were extended to other tumor cell lines as well as to primary osteosarcoma cell cultures [26]. Thirteen different human malignant cell cultures were tested. Nine of these synthesized detectable levels of SMC-CF-like proteins as determined by immunoprecipitation with SMC-CF antiserum but not by control serum. These included one cell line derived from a melanoma, two cell lines derived from glioblastoma, two from fibrosarcoma, one from a rhabdomyosarcoma, one osteosarcoma cell line and two primary osteosarcoma cell cultures. All of the cells that synthesized SMC-CF related proteins produced high levels of chemotactic activity. In eight of nine cell lines, all of the chemotactic activity could be blocked by SMC-CF antisera. The four cell lines that did not produce SMC-CF-like proteins were derived from a bladder carcinoma, epidermoid carcinoma, leiomyosarcoma, and an osteosarcoma. Significantly, none of these lines produced large amounts of chemotactic activity. Thus, a strict correlation was observed between the production of monocyte chemotactic activity and the synthesis of SMC-CF-like proteins. Other monocyte chemotactic proteins can be elaborated by tumor cells. Transforming growth factor-beta, which is produced by both normal and malignant cells, is a potent stimulator of monocyte chemotaxis [27]. However, transforming growth factor-beta is secreted in a latent form and must be activated before it can bind to specific receptors. Thus, SMC-CF-like proteins may account for most, if not all, of the non-latent monocyte chemotactic activity produced by tumor cells. The above studies also suggest that there is no obvious relationship between the tissue origin of transformed cells and the production of SMC-CF-like proteins.

Leonard and his co-workers [28] recently identified a human monocyte chemotactic protein which they termed MCP-1. This protein was purified from U-105 MG glioma cells and found to exist in two forms: $M_r = 13{,}000$ and 15,000. The cDNA sequence predicted a protein with $M_r = 8700$ and was identical to the cDNA sequence of a monocyte chemotactic factor produced by the promylocytic cell line, THP-1 [29]. Northern blot analysis indicated that the gene encoding this protein is also expressed in phytohemagglutinin (PHA) stimulated peripheral blood mononuclear leukocytes. One of the important results from these studies was the finding that MCP-1 belongs to a family of inflammatory cytokines that share nucleotide and structural similarities. These include the JE product, which is secreted by fibroblasts in response to growth factor stimulation [30]. The JE cDNA sequence shares 68% nucleotide sequence homology with MCP-1. The difference in nucleotide sequence could be due to evolutionary divergence between mice and humans. However, it remains to be demonstrated whether the JE product and MCP-1 are functionally related. Furutani and colleagues [29] have noted that other putative inflammatory proteins share nucleotide sequence homology with MCP-1: (a) an inducible lymphocyte gene, pLD78 (36% homology); (b) macrophage inflammatory protein, MIP (36% homology); the T cell-specific molecule, RANTES (28% homology); and the T cell-specific gene, TCA-3.0 (25% homology).

The amino acid composition and cDNA sequence indicate that MCP-1 and SMC-CF are the same

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proteins ([25, 29], and unpublished observations). This is consistent with recent experimental data which demonstrate that antisera to SMC-CF specifically immunoprecipitate proteins with the same apparent molecular mass as MCP-1 in U-105 MG cells, which produce MCP-1. In addition, baboon vascular smooth muscle cells and MG-63 human osteosarcoma cells, both of which produce SMC-CF, express the MCP-1 gene, while the leiomyosarcoma cell line, SKLMS, which does not produce detectable levels of SMC-CF, has extremely low levels of MCP-1 transcript [31]. We and others have noted that MCP-1-like chemotactic factors have molecular masses ranging from $M_r = 9000$ to $M_r = 16{,}000$. Recent evidence in our laboratory indicates that these proteins share a common protein core and that the various molecular masses are due to differences in O-linked glycosylation [31]. From hereon we will use the term monocyte-chemoattractant protein-1 (MCP-1) used by Leonard and his co-workers since this chemoattractant is synthesized by a variety of cell types including vascular smooth muscle cells, endothelial cells, and a number of malignant cell types.

The local production of MCP-1 by a variety of cell types may indicate that its expression represents a common mechanism by which monocytes are recruited during physiologic conditions such as wound healing and in pathologic states such as atherosclerosis or tumorigenesis. It is possible that in normal cells the production of MCP-1 is carefully regulated by paracrine factors generated during inflammatory events. In malignant cells, this regulation may be relaxed or induced secondarily by autocrine or paracrine factors produced by malignant cells. This is supported by findings that MCP-1 expression in endothelial cells is stimulated by the inflammatory cytokines, tumor necrosis factor, interferon, and interleukin-1 [32]. Other studies indicate that MCP-1 production is regulated in normal cells. MCP-1 transcripts are not detected in unstimulated peripheral blood mononuclear leukocytes, but can be induced by phytohemagglutinin or lipopolysaccharide [33]. Streiter and co-workers [34] have reported that MCP-1 is not constitutively expressed in endothelial cells, but can be detected within an hour of stimulation by lipopolysaccharide. This contrasts with results with tumor cells in which the constitutive expression of MCP-1 has been demonstrated.

The recruitment of monocytes from the peripheral vasculature and the maturation to TAM has the potential to affect tumor growth dramatically. This includes the capacity to stimulate tumor growth or, paradoxically, induce tumor regression. The capacity of macrophages to enhance or inhibit the growth of neoplastic cells is mediated by the elaboration of soluble regulatory factors [8, 9]. Although macrophages can inhibit tumors, they can also enhance tumor growth and indeed, in a few cases, appear to be actually required for tumor growth [35–37]. This is supported by *in vivo* studies in which whole body irradiation of mice prior to implantation of fibrosarcoma was associated with a relative absence of TAM and retardation of initial growth [38]. Growth-stimulating factors produced by activated macrophages can stimulate a wide variety of cell types including epithelial, endothelial, immune and connective tissue cells. A partial list of macrophage-produced growth-inducing factors includes platelet-derived growth factor, fibroblast growth factor, interleukin-1 and transforming growth factor-alpha [8]. Macrophages may also affect the behavior of tumors through the production of angiogenesis factors that increase vascular support for tumor growth [39]. Folkman [40] has demonstrated that without the support of a rich vascular bed, the ultimate size of a given tumor is limited. Thus, TAM may indirectly enhance tumor growth by inducing angiogenesis.

Macrophages can also inhibit tumor cell proliferation or exert tumoricidal effects. Inhibition of tumor growth may be mediated through production of factors such as transforming growth factor-beta (TGF-B), or alpha-interferon, which have the capacity to inhibit proliferation of tumor cells, or through the production of tumor necrosis factor-alpha, which is tumoricidal and can cause hemorrhagic necrosis *in vivo* [8, 41, 42]. Interferon and tumor necrosis factor have been noted to work cooperatively in destructive effects upon tumor cells. It is apparent that tumors grow in the presence of tumor-associated macrophages, despite the potential for macrophages to inhibit tumor cell proliferation. Although unproven, macrophages may play an important role in eliminating tumors in the early stages of tumorigenesis, but at later stages, enhance the growth of tumors that escape immune surveillance. McBride [3] has suggested that a mutual selection process may take place between tumors and tumor-associated macrophages that results in the modulation of macrophage activity beneficial to tumor growth. The characteristics of the TAM present may be more important than the actual number of macrophages infiltrating a tumor. This is supported by evidence that TAM in metastatic tumors have a different profile than non-metastatic tumors, and hence, may be functionally different [43, 44].

In summary, the production of the monocyte chemoattractant MCP-1 suggests a common mechanism for the recruitment of monocytes/macrophages and provides an explanation for monocyte/macrophage infiltration frequently observed in solid tumors. The regulated production of MCP-1 by non-transformed cells further substantiates the suggestion that MCP-1 is an important inflammatory mediator and is involved in the recruitment of monocytes in a number of pathologic or physiologic conditions.

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