The role of MIP-1α in inflammation and hematopoiesis

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Abstract: Macrophage inflammatory protein 1α (MIP-1α) is a member of the C-C subfamily of chemokines, a large superfamily of low-molecular-weight, inducible proteins that exhibit a variety of proinflammatory activities in vitro including leukocyte chemotaxis. MIP-1α is a particularly interesting chemokine, because in addition to its proinflammatory activities, it inhibits the proliferation of hematopoietic stem cells in vitro and in vivo. Here, the biologic properties of MIP-1α are reviewed in light of recent data on mice homozygous for a disruption of the MIP-1α gene. The MIP-1α null mice have no overt abnormalities of peripheral blood or bone marrow cells, indicating that MIP-1α is not necessary for normal hematopoiesis. However, the MIP-1α null mice have a reduced inflammatory response to influenza virus and are resistant to coxsackievirus-induced myocarditis. These data demonstrate that MIP-1α is required for a normal inflammatory response to these viruses. Agents that inhibit the action of MIP-1α may therefore prove useful for controlling inflammation in these and other settings. J. Leukoc. Biol. 59: 61–66; 1996.

Key Words: macrophage inflammatory protein 1α (MIP-1α) · chemokines · inflammation · stem cell inhibitor

INTRODUCTION

The chemokine superfamily is composed of small (8–11 kd), structurally related proteins that induce leukocyte chemotaxis in vitro. To date, at least 20 chemokines have been identified and many others probably await discovery. The chemokine superfamily has been divided into two subfamilies, denoted C-X-C or α and C-C or β, based on whether or not an intervening amino acid is situated between the first two of four conserved cysteine residues [1]. The two subfamilies have other differences as well. Members within a subfamily have a higher degree of structural homology and biologic similarity with one another than with members of the other subfamily, and each subfamily is encoded on different chromosomes. MIP-1α is a member of the C-C subfamily, which also includes MIP-1β, RANTES (regulated on activation, normal T cell expressed and secreted), and MCP-1 (monocyte chemoattractant protein 1). The C-C chemokines are generally chemotactic for cells of the monocyte lineage and lymphocytes. They are encoded within a relatively small region on chromosome 11 in the mouse and on chromosome 17 in humans. The C-X-C chemokines, which include interleukin-8 (IL-8), platelet factor (PF) 4, and MIP-2, are generally chemotactic for neutrophils and are also encoded within a relatively small region on chromosome 4 in humans and probably on chromosome 5 in the mouse. A third chemokine subfamily has been proposed to accommodate the recently described molecule named lymphotaxin [2]. Lymphotaxin is highly homologous to the other chemokines but differs from the other members in that it has only two cysteine residues, is chemotactic for lymphocytes but not monocytes or neutrophils, and is found in yet a third genomic location, mouse chromosome 1.

THE ROLE OF MIP-1α IN INFLAMMATION

In vitro studies

MIP-1α was initially described in 1988 as MIP-1, a partially purified 8-kd protein doublet from the conditioned medium of endotoxin-stimulated macrophages. MIP-1 induced neutrophil accumulation upon injection into the footpads of mice, hence the name macrophage inflammatory protein [3]. This protein preparation was later shown to comprise two highly related proteins, termed MIP-1α and MIP-1β [4]. MIP-1α expression can be induced in a variety of cell types, including monocytes, macrophage cell lines, mast cell lines, Langerhans cells, fibroblasts, and T lymphocytes. MIP-1α expression is induced in macrophages by lipopolysaccharide (LPS) and in monocytes by their binding to endothelial cell monolayers or to plates coated with intercellular adhesion molecule 1 (ICAM-1) [5].

The proinflammatory activities of MIP-1α overlap with, but are not identical to, the activities of other C-C chemokines. For example, both MIP-1α and MIP-1β induce migration of monocytes and T lymphocytes, but they differ in their effects on different T cell subsets. Thus, MIP-1α is

Abbreviations: CFU-S, spleen colony-forming unit; CBV3, coxsackievirus B3; EAE, experimental autoimmune encephalomyelitis; ICAM-1, intercellular adhesion molecule 1; IL-8, interleukin-8; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein 1; MIP-1α, macrophage inflammatory protein 1α; NK, natural killer; PF, platelet factor; RANTES, regulated on activation, normal T cell expressed and secreted; SCI, stem cell inhibitor; TGF-β, transforming growth factor β.

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Received August 29, 1995; accepted September 26, 1995.
primarily chemotactic for B lymphocytes and activated CD8+ T cells, whereas MIP-1β is chemotactic for activated CD4+ cells [6, 7]. MIP-1α, but not MIP-1β, induces chemotaxis of natural killer (NK) cells [8]. MIP-1α and RANTES, but not MIP-1β or MCP-1, are chemotactic for eosinophils [9] and stimulate basophils to release histamine. MIP-1α also induces ICAM-1 expression [10], mast cell degranulation [11], and production of tumor necrosis factorα (TNF-α), IL-1 and IL-6 [12].

This wide variety of activities in vitro suggest that MIP-1α may have an important role in inflammation. However, the importance of MIP-1α in vivo has been uncertain because at least two other C-C chemokines, MIP-1β and RANTES, not only share many of its biologic activities but also bind to a common receptor [13–15]. This suggests that substantial redundancy may exist among various chemokines and that each member may not be specifically required in any inflammatory response. Alternatively, changes in the expression of various chemokines may mediate subtle changes in the profile of inflammatory cells that are recruited to the site of infection and thereby fine-tune the inflammatory response appropriate to specific pathogens. The requirement of MIP-1α in inflammation has been addressed recently by loss-of-function experiments: administration of neutralizing antisera and by targeted disruption of the MIP-1α gene.

Antiserum injection

Analysis of MIP-1α expression has revealed that pulmonary alveolar macrophages are a rich source of MIP-1α [16, 17] and that its expression in whole lung is increased in response to various physical and chemical insults [18, 19]. Injection of MIP-1α–specific neutralizing polyclonal antiserum into experimental animals reduces leukocyte infiltration and associated pulmonary fibrosis in bleomycin-treated mice [20] and reduces neutrophil infiltration and associated acute lung injury in rats treated with immunoglobulin G immune complexes or LPS [21]. Antiserum injection experiments have also implicated MIP-1α in the inflammatory response to other stimuli, including granuloma formation surrounding the ova of schistosomes [22] and the recruitment of inflammatory cells into the central nervous system CNS during progression of experimental autoimmune encephalomyelitis (EAE) [23].

MIP-1α GENE DISRUPTION

To avoid the possibilities that neutralizing antisera may affect the function of more than one chemokine and that neutralization of MIP-1α with antisera may be only partial, we used embryonic stem (ES) cell technology to generate mice homozygous for a disrupted MIP-1α gene [24]. The homozygous mutant (-/-) mice were born in Mendelian proportions and had no obvious gross or histologic abnormalities, indicating that MIP-1α is not required for normal development.

Coxsackievirus infection

The observation that injection of anti-MIP-1α antisera into SJL mice prevents the induction of EAE suggested that MIP-1α may also have a role in other autoimmune-mediated diseases. To investigate this possibility, we studied the response of both wild-type (+/+ ) and -/- mice to coxsackievirus B3 (CVB3), a virus that induces myocarditis in both humans and mice [25]. Considerable evidence suggests that CVB3-induced myocarditis is mediated by an autoimmune mechanism: autoantibodies and autoreactive T cells can be demonstrated in CVB3-infected mice [26] and mice pretreated with an antibody to T cells do not develop CVB3-induced myocarditis [27]. The pathophysiology of the murine model closely resembles that of the human disease. Macrophages and polymorphonuclear cells accumulate in the heart early post infection (p.i.) and are gradually replaced by a T cell infiltrate by day 10 p.i. The MIP-1α -/- mice have no evidence of cardiac inflammation after infection with CVB3, whereas approximately 60% of age-matched +/+ mice develop myocarditis (Fig. 1). This striking result clearly demonstrates that MIP-1α is an absolute requirement for CVB3-mediated myocarditis and suggests that agents that inhibit the action of MIP-1α may prove valuable in the clinical management of myocarditis.

The absence of myocarditis in the -/- mice is not likely due to a defect in their T lymphocyte development because the relative proportions of B cells versus T cells, as well as CD4+ versus CD8+ T cells, are unchanged in the -/- mice. Moreover, splenocytes from the mutant mice are also normal in their proliferative response to the T cell–specific mitogen concanavalin A and to coxsackievirus antigen. These results indicate that the reduced inflammation observed in the null animals is probably not due to an abnormality in T cell activation and suggest that the -/- mice are unable to recruit T cells to the CVB3-infected hearts.

There is no significant difference in CVB3 viral titers between the +/+ and -/- mice, despite the dramatic difference in the mononuclear cell infiltrate. Two conclusions can be drawn from this observation. First, the inflammatory cells present in the hearts of coxsackievirus-infected mice do not significantly reduce the replication or spread of CVB3. Second, the cytolysis seen in hearts of the +/+ mice is not likely to be due to a direct cytopathic effect of the virus because the -/- mice have no cytolysis despite having viral titers indistinguishable from those of +/+ mice. Therefore, the cytolysis seen in the infected +/+ hearts is most likely due to actions of the inflammatory cells.

Influenza virus infection

Previous reports of MIP-1α expression in inflamed lungs suggested that this cytokine is important in pulmonary inflammation. To investigate the response of the -/- mice to a pneumotropie pathogen, we infected them with influenza virus. Influenza virus infection has been extensively studied in mice; antibodies provide protection from infection, whereas T cells are required for viral clearance. Studies of
mice lacking various T cell subsets have provided insight into their function in viral clearance. Nude mice, which lack both CD4+ and CD8+ T cells, are unable to clear virus and die within 3 weeks of infection, whereas mice depleted of either CD4+ or CD8+ cells can clear virus and recover. These data suggest that either class of T cells can mediate viral clearance in infected animals (reviewed in ref. 28).

MIP-1α -/- mice infected with influenza virus have significantly less pulmonary edema at necropsy than do infected age-matched +/+ control mice. The majority of -/- mice also have significantly less mononuclear cell infiltration than control mice (Fig. 2). This result provides genetic evidence that MIP-1α has a role in influenza virus–induced pneumonitis and confirms the importance of MIP-1α in the inflammatory response to viral infections.

The -/- mice have increased influenza viral titers at days 3 and 7 p.i. compared with age-matched +/+ controls, although both groups are able to clear virus completely by day 21 p.i. This increase in viral titers and the reduced inflammatory infiltrate in the -/- mice suggest that MIP-1α may recruit immunocompetent T lymphocytes, which are required for viral clearance. This hypothesis is consistent with the observations that MIP-1α is a chemoattractant of both CD8+ and CD4+ T cells in vitro, and that it facilitates the binding of CD8+ T cells to the endothelium [6, 7].

The increase in influenza viral titers in the -/- mice contrasts with our finding that CVB3-infected -/- mice do not have higher viral titers than +/+ controls. Nevertheless, both these observations are consistent with previous studies of CVB3 and influenza infection of mice lacking T cells. Thus, nude mice are unable to clear influenza virus, whereas mice depleted of T cells by antiserum treatment do not have increased CVB3 titers compared with infected control mice. Together, these data suggest that the mechanism of viral clearance differs qualitatively in the two viral disease models.

**Differential effect of the absence of MIP-1α on inflammation**

The ability of the MIP-1α -/- mice to mount an inflammatory response depends on the stimulus. Thus, the -/- mice have reduced, but not eliminated, influenza virus–induced pneumonitis, whereas the -/- mice are highly resistant, perhaps completely resistant to CVB3–induced myocarditis. In other experiments we have conducted, the absence of MIP-1α had no significant effect on the inflammatory response. For example, the -/- mice did not differ significantly from +/+ animals in their ability to reject heart allografts or kidney transplants, although MIP-1α was expressed in the organs undergoing rejection (D.N. Cook and T.M. Coffman, unpublished observations).

Several possibilities could account for this variation in the ability of the -/- mice to respond to an inflammatory stimulus. For example, the importance of MIP-1α to the
response may depend on the presence of other cytokines expressed at the site of inflammation. The immune response to allografts is particularly brisk and a large number of proinflammatory cytokines, including MIP-1α, are expressed at the site of rejection. Together, these cytokines may mediate all of the functions of MIP-1α and thereby compensate for its absence. Therefore, the -/- mice do not differ from +/- mice in their ability to reject allografts. Inflammatory responses to pathogens that do not induce expression of these compensatory cytokines are dependent on MIP-1α to fulfill those functions. Accordingly, the MIP-1α -/- mice have a reduced inflammatory response in these settings. A careful analysis of cytokine expression in different inflammatory settings may help to determine which cytokines can functionally compensate for MIP-1α. Mice having a mutation in more than one chemokine gene, particularly those with overlapping biologic activities, such as MIP-1α and MIP-1β, should also be valuable in this regard.

THE ROLE OF MIP-1α IN HEMATOPOIESIS

The majority of hematopoietic stem cells are normally quiescent, although they can be stimulated to proliferate in vivo by myeloablatative treatment. The control of stem cell proliferation is probably achieved through a balance of both positive and negative regulatory signals. Much has been learned about molecules that increase proliferation of these important cells, but comparatively little is known of the molecules that inhibit their proliferation. The identification and characterization of these inhibitory molecules are therefore crucial to an improved understanding of hematopoietic regulation. Inhibitors may also have clinical utility because of their potential to protect the bone marrow of patients receiving drugs toxic to cycling cells. In 1990, Graham et al. isolated one such inhibitory protein from supernatants of the macrophage cell line J774.2 [29]. This molecule, designated stem cell inhibitor (SCI), reversibly inhibits the proliferation of cells giving rise to both CFU-A colonies [30] and CFU-S colonies, two assays of pluripotential bone marrow cells. Comparison of the amino acid sequence of SCI to published sequence data revealed that the gene product was identical to the previously identified MIP-1α. Thus, in addition to having proinflammatory activity, MIP-1α inhibits hematopoietic stem cell proliferation.

In vitro studies

Since its initial characterization as a stem cell inhibitor, MIP-1α has been shown to inhibit the proliferation of early progenitor cells in different clonogenic assays [31, 32], in serum-supplemented and serum-free liquid cultures [33, 34], and in long-term bone marrow cultures [35]. MIP-1α is also a stimulator of proliferation for more mature pro-

Fig. 2. Influenza virus–induced inflammation. Histologic sections of lung are shown from an influenza virus–infected +/+ mouse (A) and a -/- mouse (B).
genitors such as granulocyte-macrophage colony-forming cells [31]. Together, these observations have led to the suggestion that MIP-1α may have an important role in vivo as a regulator of hematopoiesis and may be required to maintain the quiescent state of stem cells.

In vivo studies
In addition to its ability to inhibit early progenitor cycling in vitro, MIP-1α injected into mice inhibits the proliferation of these cells in vivo [36, 37]. These experiments were encouraging in terms of the potential of MIP-1α to protect bone marrow from chemotherapeutic agents, but they did not establish whether endogenous MIP-1α in bone marrow normally functions to maintain the quiescent state of stem cells. Pharmacologic doses of MIP-1α were injected, and the effective concentration in the marrow (the presumed site of action on early progenitors) of the treated mice was unknown. Also, MIP-1α is a heparin-binding protein, and an association with the extracellular matrix (ECM), which may be important in creating a local concentration of MIP-1α and presenting the ligand to its receptor, may not occur normally for injected protein. However, non-heparin-binding mutants of MIP-1α are active in vitro (G. Graham and I. Pragnell, personal communication).

The MIP-1α-/- mice provide an experimental means to investigate directly the requirement of MIP-1α in hematopoiesis. If the inhibitory activity of MIP-1α were required to maintain bone marrow stem cells in a quiescent state, then an increase in number of early progenitors might be anticipated in the marrow of the MIP-1α-/- mice. However, no overt hematologic abnormalities are observed in the marrow of these mice. They have normal numbers of both total nucleated cells and early progenitor cells, as measured by CFU-A and CFU-S colony formation. Thus, MIP-1α is apparently not required to maintain the quiescent state of hematopoietic stem cells in normal physiology.

There are a number of possible explanations for why the number of early progenitor cells was unchanged in the MIP-1α-/- mice. First, the concentration of MIP-1α in bone marrow may be insufficient to regulate stem cell proliferation. Support for this hypothesis is provided by the observations that transforming growth factor β1 (TGF-β1) down-regulates the expression of both MIP-1α and its receptor in vitro [38] and that TGF-β1 is highly expressed in bone marrow. Alternatively, normal bone marrow may contain sufficient MIP-1α to inhibit early progenitor proliferation, but other factors required for inhibition may not be present. For example, the ability of MIP-1α to inhibit early progenitor proliferation in vitro depends on the method used to isolate the cells and the cytokine used to induce proliferation [32]. Indeed, in combination with certain cytokines, MIP-1α can have a stimulatory effect on the proliferation of early progenitors [39] (I.B. Pragnell, personal communication). Thus, the ability of MIP-1α to inhibit the proliferation of early progenitors probably depends on an environmental milieu that may not be present in normal bone marrow. Finally, other inhibitors in the marrow such as TGF-β1 may compensate for the absence of MIP-1α.

The effect of multiple inhibitory molecules on early progenitors in vitro has revealed that various inhibitors can synergize with one another to reach levels of inhibition unattainable by any single inhibitor [40]. Taken together, these data suggest that stem cell inhibition may be brought about in vivo through the actions of multiple inhibitors, each of which has a relatively small effect on its own. Genetically engineered mice lacking more than one inhibitor will provide a means of addressing this issue experimentally.

ACKNOWLEDGMENTS

The MIP-1α-/- mice were generated in the laboratory of Dr. Oliver Smithies, University of North Carolina. This work was supported by NIH grant HL37001 (O.S.) and by the Cooley’s Anemia Foundation (D.C.).

REFERENCES


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