Brief report

Macrophage inflammatory protein-1α uses a novel receptor for primitive hemopoietic cell inhibition

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Macrophage inflammatory protein-1α (MIP-1α) is a member of the chemokine family of proinflammatory mediators. In addition to its inflammatory roles, MIP-1α has been shown to be active as an inhibitor of primitive hemopoietic cell proliferation. Indeed, a dysfunction in this inhibitory process has been postulated to contribute to leukemogenesis. Research has been aimed at characterizing the receptor involved in cellular inhibition by MIP-1α. This study demonstrates that of all the β-chemokines tested, only MIP-1α is capable of inhibiting primitive hemopoietic cell proliferation. Because no MIP-1α–specific receptors have been identified, this suggests that inhibition is mediated by an uncharacterized receptor. Further evidence for the involvement of a novel receptor in this process is the equivalent potencies of MIP-1αS and MIP-1αP variants of human MIP-1α and the fact that primitive cells from bone marrow derived from individual MIP-1α receptor null mice display a full response to MIP-1α inhibition. (Blood. 2001;98:3476-3478)

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Introduction

Chemokines are members of a large and expanding family of proinflammatory mediators that is defined by the presence of variations on a conserved Cys motif.[1,2] There are currently 4 chemokine subfamilies. The 2 most populous subfamilies have 4 Cys in their mature sequences and are respectively referred to as the CC or β-chemokine family and the CXC or α-chemokine family. The other 2 subfamilies are each represented by only single members, with the C family being represented by lymphotactin and the CXXXC family by fractalkine/neurotactin. Chemokines have typically been characterized as proinflammatory mediators; however, we and others have demonstrated that chemokines, most notably macrophage inflammatory protein-1α (MIP-1α), are active in inhibiting primitive hemopoietic cell proliferation in vitro and in vivo.[3-5] Chemokines interact with target cells via members of the 7 transmembrane family of G-protein–coupled receptors.[6] There is now a systematic nomenclature for chemokine receptors, with β-chemokines binding to CCRs (CC chemokine receptor), α-chemokines to CXCRs, and C or CX3C chemokines to XCR and CX3CRs, respectively. To date, 11 CCRs, 6 CXCRs, single XCR and CX3CR receptors, and 2 more promiscuous receptors (D6 and DARC) have been identified. MIP-1α binds to CCR1, CCR3 (in the mouse), CCR5, and D6, but not to any of the other characterized receptors.[7,8]

We have been attempting to characterize the receptor responsible for inhibition of primitive murine hemopoietic cells by MIP-1α. Identification of this receptor is of importance not only for enhancing our understanding of the mechanisms of cellular inhibition by MIP-1α, but also potentially for unraveling aspects of the pathogenesis of a number of leukemias, the primitive stem/progenitor cells that display a dysfunction in their response to inhibition by MIP-1α.[9-11] Whereas CCR1 null mice have been used to demonstrate the lack of involvement of this receptor in inhibition of primitive hemopoietic cells,[12] there has been no systematic examination of the involvement of all the known MIP-1α receptors in the inhibitory effects of this chemokine. Here we show, using a range of chemokines, chemokine variants, and null mouse bone marrow, that cellular inhibition by MIP-1α is not mediated through any of the currently characterized receptors. We therefore believe that inhibition of murine stem/progenitor cells by MIP-1α involves an uncharacterized receptor.

Study design

Reagents

All chemokines were purchased from either R&D Systems Europe (Oxford, United Kingdom) or PeproTech (London, United Kingdom), with the exception of murine MIP-1α[13] and human MIP-1αP,[14] which were generated in house. AOP-RANTES was prepared as described previously.[15]

The colony-forming unit direct addition assay

Bone marrow cells were obtained by flushing from the femur and either used immediately or frozen (for all experiments on receptor null mice and their wild-type counterparts). Primitive hemopoietic cells were assayed using the in vitro colony-forming unit–agar (CFU-A) assay. This assay has been described in detail elsewhere[16,17] and detects a cell that is phenotypically indistinguishable from day-12 spleen CFU cells. Briefly, 5×10^3 fresh bone marrow cells, or 5×10^4 defrosted bone marrow cells, were plated in 1 mL 0.3% agarose/25% donor horse serum (DHS) on top of a feeder layer consisting of 0.6% agar/25% DHS/0.2 ng/mL recombinant

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murine granulocyte macrophage colony-stimulating factor/6 ng/mL recombinant human macrophage colony-stimulating factor, and 12 ng/mL stem cell factor. Inhibition was assessed by directly adding the chemokines to the assay plates and incorporating them into the feeder layer.[18] Assays were scored after 11 days, and CFU-A colonies were identified as those with a diameter greater than 2 mm.

Results and discussion

To examine receptor usage, we initially investigated a variety of β-chemokines, representing ligands for each of the currently identified β-chemokine receptors, for their ability to inhibit the primitive CFU-A cells. As shown in Table 1, murine MIP-1α is fully active as an inhibitor of CFU-A cell proliferation, with essentially complete inhibition seen at a concentration of 50 ng/mL. In contrast, as shown in Table 2, none of the other chemokines tested (at 100 ng/mL) displayed any consistent or significant inhibition of CFU-A colony formation. Thus, these data support a role for MIP-1α as an inhibitor of primitive hemopoietic cells but indicate that this activity is not shared with other β-chemokines. The failure to demonstrate inhibition by chemokines other than MIP-1α is at odds with a number of reports demonstrating inhibition of hemopoietic stem and progenitor cells with a wider range of chemokines,[19,20] but is likely to be explained by differences in the assays used in the different studies. Thus, the CFU-A assay is ideally suited to the identification of the receptor involved specifically in primitive cell inhibition by MIP-1α. Because there are currently no receptors identified that bind only MIP-1α, it appears that MIP-1α inhibits CFU-A cell proliferation through an as yet uncharacterized receptor.

We have recently characterized 2 nonallelic variants of human MIP-1α (MIP-1αS and MIP-1αP), both of which bind with similar affinities to CCR1, but only one of which (MIP-1αP) binds to murine CCR5 or D6.[14] These variants allow us to assess the roles, if any, of CCR5 and D6 in the inhibitory process. In CFU-A assays, MIP-1αP and MIP-1αS display indistinguishable potencies, with half-maximal inhibition observed at approximately 30 ng/mL (Table 1), further indicating that CCR5 and D6 are unlikely to be the inhibitory receptors.

Table 1. The effects of varying concentrations of murine and human MIP-1α on CFU-A colony formation in vitro

Chemokine, ng/mL	Colony growth (%)*	Receptors used
muMIP-1α(CCL3)		
5	51.3 ± 32.0	CCR1, CCR3, CCR5, D6
12.5	61.5 ± 15.4	
25	20.5 ± 17.8	
50	0.0 ± 0.0	
MIP-1αP		
5	118.9 ± 27.1	CCR1, CCR3, CCR5, D6
10	86.8 ± 13.5	
30	51.3 ± 16.3	
50	51.3 ± 24.8	
75	32.9 ± 4.7	
MIP-1αS		
5	71.0 ± 25.2	CCR1
10	71.0 ± 7.2	
30	35.5 ± 3.6	
50	35.5 ± 12.0	
75	30.3 ± 10.0	

*Results are expressed as the mean percentage (± SD) of colonies generated in the presence of chemokine compared with control plates. Results are representative of at least 5 experiments. MIP indicates macrophage inflammatory protein; CFU-A, colony-forming unit–agar.

Table 2. The effects of β-chemokines on CFU-A colony formation in vitro

Chemokine, 100 ng/mL	Systematic name	Colony growth (%)*	Receptors used
MIP-1β	CCL4	75.0 ± 18.8	CCR1, CCR5, D6
RANTES	CCL5	111.4 ± 26.3	CCR1, CCR3, CCR5, D6
MCP1	CCL2	112.6 ± 18.8	CCR1, CCR2, D6
MCP2	CCL8	93.4 ± 9.4	CCR1, CCR2, CCR5, D6
HCC1	CCL14	93.4 ± 9.4	CCR1
HCC2	CCL15	88.9 ± 20.8	CCR1, CCR3
HCC4	CCL16	102.2 ± 18.2	CCR1
MPIF1	CCL23	76.3 ± 16.5	CCR1
Eotaxin	CCL11	107.9 ± 25.7	CCR3, D6
MDC	CCL22	83.3 ± 17.1	CCR4
MIP-3α	CCL20	104.5 ± 34.8	CCR6
SLC	CCL21	109.2 ± 11.0	CCR7, CCR11
I309	CCL1	106.5 ± 43.1	CCR8
TECK	CCL25	101.3 ± 12.8	CCR9, CCR11
ESkine	CCL27	90.8 ± 27.7	CCR10

*Results are expressed as the mean percentage (± SD) of colonies generated in the presence of chemokine compared with control plates. Results are representative of at least 5 experiments.

The ultimate test of receptor involvement in a specific biologic function is to examine cells from receptor null mice. To this end, we examined the response of CCR1,[21] CCR3 (Humbles et al, manuscript in preparation), CCR5,[22] and D6 (Cook et al, manuscript in preparation) null bone marrow cells to inhibition by MIP-1α. As shown in Table 3, all of the null bone marrow samples displayed a full inhibitory response to MIP-1α, again indicating that none of the currently characterized MIP-1α receptors is involved in CFU-A inhibition.

It remains possible that MIP-1α can use a variety of chemokine receptors to mediate inhibition of CFU-A cells. Thus, if MIP-1α can use CCR1, CCR3, CCR5, or D6 for inhibition, the involvement of an individual receptor would not be obvious in single-receptor null mice. To test this possibility, we examined the inhibitory response of CFU-A cells from individual-receptor null mouse bone marrow in the presence of the chemokine variant AOP-RANTES.[15,23] This protein binds with high affinity to murine CCR1, CCR5 (Buser et al, manuscript in preparation), and D6 (G.J.G., unpublished data, December 1997), but is inactive as an inhibitor of primitive hemopoietic cells at concentrations up to 500 ng/mL (data not shown). Thus, for the purposes of the present study, AOP-RANTES may be regarded as a blocker of these 3 receptors. As shown in Table 3, excess AOP-RANTES had no effect on the ability of MIP-1α to inhibit CFU-A cells from any of the receptor null bone marrow samples, suggesting that in the absence of one specific receptor, MIP-1α is not using the other known AOP-RANTES–sensitive receptors to mediate inhibition.

Table 3. The effects of MIP-1α and AOP-RANTES on percentage CFU-A colony formation by wild-type and receptor null bone marrow

Receptor status	100 ng/mL MIP-1α	100 ng/mL MIP-1α + AOP-RANTES, 500 ng/mL
Wild type	16.7 ± 15.2	16.7 ± 15.2
CCR1$^{-/-}$	15.9 ± 24.9	9.1 ± 14.8
CCR3$^{-/-}$	37.0 ± 18.2	19.6 ± 20.9
CCR5$^{-/-}$	23.3 ± 9.1	24.4 ± 4.7
D6$^{-/-}$	18.8 ± 17.1	21.9 ± 21.0

Results are expressed as the mean percentage (± SD) of colonies generated in the presence of chemokine compared with control plates. Each result is representative of at least 5 independent experiments using bone marrow from individual mice. MIP indicates macrophage inflammatory protein.

Although these data do not rigorously rule out the alternative use of CCR3, the full inhibitory response of the CCR3$^{-/-}$ cells, the inability of eotaxin to work as an inhibitor, and the inability of excess eotaxin (20×) to block MIP-1α inhibition (G.J.G., unpublished observations, February 2000) argue strongly against an involvement of this receptor in the inhibitory process.

Thus, the above data are consistent with the use of a novel receptor for inhibition of primitive hemopoietic cells by MIP-1α. Although these studies have necessarily been performed using murine bone marrow, it is hoped that the results will have relevance to MIP-1α inhibition of human primitive cells, a process we have demonstrated previously to be independent of CCR1.[24]

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