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Induction of the Chemokine β Peptides, MIP-1α and MIP-1β, by Lipopolysaccharide Is Differentially Regulated by Immunomodulatory Cytokines γ-IFN, IL-10, IL-4, and TGF-β

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Abstract

The macrophage occupies a central role in the host response to invasion, exerting its control over the developing inflammatory response largely through the elaboration of an assortment of endogenous mediators including many cytokines. The β chemokine peptides, macrophage inflammatory protein [MIP]-1α and MIP-1β, are two such effectors markedly up-regulated in macrophages following exposure to bacterial lipopolysaccharide (LPS). These highly homologous peptides, like the other members of the β chemokine family, exhibit diverse but partially overlapping biological activity profiles, suggesting that the cellular participants and intensity of an inflammatory response may in part be regulated by selective expression of these chemokines. Studies reported here demonstrate that, in contrast to the "balanced" MIP-1α/MIP-1β chemokine responses of LPS-stimulated macrophage cultures in vitro, circulating levels of MIP-1β are significantly higher than those of MIP-1α following LPS administration in vivo. Further studies have revealed that several immunomodulatory cytokines known to be up-regulated in vivo as a consequence of exposure to an invasive stimulus (γ-IFN, IL-10, IL-4, and transforming growth factor [TGF]-β) down-regulated the LPS-induced release of MIP-1α by macrophages in vitro, but spared the MIP-1β response. This altered pattern of secretion may explain, at least in part, the high circulating levels of MIP-1β relative to MIP-1α observed in vivo in response to LPS challenge.

Introduction

Upon registering the presence of bacterial, parasitic, or viral invasion, host macrophages promptly achieve a state of activation that is characterized by increased phagocytic activity and enhanced release of reactive oxygen intermediates, cytokines, and a variety of other intercellular mediators and effectors (1,2). By these early responses, the macrophage not only intercedes directly against foreign invaders but also coordinates the participation of other host cell types. Broadly speaking, the magnitude, scope, and character of subsequent inflammatory and immune responses are known to differ according to the stimulus, but the factors that tailor specific response patterns are incompletely understood.

Within the cytokine network, a discrete set

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of highly homologous low-molecular-weight peptides exert potent chemotactic and activating properties (3,4). This set of peptides, referred to as the *chemokine family*, have been grouped together based upon both structural and functional similarities (5,6). Members of the chemokine family exert a wide range of clinically relevant activities including target cell–specific chemotaxis and activation (6,7) and modulation of immune responses (8,9). Members of the α (or C-X-C) subgroup of this family, which include IL-8 and *gro* α, have been characterized as potent neutrophil chemoattractants and activators and are thought to figure prominently in acute inflammatory responses (6). In contrast, members of the β (or C-C) branch, comprising macrophage inflammatory protein [MIP]-1α, MIP-1β, RANTES, JE/MCP-1, and I-309, are potent macrophage and lymphocyte chemoattractants, and as such, may play a critical role in more chronic mononuclear processes (6).

The magnitude and biological consequences of cytokine induction in vivo depend critically on the context established by the larger "cytokine network." Prior conditioning by cytokine activity within the network will markedly affect which cytokines are induced by specific stimuli, and the temporal sequencing and quantitative balance of specific cytokines produced in response to a stimulus will largely determine biological outcome. Assessing the relative abundance of induced cytokines is particularly important in the case of MIP-1α and MIP-1β, because in addition to mediating distinct biological actions (e.g., MIP-1α is chemotactic for activated $CD8^+$ lymphocytes and B cells, while MIP-1β is chemotactic for activated $CD4^+$ lymphocytes [8,9]), these two closely related chemokines can exert opposite autocrine effects on macrophages, a cell that is itself a principal component of the cytokine network. MuMIP-1α, for instance, is a positive stimulator of TNF-α secretion by macrophages, whereas muMIP-1β is a potent antagonist of MIP-1α in this regard (10). The antagonistic actions of these two peptides make it likely that the macrophage has evolved mechanisms to either coordinately or differentially regulate the expression of MIP-1α and MIP-1β, depending on the nature of the invasive stimulus and the integrated status of the cytokine network. Thus, selective production of the various chemokine peptides in vivo could be expected to lead to quantitative and even qualitative differences in the host response.

In the present work we demonstrate that although both MIP-1 peptides are concomitantly up-regulated upon exposure to lipopolysaccharide (LPS) in vitro in a ratio favoring MIP-1α, circulating levels of the two peptides following exposure to LPS in vivo exhibit a markedly different pattern, with MIP-1β levels on average 8-fold higher than those of MIP-1α. The further demonstration that several immunomodulatory cytokines (γ-interferon [IFN], interleukin [IL]-10, IL-4, and transforming growth factor [TGF]-β) potently down-regulate the LPS-induced release of MIP-1α by macrophages in vitro, while sparing the MIP-1β response, suggests a mechanism by which the in vivo pattern might arise and raises the possibility that one mechanism through which anti-inflammatory cytokines may shift the cytokine network from a proinflammatory to an anti-inflammatory state is by altering the balance of MIP-1 peptide production.

Materials and Methods

Materials

E. coli 0111:B4 LPS (Difco; Detroit, MI) was resuspended in pyrogen-free saline and stored at −20°C. Brewer's thioglycollate broth (Difco) was prepared according to the manufacturer's instructions, autoclaved, and stored at room temperature, protected from light. Murine MIP-1α and MIP-1β were expressed as recombinant peptides in yeast and were purified to homogeneity from yeast culture supernatants according to a previously published fractionation scheme (11). Recombinant murine tumor necrosis factor (TNF)-α was kindly provided by Genentech (San Francisco, CA). Murine recombinant γ-IFN was obtained from Gibco BRL (Grand Island, NY). Recombinant murine IL-4, IL-10, and TGF-β were obtained from R&D Systems (Minneapolis, MN), reconstituted according to the manufacturer's instructions, and stored in aliquots at −70°C. Rabbit anti-murine MIP-1α and rabbit anti-murine MIP-1β were generated by multiple-site immunization of New Zealand White rabbits with recombinantly derived peptides. Goat anti-murine MIP-1α and goat anti-murine MIP-1β were obtained from R&D.

Animals and Experimental Design

BALB/c, BALB/$c^{nu/nu}$, and C3H/HeN mice (female, 19–21 g) were obtained from Charles River (Kingston, NY), and C3H/HeJ mice (female, 17–21 g), from Jackson Laboratory (Bar Harbor, ME). Animals were housed in groups of

ten per cage and allowed to rest at least 1 week following arrival before being used in experimental protocols. Mice were injected intraperitoneally (ip) with sublethal doses of LPS (125 μg per mouse which is the equivalent of 6.3 mg/kg), and sacrificed 0.5, 1, 2, 4, 8 and 24 hr after LPS challenge to collect serum by cardiac puncture. Serum was analyzed for MIP-1α and MIP-1β by specific ELISAs, and TNF-α by L929 cytotoxicity assay (described below).

Cell Culture

Normal, thioglycollate-elicited mouse macrophages were obtained as described previously (12). In brief, 19–21 g BALB/c, BALB/$c^{nu/nu}$, C3H/HeN or C3H/HeJ mice were injected intraperitoneally with 2.0 ml of sterile thioglycollate broth. After 3 days, peritoneal exudate macrophages were harvested by peritoneal lavage and plated at a cell density of 1×10^6 cells/ml in 24-well (1.0 ml/well) or 48-well (0.5 ml/well) flat-bottomed tissue culture dishes. Following a 2-hr attachment period, the medium was removed by aspiration, monolayers rinsed three times with phosphate-buffered saline (PBS), and 1.0 ml fresh RPMI 1640 medium (Gibco BRL) supplemented with 10% fetal bovine serum (FBS) was added to each well. Peritoneal exudate macrophage cultures were used for stimulation experiments either immediately or following overnight incubation. Pilot experiments demonstrated no significant difference in cytokine stimulation profiles between cultures treated immediately and those treated after 24 hr. All cultures were maintained under a humidified atmosphere of 5% CO_2 in air at 37°C.

Stimulation of Macrophages by LPS and Immunomodulatory Cytokines

Just prior to the start of each stimulation experiment, medium was aspirated from confluent monolayer cultures of thioglycollate-elicited peritoneal exudate macrophages. Monolayers were washed one time with serum-free RPMI, and fresh RPMI 1640 supplemented with 1% FBS containing the indicated treatment was then added to each well. Cells were routinely cultured for 18 hr, at which time the conditioned media were collected for analysis. Supernatants were clarified by centrifugation at 10,000 × *g*, sterilized by filtration (0.22 μm), and stored frozen at −20°C until assays for MIP-1α and MIP-1β (specific ELISAs), and TNF-α (L929 cytotoxicity assay) were performed. For time-course studies, medium was removed from thioglycollate-elicited macrophage monolayer cultures by aspiration and replaced with medium containing the appropriate reagents. At appropriate time intervals, supernatant fluid was collected from each monolayer. Conditioned media were centrifuged at 10,000 × *g*, passed through a 0.22-μm filter, and then assayed in sterile serial dilution for MIP-1α, MIP-1β, and TNF-α.

Cytokine Assays

Murine MIP-1α and murine MIP-1β levels were each determined by a specific sandwich ELISA. The 96-well plates were coated for 2 hr at room temperature (or overnight at 4°C) with either goat anti-murine MIP-1α (for murine MIP-1α ELISA) or goat anti-murine MIP-1β (for MIP-1β ELISA). Coating antibodies were diluted in PBS/N_3 to 0.8 μg/ml, and 50 μl added per well (0.04 μg/well). After coating, the plates were washed 6 times with wash buffer (PBS supplemented with 0.05% Tween-20), and nonspecific binding sites blocked by incubation for at least 2 hr at room temperature after the addition of 100 μl/well blocking buffer (wash buffer supplemented with 0.6% BSA). Plates were rinsed 6 times with wash buffer, appropriate dilutions of test samples and standards (in 50 μl blocking buffer) were added to replicate wells, and plates were incubated for 2 hr at room temperature (or overnight at 4°C). Plates were then washed 6 times with wash buffer, and primary antibodies were added for 2 hr at room temperature. For both mouse MIP-1α and MIP-1β ELISAs, 0.05 μl/well of the appropriate rabbit anti-mouse MIP-1 peptide antisera in 50 μl blocking buffer was added. Plates were then washed 6 times with wash buffer, and secondary antibody (mouse anti-rabbit Ig conjugated to alkaline phosphatase [Sigma] diluted 1:5000 in blocking buffer) added for 1 hr at room temperature. Plates were washed 6 times with wash buffer, followed by two times with diethanolamine substrate buffer (pH 9.5; Pierce), and then 100 μl/well p-nitrophenyl phosphate substrate (1 mg/ml) was added in diethanolamine buffer. Color was allowed to develop, and absorbance was read at 405 nm. Standards were prepared in a 2-fold dilution series of recombinant murine MIP-1α or MIP-1β. These two chemokine sandwich ELISAs are specific (no detectable MIP-1α/MIP-1β cross-reactivity), and they consistently detect concen-

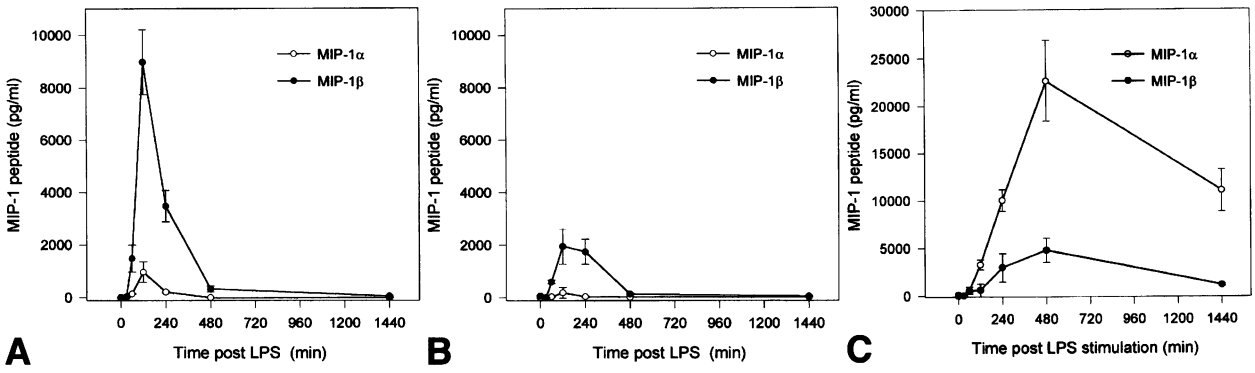

Fig. 1. The relative ratios of MIP-1α to MIP-1β released in response to LPS stimulation are different for in vivo versus in vitro models of endotoxemia. Circulating MIP-1α and MIP-1β levels following administration of a sublethal dose of LPS in (A) BALB/c and (B) BALB/c$^{nu/nu}$ mice. Mice were treated with LPS by intraperitoneal (ip) injection, and blood sampled at designated intervals. Quantification of MIP-1α (open circles) and MIP-1β (filled circles) in serum samples were determined by specific ELISA assays as described in Materials and Methods. Data are expressed as mean ± SEM (for A: n = 3 independent LPS-injection experiments; for B: n = 2 independent LPS-injection experiments). (C) Kinetics of LPS-induced MIP-1α (open circles) and MIP-1β (filled circles) appearance in murine macrophage cultures in vitro. Parallel cultures (0.5×10^6 thioglycollate-elicited peritoneal exudate macrophages) were incubated with 200 ng/ml LPS and the conditioned medium removed at specified intervals for ELISA analysis. Data are expressed as mean ± SEM (n = 3 independent experiments).

trations of murine MIP-1α or murine MIP-1β above 50 pg/ml.

TNF-α levels in the supernatant fluid of macrophage cultures were determined by an L929 cell cytotoxicity assay described previously (13,14). Briefly, L929 cells were suspended in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS and plated at 2×10^4 cells per well in 96-well flat-bottomed microtiter plates. After 24 hr, media were aspirated and then replaced with medium containing actinomycin D (1 μg/ml) and the samples to be assayed. Plates were incubated for 16 hr, at which time 10 μl of a 7 mg/ml solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) was added to each well. After 4 hr, the 570 nm absorbance was recorded, and cell viability was determined by the extent to which MTT is converted to a colored formazan product. Absorbance values were converted to units/ml by comparison with a standard curve for murine recombinant TNF-α.

Results

Circulating Levels of MIP-1β Are 4- to 10-fold Higher Than Those of MIP-1α in Mice Injected with LPS

The proinflammatory nature of the MIP-1 peptides led us to examine whether the kinetics of MIP-1 peptide release in response to LPS administration in vivo were similar to those reported in the conditioned medium of macrophages following stimulation by LPS in vitro. Mice were injected with LPS, sacrificed at intervals, and serum collected by cardiac puncture for quantification of MIP-1α and MIP-1β by ELISA. Circulating levels of MIP-1α and MIP-1β peaked early, at 2 hr post-injection (Fig. 1A). At all time points sampled, with the exception of the 24 hr time point (at which time both MIP-1α and MIP-1β were below the limit of detection), sera contained higher levels of MIP-1β than MIP-1α; although quite low, there was still detectable circulating MIP-1β at 8 hr post-injection when no MIP-1α was measurable. These kinetics of MIP-1α and MIP-1β appearance in the circulation following LPS administration in vivo are markedly different from the kinetics of MIP-1 peptide accumulation in media conditioned by macrophages cultured in vitro and stimulated with LPS (Fig. 1C), where the net release of both MIP-1α and MIP-1β peaked at approximately 8 hr, and the amount of MIP-1α released into the culture medium was always 4- to 10-fold higher than that of MIP-1β. TNF-α levels were assessed in these serum samples, and as expected, TNF-α was detectable at 30 min post-injection, and levels peaked at 1 hr.

The marked difference in the MIP-1α-to-MIP-1β ratio found in vivo following LPS-injec-

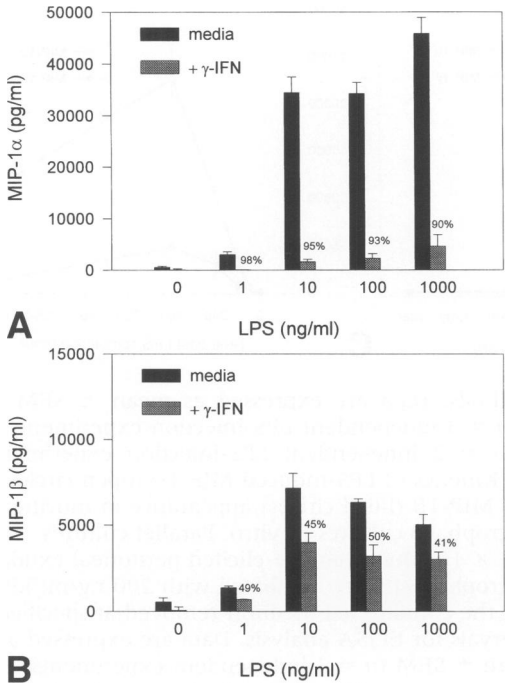

Fig. 2. Costimulation with γ-IFN completely blocks the LPS-induced release of MIP-1α by murine thioglycollate-elicited peritoneal exudate macrophages in vitro, while sparing the MIP-1β response. (A) Macrophages (1×10^6 cells) were incubated for 18 hr in medium supplemented with the indicated amount of LPS, either in the absence (solid bars) or presence (cross-hatched bars) of γ-IFN (200 units/ml). Culture medium was then removed and the MIP-1α content quantified by specific ELISA. Data are from a representative experiment (of 3) and are expressed as the mean ± SD of values obtained from duplicate macrophage stimulations. The % values noted above each cross-hatched bar refers to % suppression in γ-IFN-treated cultures compared with control cultures in which γ-IFN was not included. (B) As in (A), macrophages (1×10^6 cells) were incubated for 18 hr with the indicated amount of LPS either in the absence (solid bars) or presence (cross-hatched bars) of γ-IFN (200 units/ml). Culture medium was then removed and the MIP-1β content quantified by specific sandwich ELISA. Data are from a representative experiment and are expressed as the mean ± SD.

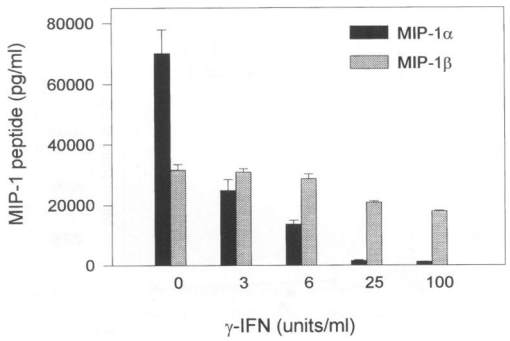

Fig. 3. The suppressive effect of γ-IFN on LPS-induced MIP-1α release is dose-dependent. Macrophages (1×10^6 cells) were incubated for 18 hr with LPS (10 ng/ml) either in the absence or presence of γ-IFN at the indicated doses. Culture medium was then removed and the MIP-1α (solid bars) content quantified by ELISA. Data are from a representative experiment (of 3) and are expressed as the mean ± SD of values obtained from duplicate macrophage stimulations. Lesser relative suppression of MIP-1β release was observed in γ-IFN-treated cultures (cross-hatched bars).

tion to that found in vitro following LPS-stimulation of macrophage monolayers suggested that the macrophage might not be the major source of LPS-induced MIP-1β in our in vivo model of endotoxemia. Activated T cells are known to produce significant quantities of both MIP-1α and MIP-1β, and therefore, serum levels of the two peptides were assessed in T cell–deficient BALB/$c^{nu/nu}$ mice. The kinetics of MIP-1 peptide release into the circulation in BALB/$c^{nu/nu}$ mice was similar to that seen in wild-type BALB/c mice (Fig. 1B). Comparison of Figure 1A and B (identical *y* axis scale), however, reveals that the magnitude of serum appearance of both MIP-1α and MIP-1β was markedly lower in the T cell–deficient mice.

γ-IFN Differentially Regulates LPS Induction of MIP-1α and MIP-1β

Although stimulation of thioglycollate-elicited peritoneal exudate macrophages with endotoxin alone results in the concommitant induction of both MIP-1α and MIP-1β peptides (with MIP-1α being produced in 4-fold excess of MIP-1β), costimulation of macrophages with endotoxin plus γ-IFN, a potent proinflammatory agonist, resulted in a markedly different outcome. Pretreatment with γ-IFN completely blocked subsequent induction of MIP-1α by LPS at all concentrations of LPS examined (Fig. 2A), when the production of MIP-1β, although diminished somewhat, always remained ≥50% of that released by macrophages treated with LPS alone (Fig. 2B). The decrease in MIP-1α protein accumulation was dose-dependent and was apparent when macrophage cultures were treated with as little as 6 units of γ-IFN/ml (Fig. 3). γ-IFN suppression of LPS-induced MIP-1α release did not require the presence of γ-IFN for the full course of the LPS treatment period, as evidenced by wash-out ex-

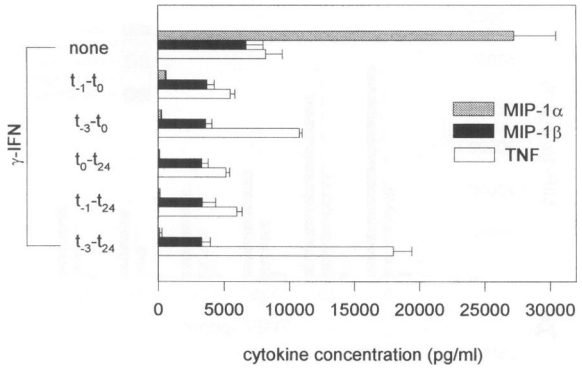

Fig. 4. Kinetics of γ-IFN down-regulation of LPS-induced MIP-1α expression. Macrophages (1×10^6 cells) were incubated with medium alone or with γ-IFN (200 units/ml) for the times indicated on the left axis, and at time zero (t_0) LPS was added to a final concentration of 100 ng/ml. Supernatant fluids were collected 24 hr later and assayed for MIP-1 peptides by separate ELISAs. Data are from a representative experiment (of 2) and are expressed as the mean ± SD of values obtained from duplicate macrophage stimulations. As is evident from this figure, pretreatment of macrophages for as little as 1 hour leads to a marked drop in the amount of MIP-1α released in response to LPS stimulation, with less affect on MIP-1β.

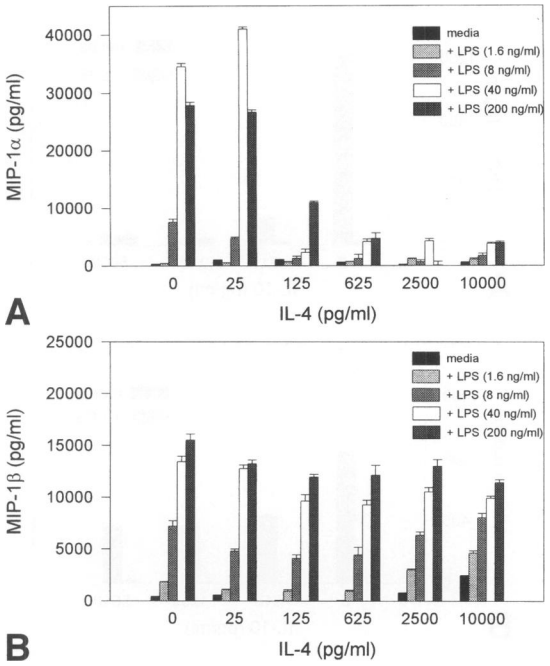

Fig. 5. Costimulation of macrophages with IL-4 does not inhibit the release of MIP-1β by LPS-stimulated macrophages in vitro at concentrations that completely shut off LPS-induced production of both MIP-1α and TNF-α. (A, B) Macrophages (1×10^6 cells) were incubated with the indicated amount of IL-4 either in the absence (solid bars) or presence of various concentrations of LPS. Culture medium was collected at 18 hr and the MIP-1α (A) and MIP-1β (B) content quantified by separate ELISAs. Data are from a representative experiment (of 2) and are expressed as the mean ± SD of values obtained from duplicate macrophage stimulations.

periments in which the macrophages were pretreated for either 1 or 3 hr with γ-IFN, washed thoroughly, and then stimulated with LPS (Fig. 4). Similar results were observed with resident peritoneal macrophages and RAW 264.7 cells (data not shown). For comparison, TNF-α levels were measured in all supernatants, and as expected, γ-IFN synergized with LPS to induce markedly higher levels of TNF-α than if cells were treated with LPS alone (see Fig. 4, t_{-3}–t_{24} pretreatment with γ-IFN). When a lower dose of LPS was used in stimulation experiments the enhancement of LPS-induced TNF release upon costimulation with γ-IFN was more pronounced, while the effects on MIP-1 peptide production remain unchanged (data not shown).

Th2 Cytokines IL-10 and IL-4 Also Differentially Regulate LPS-induced Release of MIP-1α and MIP-1β by Macrophages

The observation that one of the classic Th1 cytokines, γ-IFN, differentially modulated the LPS-induced release of the MIP-1 peptides suggested that the two β chemokines, although highly homologous, might be participating in discrete aspects of the host response to inflammation. In sharp contrast to Th1 cytokines, which augment host inflammatory responses, Th2 cell–derived lymphokines are now recognized as providing some of the negative regulatory signals required to shut down the response. We therefore initiated studies to assess what effect IL-4 (and IL-10 as well) had on the relative ratio of MIP-1α to MIP-1β released by LPS-stimulated macrophages. Costimulation of thioglycollate-elicited macrophage cultures with LPS and IL-4 completely prevented MIP-1α expression (Fig. 5A) without markedly affecting MIP-1β expression (Fig. 5B), thereby shifting the balance of MIP-1 peptide production from MIP-1α to MIP-1β. We next examined the effects of costimulating macrophages with LPS and IL-10. Similar to observations with IL-4, costimulation with IL-10 did not inhibit the release of MIP-1β by LPS-stimulated macrophages in vitro (Fig. 6B), even at

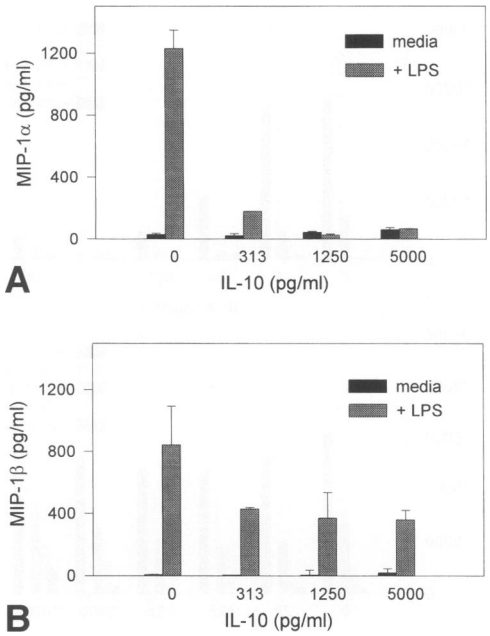

Fig. 6. IL-10 completely blocks LPS-induced release of MIP-1α, while only partially inhibiting release of MIP-1β. (A, B) Macrophages (1×10^6 cells) were incubated for 18 hr with 10 ng/ml LPS either in the absence (solid bars) or presence (cross-hatched bars) of the indicated amount of IL-10. Culture medium was then removed and the MIP-1α content quantified by murine MIP-1α ELISA (A) or murine MIP-1β ELISA (B). Data are from a representative experiment (of 3) and are expressed as the mean ± SD of values obtained from triplicate macrophage stimulations.

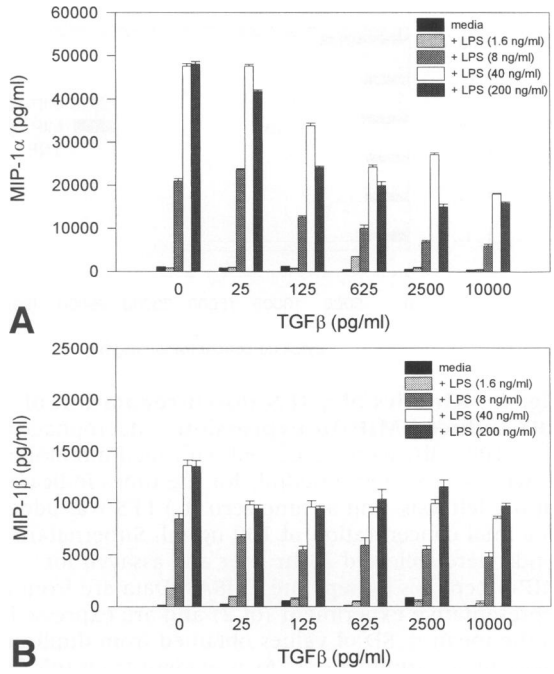

Fig. 7. TGF-β dose-dependently inhibits LPS-induced MIP-1α release in vitro, but is without significant effect on MIP-1β. (A, B) Macrophages (1×10^6 cells) were incubated for 18 hr with the indicated amount of TGF-β either in the absence (solid bars) or presence of varying concentrations of LPS. Culture medium was then removed and the MIP-1 peptide content quantified by murine MIP-1α ELISA (A) or murine MIP-1β ELISA (B). Data are from a representative experiment (of 3) and are expressed as the mean ± SD of values obtained from triplicate macrophage stimulations.

concentrations effective to completely block the production of both MIP-1α (Fig. 6A) and TNF-α (data not shown).

TGF-β, a "Suppressor" Cytokine, Also Fails to Down-modulate the LPS-induced Release of MIP-1β

Since both Th1 and Th2 cytokines were observed to down-modulate LPS-induced MIP-1α release while sparing MIP-1β release, we hypothesized that there might be a global difference in the ability of macrophages to respond to negative immunomodulatory cytokine signals specifically with respect to MIP-1β. To test this, we examined whether TGF-β, another cytokine capable of suppressing many host inflammatory responses (e.g., TNF-α release by LPS-stimulated macrophages), but one which does not fall within either the Th1 or Th2 class, would down-modulate LPS-induced production of MIP-1β. Costimulation of macrophages with TGF-β failed to block the release of MIP-1β by LPS-stimulated macrophages in vitro (Fig. 7B). But, surprisingly, treatment with TGF-β did not completely down-regulate LPS-induced release of MIP-1α (Fig. 7A). Each supernatant was assayed for bioactive TNF-α by L929 cytotoxicity assay as a control, and as expected from previously published work (15), complete suppression was observed (data not shown).

Discussion

One of the hallmarks of both acute and chronic inflammation is the recruitment and activation of several successive populations of responding cells. These processes are largely controlled by the coordinated expression and release by macrophages and other inflammatory cells of a large repertoire of endogenous mediators. Prominent

among these proinflammatory mediators are the peptide cytokines, which include the β chemokines MIP-1α and MIP-1β. Regulatory and counterregulatory pathways governing the expression of chemokine peptides have been only partially characterized. Here, we have demonstrated that LPS-induced MIP-1 peptides accumulate differently in vivo versus in vitro. The observation that circulating levels of MIP-1β are significantly higher (at least 8-fold) than those of MIP-1α in a murine model of endotoxemia was unexpected, as supernatants conditioned by endotoxin-stimulated macrophages in vitro contained MIP-1α in greater than 4-fold excess over MIP-1β. This disparity illustrates the need to cross-check in vitro response profiles against the actual scope of cytokine responses in vivo.

Several potential explanations exist for the altered pattern of MIP-1 peptide appearance in mice administered LPS. Circulating cytokine levels reflect the integrated output of many cell types, and activation of cell types other than the macrophage might lead to the release of large amounts of MIP-1β, relative to MIP-1α, into the circulation, tipping the balance in favor of MIP-1β. Because activated T cells are known to be a rich source of MIP-1 peptides, T cell–deficient mice were injected with LPS and the serum levels of MIP-1α and MIP-1β quantitated by ELISA. Although the levels of circulating MIP-1 in these T cell–deficient mice were markedly decreased (5-fold on average), the ratio between the two peptides remained unchanged, arguing against T cells as the source of large amounts of MIP-1β relative to MIP-1α. The lower overall accumulation of MIP-1 peptides did, however, indicate that, at least in this mouse model of endotoxemia, T cells are either a major source of both MIP-1α and MIP-1β, or T cells act by unknown mechanisms to markedly upregulate the production of both peptides by other cell types.

The altered pattern of MIP-1 peptide appearance in vivo could alternatively result from the action of immunomodulatory cytokines up-regulated as a consequence of exposure to the invasive stimulus. γ-IFN is known to be up-regulated in animal models of sepsis, as well as in septic patients, and therefore the effect of co-stimulation with γ-IFN, generally considered an enhancer of macrophage inflammatory responses, on the magnitude of the LPS-induced MIP-1 peptide response was examined. Pretreatment with γ-IFN completely blocked the induction of MIP-1α by LPS at all concentrations of LPS examined, whereas the production of MIP-1β was much less affected, never dropping to below 50% of the amount released upon stimulation with LPS alone. This essentially reproduces, under in vitro conditions, the ratio of MIP-1α to MIP-1β that we had observed in vivo in response to LPS administration. The suppressive action of γ-IFN with respect to MIP-1α induction might afford a counterbalance by which the proinflammatory biologic potency of MIP-1α would not be brought into play in an ongoing host response that has already produced γ-IFN as a proinflammatory conditioning cytokine. Alternatively, this differential induction might have evolved to allow the biological activity of MIP-1β to predominate in the absence of MIP-1α. A similar suppressive effect of γ-IFN on LPS-induced expression of two other chemokine peptides, JE/MCP-1 and KC/GRO, has already been demonstrated (16), suggesting that γ-IFN may be an important immunomodulatory factor that differentially regulates the expression of several members of the chemokine family. One could envision that the activity of γ-IFN to down-modulate the release of MIP-1α, while allowing the release of MIP-1β to remain essentially unchanged, might be critically important in select circumstances. For example, MIP-1α, in addition to its chemoattractant properties, is known to activate macrophages, eosinophils, and basophils (6). Such cellular-activating capabilities, although adaptive early in the course of an inflammatory response, might become detrimental once the invading pathogen was destroyed and tissue repair processes initiated. At such times, host mechanisms might be brought into play to minimize the release of activation-inducing cytokines, while continuing to maintain the capacity to attract resting monocytes and lymphocytes. MIP-1β, which is notably lacking in the cellular activating properties described for MIP-1α, is an ideal candidate. Similarly, it might be advantageous to maintain levels of MIP-1β, in the absence of activating cytokines like MIP-1α, during the wound-healing process or during granuloma formation. In both such instances, γ-IFN is expressed at high levels within the microenvironment and would act to down-modulate MIP-1α expression while sparing MIP-1β responses (17,18).

Th2 cell–derived lymphokines are now recognized for their role as important negative regulators of inflammatory responses. They exert their negative effects, in part, by suppressing the production of a broad range of proinflammatory cytokines, including IL-1, TNF-α,

IL-6, and IL-8 (19,20). Such Th2 effects are believed to mitigate some of the tissue damaging effects of immune responses mediated by Th1 cytokines. Since γ-IFN, a Th1 cytokine, was observed to down-modulate the appearance of LPS-induced MIP-1α but not MIP-1β, we decided to examine how costimulation with Th2 cytokines might affect the ratio of MIP-1α to MIP-1β. A previous study demonstrated that IL-4 down-modulates LPS-induced expression of MIP-1α mRNA (21). We found that both IL-4 and IL-10 down-modulated MIP-1α at the level of secreted protein while sparing MIP-1β responses. Thus, both Th1 and Th2 cytokines act similarly to down-modulate the expression of a proinflammatory chemokine, MIP-1α, while sparing its counterpart.

TGF-β, another pleiotropic cytokine known to exert potent regulatory effects on a broad spectrum of cell types, was also examined for its activity on LPS-induced MIP-1 peptide expression. With polymorphonuclear leukocytes and endothelial cells, TGF-β treatment both suppresses the release of reactive oxygen and nitrogen intermediates and inflammatory cytokines, and counteracts the effects of inflammatory cytokines (22). Indeed, TGF-β knockout mice develop a severe inflammatory syndrome, characterized by a massive infiltrate of mononuclear cells into the vital organs within 3 weeks of birth (23,24). As expected, costimulation of macrophages with TGF-β and LPS led to decreased production of MIP-1α without affecting the expression of MIP-1β.

As a general feature of cytokine biology, synthesis and release of a given mediator depends critically on the combined interaction of many other participants in the cytokine network. The requirement, for instance, of precisely sequenced or contemporaneous signals may serve to protect the host against the unintended elicitation of too vigorous a tissue response. The chemokines in general, and MIP-1α and MIP-1β in particular, manifest qualities that could be important within the self-regulatory properties of the cytokine network. They appear to have evolved to selectively recruit specific cell types rather than globally eliciting immune effector cells. Combined with their selective activation of specific effector populations, this would tend to reinforce certain limbs of the host response while minimizing others, thereby tailoring the host response to the specific invasive challenge.

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