Interferon-γ Differentially Regulates Monocyte Matrix Metalloproteinase-1 and -9 through Tumor Necrosis Factor-α and Caspase 8*  

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Tumor necrosis factor-α (TNFα) and granulocyte macrophage colony-stimulating factor (GM-CSF) individually enhance monocyte matrix metalloproteinase-9 (MMP-9) but induce MMP-1 only when added in combination. Because interferon-γ (IFNγ) is also found at inflammatory sites, we determined its effect on monocyte MMPs in the presence or absence of TNFα and GM-CSF. IFNγ alone did not stimulate monocyte MMP-9 or MMP-1; however, in the presence of GM-CSF it induced MMP-1 and enhanced MMP-1 stimulated by GM-CSF and TNFα. IFNγ induced MMP-1 in the presence of GM-CSF through the stimulation of TNFα production by a mechanism involving both p38 and ERK1/2 MAPKs, in which GM-CSF-stimulated ERK1/2 whereas IFNγ activated p38. In support of this conclusion TNFα neutralizing antibody and antibodies against TNF receptor I and II blocked the induction of MMP-1 by GM-CSF and IFNγ. In contrast to its effects on MMP-1, IFNγ inhibited TNFα-induced MMP-9 through a caspase 8-dependent pathway as demonstrated by the restoration of MMP-9 with caspase 8 inhibitors. Moreover, the phosphorylation of STAT1 by IFNγ was blocked by an inhibitor of caspase 8, indicating that STAT1 had a suppressive effect on MMP-9. Caspase 8-mediated phosphorylation of STAT1 through p38 MAPK as shown by the inhibition of IFNγ-induced phosphorylation of p38 by caspase 8 inhibitors. Activation of caspase 8 by IFNγ did not result in increased apoptosis. Thus IFNγ in the presence of GM-CSF and/or TNFα differentially regulates monocyte MMPs through induction of TNFα and a novel mechanism involving caspase 8 that is independent of apoptosis.

Monocytes/macrophages are prominent at sites of inflammation associated with extensive connective tissue destruction. They are thought to contribute to the loss of connective tissue components at these sites through the production of matrix metalloproteinases (MMPs). MMPs are comprised of a family of extracellular matrix degrading enzymes that include the interstitial collagenases, gelatinases, stromelysins, matrilysin, metalloelastase, and membrane-type MMPs (1–3). MMP-1/interstitial collagenase and MMP-9/92-kDa gelatinase are major MMPs produced by monocytes. Fibrillar collagens, such as type I and II collagens, are degraded primarily by interstitial collagenases whereas MMP-9 is involved in degrading fibrillar collagens that have been denatured by the initial cleavage by MMP-1. Additionally, MMP-9 cleaves substrates, such as laminin and type IV collagen, that comprise the basement membrane.

As monocytes migrate into an inflammatory site they are exposed to multiple cytokines that influence the MMPs produced by these cells. The Th2 cytokines, IL-4 and IL-10, have been shown to suppress monocyte MMP production (4–7). Inhibition of monocyte MMPs by these Th2 cytokines is related in large part to their suppression of prostaglandin H synthase-2 or cyclooxygenase-2 resulting in the loss of prostaglandin E2 required for the induction of MMPs (4, 6, 8). A number of cytokines have also been shown to induce or enhance MMP production by monocytes. Depending on the specific cytokine or combination of cytokines, monocyte MMP production can be differentially regulated. For example, TNFα, IL-1β, or GM-CSF, when added individually to monocytes, enhance the expression of 92-kDa gelatinase (MMP-9) but not interstitial collagenase (MMP-1) (9, 10). However, the combination of TNFα with GM-CSF induces the production of MMP-1 by monocytes through a prostaglandin-dependent pathway and synergistically enhances MMP-9 through a prostaglandin-independent mechanism (10).

Another cytokine produced by activated T cells and NK cells at inflammation sites is IFNγ and has classically been considered an activator of monocytes. However, IFNγ has been shown previously (9, 11, 12) to inhibit the production of MMPs by monocytes/macrophages stimulated with Con A or LPS. Because it is most likely that IFNγ interacts in vivo with monocytes in the context of other cytokines, we hypothesized that its effect on monocyte MMPs may be different in the presence of other cytokines than with Con A or LPS. We therefore examined the effect of IFNγ on the production of monocyte MMPs in the presence or absence of TNFα and/or GM-CSF, as well as the mechanisms through which IFNγ might mediate its effects.

Here we report that IFNγ in the presence of TNFα and/or GM-CSF differentially regulates the production of monocyte MMP-9 and MMP-1. Although IFNγ alone does not stimulate monocyte MMP production, it induces MMP-1 when combined with GM-CSF and enhances the production of MMP-1 induced by GM-CSF and TNFα. This occurs as a result of IFNγ-mediated caspase-8, GADD45, glyceraldehyde-3-phosphate dehydrogenase; IRF1, interferon regulatory factor; TNFR, tumor necrosis factor receptor.

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1 The abbreviations used are: MMP, matrix metalloproteinase; MMP-1, interstitial collagenase; MMP-9, 92-kDa gelatinase; STAT1, signal transducer and activator of transcription; IL, interleukin; TNF, tumor necrosis factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; LPS, lipopolysaccharide; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; Z, benzoyloxy carbonyl; FMK, fluoromethyl ketone; RT, reverse transcriptase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IRF, interferon regulatory factor; TNFR, tumor necrosis factor receptor.

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ated increase in monocyte TNFα production when combined with GM-CSF and involves both p38 and ERK1/2 mitogen-activated protein kinases (MAPKs). In contrast, IFNγ inhibits TNFα-induced MMP-9 production through a novel mechanism involving caspase 8 regulation of STAT1 through p38 MAPK that is independent of apoptosis.

**EXPERIMENTAL PROCEDURES**

**Purification of Human Monocytes**—Human peripheral blood cells were obtained by leukapheresis of normal volunteers at the Department of Transfusion Medicine at the National Institutes of Health. These cells were diluted in endotoxin-free phosphate-buffered saline with 5 mM Ca2+ and Mg2+ (BioWhittaker, Walkersville, MD) supplemented with 2 mM L-glutamine (Mediatech, Washington, D.C.) and 10 μg/ml gentamicin sulfate (BioWhittaker, Walkersville, MD). IFNγ (1 × 10⁴ units/mg), TNFα (1 × 10⁴ units/mg), and GM-CSF (1 × 10⁴ units/mg) were obtained from PeproTech (Rocky Hill, NJ). Apoptotic monocytes were determined by fluorescence-activated cell sorter analysis following staining with annexin V-fluorescein isothiocyanate (Trevenien Inc., Gaithersburg, MD). TNFα, TNFR-I, and TNFR-II blocking antibodies (R & D systems, Minneapolis, MN) and caspase inhibitors including Z-DEVD-FMK (caspases 3, 6, 7, 8, 10), Z-YVAD-FMK (caspase 1), Z-LEHD-FMK, Z-VEID-FMK (caspase 6), Z-LETD-FMK (caspase 8), Z-LEHD-FMK, and Z-VAD-FMK (a general inhibitor of all caspases) (ENZYMEP Systems Products, Livermore, CA and Calbiochem) were added to some of the cultures. Unless stated otherwise, following purification the monocytes were adhered for 30 min prior to the addition of reagents. Each experiment was repeated a minimum of three times with different donors.

**Western Analysis of MMP-1 and MMP-9**—For detection of MMP-1 and MMP-9, the conditioned media from monocyte cultures (5 × 10⁶ cells/ml of Dulbecco’s modified Eagle’s medium (BioWhittaker, Walkersville, MD) supplemented with 2 mM L-glutamine (Mediatech, Washington, D.C.) and 10 μg/ml gentamicin sulfate (BioWhittaker, Walkersville, MD), IFNγ (1 × 10⁴ units/mg), TNFα (1 × 10⁴ units/mg), and GM-CSF (1 × 10⁴ units/mg) were obtained from PeproTech (Rocky Hill, NJ). Apoptotic monocytes were determined by fluorescence-activated cell sorter analysis following staining with annexin V-fluorescein isothiocyanate (Trevenien Inc., Gaithersburg, MD). TNFα, TNFR-I, and TNFR-II blocking antibodies (R & D systems, Minneapolis, MN) and caspase inhibitors including Z-DEVD-FMK (caspases 3, 6, 7, 8, 10), Z-YVAD-FMK (caspase 1), Z-LEHD-FMK, Z-VEID-FMK (caspase 6), Z-LETD-FMK (caspase 8), Z-LEHD-FMK, and Z-VAD-FMK (a general inhibitor of all caspases) (ENZYMEP Systems Products, Livermore, CA and Calbiochem) were added to some of the cultures. Unless stated otherwise, following purification the monocytes were adhered for 30 min prior to the addition of reagents. Each experiment was repeated a minimum of three times with different donors.

**RESULTS**

**Regulation of MMP by Interferon-γ**—The association of interferon-γ (IFNγ) with the cytokine TNFα, play a role in the pathogenesis of chronic inflammatory lesions suggests that it may act in concert with these cytokines to modulate monocyte MMPs. Therefore, we tested the effect of IFNγ on the production of monocyte MMPs in the presence or absence of TNFα and GM-CSF. IFNγ alone failed to influence the production of MMP-1 and MMP-9 by monocytes (Fig. 1). However, synthesis of monocyte MMP-1, as detected by Western analysis, was induced when IFNγ was combined with GM-CSF but not TNFα (Fig. 1). Moreover, the stimulation of monocyte MMP-1 by the combination of GM-CSF and TNFα was significantly enhanced by IFNγ (Fig. 1). In
contrast, as shown previously (9), IFNγ suppressed the enhancement of MMP-9 by TNFα. Although the lowest concentration of IFNγ utilized in this experiment was 10 ng/ml, inhibition of TNFα-induced MMP-9 was also observed with 0.5 to 10 ng/ml of IFNγ (data not shown). However, the GM-CSF-mediated increase in MMP-9 was further enhanced by IFNγ, whereas the 1.8-fold increase, as determined by densitometry, in MMP-9 stimulated by the combination of TNFα and GM-CSF was slightly decreased by IFNγ. These results demonstrate that whereas IFNγ alone has no effect on monocyte MMP-1, it can significantly affect the induction or enhancement of MMP-1 when combined with other cytokines, whereas IFNγ can have stimulatory or inhibitory effects on MMP-9 depending on the specific cytokines present.

**Effect of Time of Addition of IFNγ on MMP-1 and MMP-9**

**Induction**—In light of the ability of IFNγ to induce or enhance MMP-1 when added with GM-CSF or GM-CSF plus TNFα, respectively, IFNγ was added at various times with respect to the other cytokines to determine the time course of the IFNγ modulatory effects. As shown in Fig. 2A, when IFNγ was added at the indicated times with GM-CSF the optimal induction occurred if these two cytokines were added at the same time. When IFNγ was added 1 h prior to GM-CSF it was not as effective at inducing MMP-1, and when added 4.5 h after GM-CSF the effect of IFNγ was essentially lost. Similarly, when IFNγ was added 1 h before the combination of GM-CSF and TNFα it further enhanced MMP-1 production, but the optimal response, as with IFNγ and GM-CSF, occurred when all three cytokines were added at the same time. As with GM-CSF, the effect of IFNγ was lost when it was added 4.5 h after the combination of GM-CSF and TNFα. Examination of the time-dependent effects of IFNγ on GM-CSF-induced MMP-9 revealed a kinetic pattern similar to that observed with MMP-1 (Fig. 2B). In contrast, when added with GM-CSF and TNFα, IFNγ, in general, suppressed MMP-9 production. Because the suppressive effects of IFNγ on GM-CSF and TNFα-induced MMP-9 were not lost by 4.5 h, and may be related to its suppression of TNFα-stimulated MMP-9 as shown in Fig. 1, we examined the effect of IFNγ on TNFα-induced MMP-9 at later time points. As shown in Fig. 2C, IFNγ suppressed TNFα-induced MMP-9 when added as late as 4 to 8 h after TNFα. The failure of IFNγ to inhibit MMP-9 after 8 h was related to the induction of MMP-9 mRNA above control (media only) levels by TNFα between 8 to 12 h (data not shown).

**IFNγ and GM-CSF Stimulate Monocyte MMP-1 and MMP-9 through the Induction of TNFα**—A potential mechanism by which IFNγ may stimulate MMP-1 in the presence of GM-CSF and enhance MMP-9 may be because of induction of another cytokine(s). We tested this possibility through the addition of neutralizing antibodies. As shown in Fig. 3A, neutralizing antibodies against TNFα blocked the induction of MMP-1 and the enhancement of MMP-9 by IFNγ and GM-CSF. Moreover, antibodies against TNFRI and -II also significantly decreased MMP-1 and MMP-9 production indicating that TNFα utilized both receptors in the stimulation of monocyte MMPs. These findings suggested that IFNγ, when added with GM-CSF, stimulated TNFα production that, in turn, resulted in the induction of MMP-1 and enhancement of MMP-9. As shown in Fig. 4, A and B, whereas GM-CSF or IFNγ caused a slight increase in TNFα mRNA and protein, when these cytokines were added in combination to monocytes there was an 11-fold increase in
TNFα mRNA and protein. Stimulation of TNFα production was detected 7 h after the addition of GM-CSF and IFNγ with a substantial increase in TNFα occurring between 17 and 28 h (Fig. 5A). Addition of IFNγ at varying times relative to GM-CSF revealed that when IFNγ was added 4.5 h after GM-CSF its ability to induce TNFα was reduced by ~67% (Fig. 5B). These findings demonstrate that the effect of IFNγ on monocyte MMP production when combined with GM-CSF is mediated through the stimulation of TNFα and explain why this effect is lost after 4.5 h.

**GM-CSF and IFNγ Induce TNFα through p38 and ERK1/2 MAPKs**—To determine the possible mechanism(s) by which GM-CSF and IFNγ induce TNFα, we examined the role of MAPKs in the regulation of TNFα. Addition of the p38 inhibitor, SB203580, or the ERK1/2 inhibitor, PD98059, suppressed the level of TNFα detected in the media (Fig. 6A). PD98059 caused the greatest inhibition of TNFα. Examination of the activation of p38 and ERK1/2 revealed that GM-CSF increased ERK1/2 phosphorylation whereas IFNγ enhanced the activation of p38 (Fig. 6B). These results demonstrate that both p38 and ERK1/2 are required for TNFα production by IFNγ and GM-CSF.

**Caspase 8 Inhibitors Reverse IFNγ-mediated Inhibition of TNFα-induced MMP-9**—As shown in Fig. 1, unlike its ability to increase MMP-1 and MMP-9 in the presence of GM-CSF, IFNγ suppressed TNFα-induced MMP-9 production. Because recent studies have shown that IFNγ can activate caspase 8 in other cell types (16, 17), we examined whether activation of caspasases might be the mechanism through which IFNγ suppresses TNFα-mediated stimulation of monocyte MMP-9. Addition of an inhibitor of caspases 6, 8, 9, and 10 to TNFα- and IFNγ-treated monocytes resulted in a significant restoration of MMP-9 as shown by Western (Fig. 7A) and zymogram analysis (Fig. 7B). An inhibitor of caspases 3, 6, 7, 8, and 10 also restored MMP-9 production, although to a lesser degree, because this is primarily a caspase 3 inhibitor (Fig. 7A). In contrast, an inhibitor of caspases 1 (interleukin 1β-converting enzyme) and 4 failed to restore MMP-9 production (Fig. 7A).

We further examined the individual inhibitors against caspase 6, 8, and 9, as well as a general caspase inhibitor. The inhibitor of caspase 8 activity, as well as a general inhibitor (all), but not caspase 9, reversed the inhibition by IFNγ- or TNFα-induced MMP-9 production (Fig. 7B). This was shown to be because of inhibition at the transcriptional level as demonstrated by restoration of MMP-9 mRNA by an inhibitor of caspase 8 (Fig. 7C).

**Stimulation and Activation of Caspase 8 by IFNγ**—The caspase inhibitor studies indicated that IFNγ was mediating its inhibitory effect on TNFα-stimulated MMP-9 through a caspase pathway. To examine this possibility we next determined the effects of IFNγ on the activation of caspase 8, because it is an apical caspase involved in downstream effects on other caspasases, and it was shown by our inhibitor studies to be involved in restoration of MMP-9. Western analysis revealed that IFNγ or TNFα increased the 55-kDa proenzyme form of caspase 8, as well as the first cleavage products, which are 45/43-kDa fragments (Fig. 8A). However, only in the presence of IFNγ were significant amounts of the p25-kDa fragment detected, which indicates an increase of the active p18 fragment of caspase 8 that was not detected with this antibody, as both p25- and p18-kDa fragments are derived from cleavage of the p43-kDa fragment. However, the p18 fragment was detected with a specific antibody against this fragment and was only induced in the presence of IFNγ (Fig. 8B). In contrast, stimulation with TNFα alone failed to result in substantial amounts of the p25 or p18 fragment of caspase 8.

**Caspase 8 Inhibition Suppresses IFNγ-induced STAT1 Phosphorylation**—IFNγ mediates many of its effects on cellular function through the phosphorylation of STAT1. Moreover, recently IFNγ-induced phosphorylation of STAT1 has been shown to be involved in the inhibition of TNFα-stimulated MMP-9 in human Ewing’s sarcoma EW-7 cells (18). We there-
through which IFN-STAT1 through p38 MAPK—To determine the mechanism
of STAT1. As shown in Fig. 10A, IFN-α/H9253 in Fig. 9, IFN-α stimulated phosphorylation of STAT1. These findings indicate caspase 8 is involved in the regulation of the phosphorylation of STAT1.

IFN-γ-activated Caspase 8 Regulates Phosphorylation of STAT1 through p38 MAPK—To determine the mechanism through which IFN-γ activation of caspase 8 regulates STAT1 phosphorylation we initially examined the potential role of p38 and ERK1/2 MAPKs in IFN-γ-mediated phosphorylation of STAT1. As shown in Fig. 10A, IFN-γ stimulated the phosphorylation of p38, as also shown in Fig. 6B; however, IFN-γ did not increase the phosphorylation of ERK1/2. Involvement of p38 in the phosphorylation of STAT1 was demonstrated through the use of SB203580, a specific inhibitor of p38 MAPK, which suppressed the phosphorylation of STAT1 by IFN-γ (Fig. 10B). In contrast, PD98059, an inhibitor of ERK1/2, had no affect on the phosphorylation of STAT1.

Caspase 8 Regulates the Activation of p38 by IFN-γ and TNFα—Because IFN-γ induction of p38 activity was involved in the phosphorylation of STAT1 we next examined the effect of caspase 8 inhibition on the phosphorylation of p38 by IFN-γ and the combination of TNFα and IFN-γ. As shown in Fig. 11A, IFN-γ-induced phosphorylation of p38 was suppressed by inhibition of caspase 8. In contrast, inhibitors of caspase 6 and 9 had little affect on the phosphorylation of p38 by IFN-γ (Fig. 11A). Additionally, the phosphorylation of p38 by TNFα plus IFN-γ was suppressed by an inhibitor of caspase 8 and a general caspase inhibitor (Fig. 11B).

Monocyte Apoptosis Is Unaffected by IFN-γ—Activation of caspase 8 is generally associated with apoptosis that could account for the suppression of MMP-9 stimulated by TNFα. However, analysis of annexin V, a marker of early apoptosis, demonstrated that IFN-γ did not cause apoptosis of monocytes (Fig. 12). Although the level of apoptosis in control cells in this representative experiment was 17%, the levels of apoptotic cells cultures treated with IFN-γ and/or TNFα ranged from 6 to 13%. These results indicate that stimulation of monocytes with either IFN-γ or TNFα does not increase apoptosis as com-
pared with control cells. Moreover, the number of dead cells, as detected by propidium iodide, was not increased by either IFN$\gamma$/H9253 or TNF$\alpha$/H9251 (data not shown). These findings demonstrate that the inhibitory effect of IFN$\gamma$/H9253 on TNF$\alpha$/H9251-stimulated MMP-9 is not because of apoptosis of the monocytes.

**DISCUSSION**

The findings presented here demonstrate that although IFN$\gamma$ alone does not affect the production of MMP-1 or MMP-9 by monocytes; however, in the presence of GM-CSF or TNF$\alpha$ it differentially regulations the production of these MMPs. IFN$\gamma$ in combination with GM-CSF induces the production of MMP-1. This occurs as a result of the stimulation of TNF$\alpha$ synthesis by the combination of GM-CSF and IFN$\gamma$ that, in turn, acts with GM-CSF to stimulate MMP-1 production. In contrast to MMP-1, IFN$\gamma$ inhibits the stimulation of MMP-9 by TNF$\alpha$ through a caspase 8/p38/STAT1 pathway.

The differential effects of IFN$\gamma$ on monocyte MMP produc-
Moreover, the stimulation of monocyte TNF α production of TNF α/IFN γ priming of monocytes enhances LPS- and bacillus Calmette-Guerin-induced production of TNF α/IFN γ/H9253. Monocytes were stimulated for 18 h with IFN γ/H9253. To determine apoptotic cells the monocytes were stained for annexin V and then analyzed by fluorescence-activated cell sorter. Control profiles for each condition are shown in the dotted lines and the shaded areas indicate the annexin V staining.

stimulation suggested several mechanisms are potentially involved in the regulation of these enzymes. Previous studies (19, 20) have shown that IFN γ can influence TNF α production by monocytes. IFN γ priming of monocytes enhances LPS- and bacillus Calmette-Guerin-induced production of TNF α/IFN γ (19, 20). Additionally, treatment of LPS-desensitized monocytes with IFN γ induces TNF α production (21). Our findings demonstrate that combining IFN γ with GM-CSF results in a significant induction of TNF α mRNA and protein. The increase in TNF α in the presence of GM-CSF results in the induction of MMP-1 and also causes the enhancement of MMP-1 when IFN γ is added with TNF α and GM-CSF. This conclusion is supported by the ability of neutralizing antibodies against TNF α, TNFR-I, or TNFR-II to block the induction of MMP-1 by IFN γ and GM-CSF. Moreover, the stimulation of monocyte TNF α by the combination of IFN γ and GM-CSF was shown to involve both the p38 and ERK1/2 MAPKs.

Unlike MMP-1 in which TNF α fails to induce this enzyme in monocytes, TNF α alone induces MMP-9. However, as shown here and previously (9), IFN γ suppresses the induction of MMP-9 by TNF α. In contrast, when IFN γ was added with GM-CSF it enhanced MMP-9 production through the induction of TNF α. This may result from the failure to totally inhibit the additive effect of GM-CSF with the endogenously produced TNF α that is significantly enhanced beginning at 7 h after the addition of IFN γ. Additionally, as opposed to experiments in which IFN γ inhibited MMP-9 when added at the same time as TNF α, the GM-CSF- and IFN γ-mediated increase of TNF α occurred several hours after the major suppressive effects of IFN γ on the induction of MMP-9. Thus, production of TNF α induced from 7 to 28 h after IFN γ stimulation resulted in further enhancement of GM-CSF-stimulated MMP-9 as measured at 48 h.

To determine the mechanism by which IFN γ inhibited the induction of MMP-9 by TNF α our studies focused on caspases. Recent studies (16, 17) have shown that IFN γ can increase caspase 8 activity in Fanconi anemia group C hematopoietic progenitor cells and breast tumor cells, which results in apoptosis of these cells. Caspase 8 is an apical caspase that activates caspase 3 and caspase 7 with caspase 3, in turn activating caspase 6 and caspase 2. These activated caspases cleave structural elements of the cytoplasm and nucleus and certain protein kinases that, in general, result in the disruption of survival pathways leading to apoptotic cell death (22, 23). Our findings demonstrate that IFN γ also activates caspase 8 in monocytes. However, IFN γ activation of caspase 8 in monocytes does not lead to apoptosis. Of particular interest is the finding that inhibitors of caspase 8 reversed the inhibition of MMP-9 by IFN γ/H9253-activated monocytes.

The proposed mechanism by which IFN γ inhibits MMP-9 through a caspase 8 pathway is outlined in Fig. 13. We show that caspase 8 activated by IFN γ increases the phosphorylation of p38 MAPK that, in turn, leads to the serine phosphorylation of STAT1. Evidence for this part of the pathway is supported by the ability of inhibitors of caspase 8 to block IFN γ-mediated phosphorylation of p38 MAPK and STAT1 and the suppression of STAT1 phosphorylation by the p38 inhibitor SB203580. Regulation of p38 MAPK by caspase 8 has been demonstrated previously (24) in a caspase 8-deficient cell line. Additionally, the activation of MEKK-1, the upstream activator of the Jun N-terminal kinases, by caspases provides further evidence for the involvement of caspases in the regulation of MAPKs (25, 26). IFN γ-mediated phosphorylation of STAT1 has been shown to require p38 MAPK (27) but has also been reported to occur through a different signaling pathway (28). Our studies agree with the requirement of p38 for IFN γ-mediated phosphorylation of STAT1. The differences between these studies may be because of variations in the signaling pathways in the cell types used.

The proposed mechanism by which phosphorylated STAT1 inhibits TNF α induction of MMP-9 is based on data from previous studies. It has been shown in TNF α-treated human Ewing’s sarcoma EW-7 cells, transfected with the MMP-9 promoter, that phosphorylation of STAT1 by IFN γ can inhibit MMP-9 production through the induction of interferon regulatory factor-1 (IRF-1); IRF-1 suppresses TNF α-induced MMP-9 expression by binding to IFN γ-stimulated responsive elements that overlap NF-κB binding sites on the MMP-9 promoter (18). Thus IRF-1 inhibits MMP-9 by completing with TNF α-induced NF-κB binding to the MMP-9 promoter. Further support for the suppressive role of STAT-1 on MMP-9 production comes from experiments in which reconstitution of STAT1 in STAT1-defi-
cient tumor cells decreased expression of MMP-9 (29). Our findings are in agreement with these studies and provide an additional signaling component, caspase 8, as a regulator of STAT1 phosphorylation leading to the IFNγ-induced suppression of MMP-9. These data demonstrate a new and novel effect of IFNγ on the regulation of MMPs, at least in part, through a caspase 8 pathway that is independent of apoptosis.

In summary, the findings presented here demonstrate that the effect of IFNγ on the regulation of monocyte involvement in connective tissue turnover is complex and is dependent on the cytokines present at an inflammatory site. Of significance is (a) the ability of IFNγ, in combination with GM-CSF, to stimulate the production of TNFα causing the induction of MMP-1 and (b) the activation of caspase 8 by IFNγ resulting in the stimulation of a p38-STAT1 pathway that inhibits TNFα-stimulated MMP-9 production. The mechanism by which caspase 8 activates p38 is currently under investigation.

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REFERENCES
1. Nagase, H., and Woessner, J. F. (1999) J. Biol. Chem. 274, 21491–21494
2. Stetler-Stevenson, W. G., and Yu, A. E. (2001) Semin. Cancer Biol. 11, 143–152
3. Sternlicht, M. D., and Werb, Z. (2001) Annu. Rev. Cell Dev. Biol. 17, 403–436
4. Corcoran, M. L., Stetler-Stevenson, W. G., Brown, P. D., and Wahl, L. M. (1992) J. Biol. Chem. 267, 515–519
5. Lacraz, S., Nicol, L. P., Galve-de Rochemonteix, B., Baumberger, C., Dayer, J. M., and Welgus, H. G. (1992) J. Clin. Invest. 90, 382–388
6. Mertz, P. M., DeWitt, D. L., Stetler-Stevenson, W. G., and Wahl, L. M. (1994) J. Biol. Chem. 269, 21322–21329
7. Lacraz, S., Nicol, L. P., Chicheportiche, R., Welgus, H. G., and Dayer, J. M. (1995) J. Clin. Invest. 96, 2304–2310
8. Mertz, P. M., Corcoran, M. L., McCluskey, K. M., Zhang, Y., Wang, H. L., Lotze, M. T., DeWitt, D. L., Wahl, S. M., and Wahl, L. M. (1996) Cell Immunol. 173, 252–260
9. Saren, P., Welgus, H. G., and Kovanen, P. T. (1996) J. Immunol. 157, 4159–4165
10. Zhang, Y., McCluskey, K., Fujii, K., and Wahl, L. M. (1998) J. Immunol. 161, 3071–3076
11. Wahl, L. M., Corcoran, M. E., Merzenhagen, S. E., and Finbloom, D. S. (1990) J. Immunol. 144, 3518–3522
12. Shapiro, S. D., Campbell, E. J., Kobayashi, D. K., and Welgus, H. G. (1990) J. Clin. Invest. 86, 1204–1210
13. Wahl, L. M., Katen, I. M., Wilder, R. L., Winter, C. C., Harauzi, B., Scher, I., and Wahl, S. M. (1984) Cell Immunol. 85, 373–383
14. Wahl, L. M., and Smith, P. (1991) in Current Protocols in Immunology. (Coligan, J. E., Kruisbeek, A. M., Margules, D. H., Shreff, E. M., and Stobo, W., eds) pp. 7.6.1–7.6.6, John Wiley & Sons, Inc., New York
15. Wahl, S. M., McCartney-Francis, N., Hunt, D. A., Smith, P. D., Wahl, L. M., and Katen, I. M. (1987) J. Immunol. 138, 1342–1347
16. Rathbun, R. K., Christiansen, T. A., Faulkner, G. R., Jones, G., Keeble, W., O’Dwyer, M., and Bagby, G. C. (2000) Blood 96, 4204–4211
17. Ruiz-Ruiz, C., Munoz-Finedo, C., and Lopez-Rivas, A. (2000) Cancer Res. 60, 5673–5680
18. Sanceau, J., Boyd, D. D., Seiki, M., and Bauvois, B. (2002) J. Biol. Chem. 277, 35766–35775
19. Hayes, M. P., Freeman, S. L., and Donnelly, R. P. (1995) Cytokine 7, 427–435
20. Quiding-Jarbrink, M., Smith, D. A., and Bancroft, G. J. (2001) Infect. Immun. 69, 5661–5670
21. Random, F., Duche, W. D., Bundschuh, D. S., Hartung, T., Wendel, A., and Volk, H. D. (1997) J. Immunol. 158, 2911–2918
22. Rokhlin, O. W., Glover, R. A., and Cohen, M. B. (1998) Cancer Res. 58, 5870–5877
23. Hitata, H., Tukahashi, A., Kobayashi, S., Yonehara, S., Sawai, H., Okazaki, T., Yamamoto, K., and Sasada, M. (1998) J. Exp. Med. 187, 587–600
24. Joo, P., Koo, C. J., Yuan, J., and Bilenis, J. (1996) Curr. Biol. 8, 1001–1008
25. Cardone, M. H., Salvesen, G. S., Widmann, P., Johnson, G., and Frisch, S. M. (1997) Cell 90, 315–323
26. Widmann, C., Gerwins, P., Johnson, N. L., Jarpe, M. B., and Johnson, G. L. (1998) Mol. Cell Biol. 18, 2416–2429
27. Goh, K. C., Hase, S. J., and Williams, B. R. (1999) EMBO J. 18, 5601–5608
28. Kovalik, P., Stoiber, D., Eyers, P. A., Menghini, R., Neininger, A., Gaestel, M., Cohen, P., and Decker, T. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 13956–13961
29. Huang, S., Bucana, C. D., Van Arsdaal, M., and Fidler, I. J. (2002) Oncogene 21, 2504–2512
