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Inhibition of matrix metalloproteinase-9 by interferons and TGF-β1 through distinct signalings accounts for reduced monocyte invasiveness

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Abstract Cytokines may provide signals for regulating human monocyte matrix metalloproteinase-9 (MMP-9) activity. In this study, we investigated the roles of interferons (IFN) type I/II and transforming growth factor-β1 (TGF-β1) in MMP-9-mediated invasiveness. MMP-9 antibody and inhibitor, IFNs and TGF-β1 inhibited monocyte transmigration through Matrigel. IFNs and TGF-β1 downregulated MMP-9 mRNA, protein and activity levels. The inhibitory action of IFNs was associated with the STAT1/IRF-1 pathway since the JAK inhibitor AG490 blocked STAT1 phosphorylation, IRF-1 synthesis and counteracted the blockade of MMP-9 release. TGF-β1-mediated MMP-9 inhibition appeared STAT1/IRF-1-independent but reversed by the protein tyrosine kinase inhibitor tyrphostin 25. Our data point out the importance of IFNs and TGF-β1 in the control of monocyte MMP-9-mediated extravasation.
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Keywords: IRF-1; Transmigration; MMP-9; STAT1; PTK, signaling

1. Introduction

Monocytes infiltrating injured tissues (inflammation, wound healing, tumors) play a crucial role by releasing cytokines, chemokines and matrix metalloproteinases (MMPs) which regulate cancer and inflammation [1–3]. Through their hydrolytic abilities, MMPs are involved in a variety of biological processes, such as proliferation, migration and invasion of cells, tumor metastasis and angiogenesis [3]. Several investigations suggest that MMP-9 (gelatinase B) production by monocytes is associated with their differentiation into macrophages [4–7]. MMP-9 is synthesized as a preproenzyme, secreted as a proform (proMMP-9, 92 kDa) and its activation requires proteolytic removal of a fragment of the N-terminal domain [3]. MMP-9 is capable of degrading extracellular matrix substrates (type I, IV, V, VII, XI collagens, fibrin and laminin) and non-matrix substrates [3,8,9]. Recent data demonstrated that proMMP-9 binding to collagen type IV results in an enzymatic activation of proMMP-9 without loss of its NH2-terminal propeptide [10].

Based on the evidence that MMP-9 is of critical importance in cell invasion, current trials focus on the identification of biomarkers that could inhibit MMP-9 [11]. The promoter of MMP-9 possesses several functional enhancer element binding sites including three AP-1 sites, a non consensus NF-κB site, an Ets site, an SP-1 site and a retinoblastoma element [12,13]. Tumor necrosis factor-α (TNF-α) stimulates MMP-9 gene transcription in monocytes through activation of NF-κB [14,15]. On the basis of their physicochemical and biological properties, the interferon (IFN) family is subdivided into type I IFNs (α,β) and type II (γ). Cellular effects of IFNs include inhibition of cell proliferation, and effects on apoptosis, immunomodulation, angiogenesis and leukocyte trafficking [16–18]. IFN-γ inhibits MMP-9 release in human monocytes treated with exogenous TNF-α [19,20]. Transforming growth factor-β1 (TGF-β1) is a multifunctional cytokine which inhibits cell proliferation, induces cell migration and angiogenesis [21]. Discrepant data have been reported with regard to transforming growth factor (TGF)-β1 action on monocyte MMP-9 expression. TGF-β1 could upregulate MMP-9 release in human TNF-α-treated monocytes and THP-1 cells [22,23] or downregulate MMP-9 in monocytes [24].

IFN-γ and TGF-β1 induce maturation of monocytes and their precursors into macrophages [25–27]. Although without any effect on monocyte maturation, IFNs type I (α and β) may activate signaling pathways common to IFN-γ [28]. The objective of the present study was to investigate the effects of TGF-β1 and IFNs on MMP-9 expression and transmigratory properties of human monocytes. In this study, we demonstrated that inhibition of MMP-9 in human monocytes by IFN or TGF-β1 accounts for their reduced invasiveness. We further explored the mechanisms by which IFNs and TGF-β1 downregulate MMP-9 in monocytes, and we found that these cytokines inhibited MMP-9 expression through separate signaling pathways.

2. Materials and methods

2.1. Reagents

Recombinant human (rh) TGF-β1, rh IFN-γ (2×10^7 U/mg) and rh proMMP-9 were supplied by R&D (Abindgton, UK). Rh IFN-α2a (2×10^8 U/mg) and rh IFN-β (2×10^8 U/mg) were provided by

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Abbreviations: IFN, Interferon; MMP, matrix metalloproteinase; TGF, transforming growth factor; TNF, tumor necrosis factor

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Hoffman-La Roche (Basel, Switzerland) and by Ares-Serono (Geneva, Switzerland), respectively. Amino-phenyl mercuric acid (APMA), bisindolylmaleimide, H-89, indomethacin, wortmannin, and tyrphostin 25 were obtained from Sigma Chemicals Co. (Saint-Louis, MO). AG490 (tyrphostin B42), PP2 and MMP-9 inhibitor ((2R)-[4-biphenylsulfonylamino]-*N*-hydroxy-3-phenylpropionamide) were from Calbiochem (Darmsdat, Germany). SB203580 was from BioMol (Plymouth Meeting, PA).

2.2. Antibodies

Irrelevant mouse (m)IgGs and monoclonal antibodies (mAbs) (FITC or PE) specific for CD11b (mIgG1, BEAR 1) and CD14 (mIgG1, 134620) were obtained from Coulter/Beckman (Luminy, France). Abs specific for phosphorylated-Y701 STAT1 (goat polyclonal IgG, Tyr 701), STAT1 (rabbit polyclonal IgG, E-23) and IRF-1 (rabbit polyclonal IgG, C-20) were from Santa Cruz Biotechnology (Tebu, France). Neutralizing anti-MMP-9 (mIgG1, AZC02) was from R&D (Abingdon, UK). Anti-actin (mIgG1, C4) was from ICN Biomedicals (Ohio, USA).

2.3. Obtention of isolated monocytes and culture conditions

Human peripheral blood monocytes were isolated by Ficoll–Hypaque density gradient (1.077 g/mL) centrifugation and further adherence to plastic as described [26]. As assessed by flow cytometry analysis of the CD14 antigen, adherent cells contained $\geqslant 95\%$ monocytes. Cells ($0.5–1 \times 10^6$/mL) were cultured in RPMI 1640 medium/10% FCS at 37 °C, in the absence or presence of 1000 U/mL IFN-α/-β/-γ or 10 ng/mL TGF-β1 or various pharmacological inhibitors. In some experiments, cells were pretreated with the antagonist GW9662 or AG490 for 30 min before adding stimuli. After various periods of incubation, cells were collected, washed twice, counted with a Coulter Counter channelizer 256 (Coulter/Beckman). Differentiation of cells into macrophages was determined according to the change in morphology (by staining of cytocentrifuged cells with the Hemacolor kit from Merck and light microscope examination). Cell viability was assessed by trypan blue exclusion and the Coulter Counter.

2.4. Flow cytometry analysis

Intact cells were immunostained as described [26]. Analysis was performed in a FACS flow cytometer analyzer (Becton-Dickinson, Mountain View, CA).Values are given as percentages of positive cells.

2.5. ELISA

The culture supernatants from treated cells were tested for MMP-9 and TNF-α contents using commercial ELISA kits (R&D, Abingdon, UK). Controls included FCS-supplemented RPMI 1640 medium incubated under the same conditions. Detection level for TNF-α and MMP-9 was 10 pg/mL.

2.6. RNA isolation, cDNA synthesis and PCR

RNA extraction from treated cells and cDNA synthesis were conducted as described [29]. The cDNAs for human β_2-microglobulin (165 bp) and MMP-9 (296 bp) were amplified by PCR and primers were designed according to published sequences [29]. MMP-9 cDNA (296 bp) was amplified using the sense primer 5′-GGA GAC CTG AGA ACC AAT CTC-3′ and the antisense primer 5′-TCC AAT AGG TGA TGT TGT CGT-3′. β_2-microglobulin cDNA (165 bp) was amplified using the sense primer 5′-CAT CCA GCG TAC TCC AAA GA-3′ and antisense 5′-GAC AAG TCT GAA TGC TCC AC-3′. The PCR products were visualized by electrophoresis in 2% agarose gel containing 0.2 μg/mL ethidium bromide. The NIH Image 1.63 software was used for the analysis.

2.7. Zymography

Analysis of MMP-9 activity was carried out in 7.5% (w/v) SDS–PAGE containing 0.1% gelatin (w/v) as described elsewhere [29]. Recombinant proMMP-9 (1 ng) and culture supernatants were preincubated for 60 min at room temperature with 0.5 mM APMA which activates the proform (92 kDa) to the activated form (82 kDa). The NIH Image 1.63 software was used for the analysis of the bands after acquisition in an Appligen densitometer (Oncor).

2.8. Western blot analysis

Following stimulation of monocytes with IFN (1000 U/mL) or TGF-β1 (10 ng/mL) for 18 h, total cell extracts were prepared as described [30], separated on 12% polyacrylamide–SDS gels, transferred to nitrocellulose and blotted as described [30]. Membranes were hybridized with Abs for phosphorylated-STAT1 (pY-STAT1), STAT1, IRF-1 and actin as described [30] using chemiluminescence technique developed by Santa Cruz. Immunoreactive protein bands were detected by autoradiography on Hyperfilms (Kodak). The NIH Image 1.63 software was used for the quantitation of the bands.

2.9. In vitro invasion assay

Invasion was measured by assessement of monocyte migration rate through an artificial membrane in Transwell cell cultures chambers (Corning Costar, 8 μm pore size). Briefly, membranes were precoated with 25 μg of Matrigel (BD Biosciences, MA) on the upper surface. Freshly isolated monocytes (1×10^5 cells in 100 μl RPMI) were seeded into the upper well of the chamber, while the lower well was filled up to the top (600 μl) with RPMI + 10% FCS as a chemoattractant. The neutralizing MMP-9 antibody, its control isotype mIgG1, MMP-9 inhibitor, IFN-α/β/-γ or TGF-β1 were added to the cells. After 18 h at 37 °C in 5% CO_2, cells that had migrated through the matrigel-coated filters were recovered from the lower compartment and counted using the Cell Coulter Counter. Results from duplicates wells were expressed as means ± S.D.

2.10. Statistical analysis

Values are represented as means ± S.D. of *n* separate experiments. The statistical significance of differences was evaluated by ANOVA using the StatView® Student v1.0 software.

3. Results

3.1. Synthesis and release of MMP-9 by cultured monocytes

Total RNA from cultured monocytes was isolated, reverse-transcribed and subjected to PCR amplification. Samples were standardized for total cDNA content by assessing the presence of identical amounts of β_2-microglobulin transcripts. As shown in Fig. 1A, MMP-9 PCR products were not detected in freshly isolated monocytes (lane 1). Induced levels of MMP-9 transcripts were already evident in monocytes cultured for one day (Fig. 1A, lane 2) and persisted at day 7 (Fig. 1A, lane 4).

Using ELISAs, kinetic studies indicated a rapid and concomitant release in TNF-α and MMP-9 proteins in supernatants of cultured monocytes (Fig. 1B). MMP-9 production by 24-h cultured monocytes was abolished in presence of neutralizing TNF-α Ab but not with its isotype used as control. Fig. 1B ascertaining the pivotal role of endogenous TNF-α in monocyte MMP-9 induction. Zymography analysis of the conditioned media from cultured monocytes showed the presence of a gelatinase activity at 92-kDa consistent with the pattern of proMMP-9 (Fig. 1C, compare lanes 2 and 4) and APMA treatment resulted in conversion of proMMP-9 to the active form of 82-kDa size (Fig. 1C, lanes 3 and 5). Thus, the upregulation of MMP-9 mRNA levels in cultured monocytes is consistent with the induced levels of MMP-9 protein and activity.

3.2. IFNs and TGF-β1 inhibit monocyte MMP-9 synthesis and release

As described [25,26], IFN-γ and TGF-β1 accelerate the spontaneous differentiation of monocytes into macrophages whereas IFNs type I (α and β) did not affect monocyte maturation (data not shown). Day 2-PCR reactions indicated that

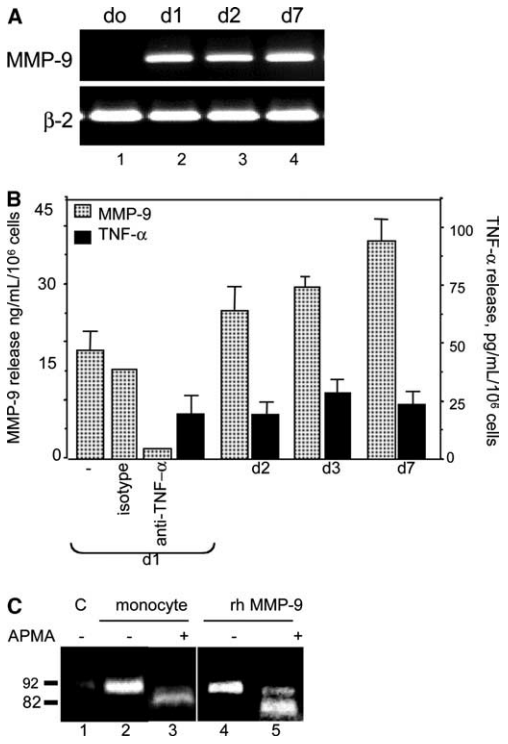

Fig. 1. *MMP-9 synthesis and release by in vitro cultured monocytes*. Freshly isolated monocytes (1×10^6) were cultured for 7 days. (A) PCR analyses were performed as described in Section 2 using 2% agarose gels. (B) Time course of MMP-9 and TNF-α production in the culture supernatants of monocytes determined by ELISA. Data are expressed as means ± S.D. ($n = 4$). (C) Analysis of gelatinolytic activity in the culture media of day 2-monocytes. (C) Control culture medium without cells; activation of monocyte proMMP-9 (lane 2) and rh proMMP-9 (lane 4) by APMA (0.5 mM) results in the 82-kDa active forms (lanes 3 and 5).

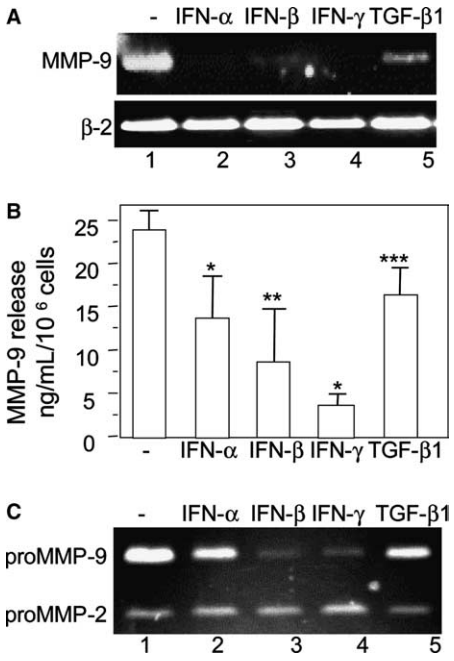

Fig. 2. *Effects of IFNs and TGF-β_1 on monocyte MMP-9 expression*. Monocytes (1×10^6) were cultured for two days in the absence or presence of IFNs (1000 U/mL) or TGF-β1 (10 ng/mL). (A) PCR analyses of the effects of IFNs and TGF-β1 on MMP-9 and β2-microglobulin transcripts. (B) MMP-9 production measured by ELISA. Data represent the mean of five independent experiments. Values are expressed as means ± S.D. $^{*}P < 0.005$ for IFN-α and IFN-γ compared with control; $^{**}P < 0.080$ for IFN-β compared with control; $^{***}P < 0.0004$ for TGF-β1 compared with control. (C) Analysis of proMMP-9 and proMMP-2 gelatinolytic activities in the culture media of untreated and treated monocytes.

the levels of MMP-9 transcripts were downregulated with both types of IFNs and TGF-β1 (Fig. 2A lanes 2–5). Furthermore, we analyzed the ability of monocytes, before and after treatment, to release proMMP-9 into the culture medium. ELISA data showed that IFN-α/-β as well as the two differentiating cytokines TGF-β1 and IFN-γ blocked the production of MMP-9 protein (Fig. 2B) and this was associated with reduced gelatinolytic activity of MMP-9 (Fig. 2C). The correlation observed between the levels of MMP-9 transcript and protein at day 2 showed that TGF-β1 was much less effective on MMP-9 expression compared with IFNs. Monocytes produced a 72-kDa gelatinolytic activity representing proMMP-2 at very low level compared with MMP-9, which was unaffected by IFN or TGF-β1 treatment (Fig. 2C). IFN- and TGF-β1-mediated inhibition of MMP-9 release was time- and dose-dependent with maximal IFN and TGF-β1 effects being obtained at 1000 U/mL and 10 ng/mL, respectively (data not shown). These experiments indicate that IFNs and TGF-β1 inhibited MMP-9 protein level which is likely the result of downregulated MMP-9 mRNA levels.

3.3. Comparative effects of IFNs and TGF-β1 on TNF-α release

Whether IFNs or TGF-β1 inhibited endogenous TNF-α release was investigated. A previous study already reported the stimulatory effect of IFN-γ on TNF-α release by human monocytes whereas IFN-α inhibited such production [31]. As seen in Fig. 3, day 2-monocytes released detectable levels of TNF-α (around 20 pg/mL/10^6 cells). TGF-β1 as well as IFN-α/-β did not influence TNF-α production. Consistent with [31], IFN-γ enhanced TNF-α release (upto 160 pg/mL/10^6 cells) (Fig. 3). These data thus indicate that MMP-9 inhibition was not related to a diminished production of endogenous TNF-α, $\alpha v\delta$ that IFN-γ at 1000 U/mL was still capable of reversing the activating effect of 160 ng/mL TNF-α toward MMP-9.

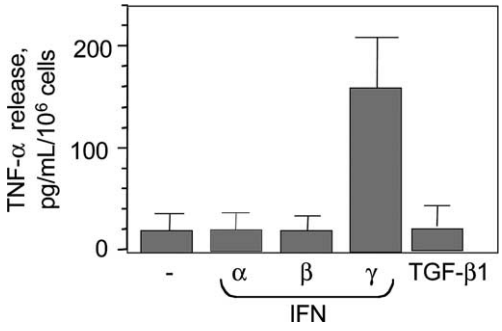

Fig. 3. *Effects of IFNs and TGF-β_1 on monocyte TNF-α release*. Monocytes (1×10^6) were cultured for two days in the absence or presence of IFNs (1000 U/mL) or TGF-β1 (10 ng/mL) and culture supernatants were assessed for TNF-α production measured by ELISA. Data represent the mean of 4–13 independent experiments. Values are expressed as means ± S.D.

3.4. IFNs inhibit MMP-9 expression through the STAT1/IRF-1 signaling

We previously showed that IFNs type I and II inhibited MMP-9 expression in a model of tumor epithelial cell line by activating the STAT1/IRF1 signaling [30]. Since TGF-β1 could interfere with IFN signaling [32], we assessed whether it could inhibit MMP-9 through the STAT/IRF-1 pathway. IFNs activate STAT1 through tyrosine phosphorylation, dimerization, nuclear translocation and DNA binding [28]. Activated STAT1 induces IRF-1 transcription [33]. Here, IFN treatment induced tyrosine phosphorylation of STAT1 in whole cell lysates from monocytes (Fig. 4A, left panel). As described [34], STAT1 levels were upregulated following IFN stimulation (Fig. 4A, left panel). IFN stimulation led to an increase in the levels of IRF-1 in whole cell lysates (Fig. 4A, left panel). In contrast, TGF-β1 neither induced STAT1 tyrosine phosphorylation nor enhanced IRF-1 protein (Fig. 4A, left panel). These data therefore indicated that IRF-1, downstream mediator of STAT1, was efficiently induced by IFNs but not by TGF-β1.

AG490 inhibits IFN-mediated JAK activity involved in STAT1 tyrosine phosphorylation [35]. Since AG490 concentrations in culture medium >5 μM resulted in cell toxicity, monocytes were therefore preincubated for 30 min with 5 μM AG490 and subsequently treated with IFNs or TGF-β1.Western analysis indicated that AG490 downregulated tyrosine phosphorylated STAT1 and IRF-1 protein levels in IFN-treated monocytes (Fig. 4A, right panel). In contrast, the basal levels of IRF-1 in unstimulated and TGF-β1-stimulated cells remained almost unchanged (Fig. 4A, right panel). In parallel, AG490 diminished the IFN-mediated inhibition of MMP-9 release by monocytes, but was without effect on TGF-β1-mediated MMP-9 inhibition (Fig. 4B). Together, these data indicate that IFN-mediated MMP-9 inhibition in monocytes, but not TGF-β1-mediated MMP-9 inhibition, was associated with the STAT1/IRF-1 pathway.

3.5. Effects of pharmacological inhibitors on TGF-β1-mediated MMP-9 inhibition

We furthermore examined the abilities of various pharmacological inhibitors, i.e., tyrphostin 25 which is a highly specific protein tyrosine kinase (PTK) inhibitor, SB203580 a specific inhibitor for p38 MAPK, indomethacin an inhibitor of the cyclooxygenase pathway, H-89 an inhibitor of cAMP-dependent protein kinase, wortmannin that acts as a selective and irreversible inhibitor of PI3-kinase and the highly specific PKC inhibitor bisindolylmaleimide, to reverse the inhibitory effect of TGF-β1 on MMP-9 release. As shown in Fig. 5, H-89, indomethacin, bisindolylmaleimide and wortmannin were without effect on TGF-β1-mediated MMP-9 inhibition. SB205380 instead slightly reenforced the inhibitory effect of TGF-β1 (Fig. 5). In contrast, tyrphostin 25 markedly prevented the inhibition of MMP-9 production by TGF-β1 (Fig. 5) thus suggesting PTK involvement in the pathway leading to MMP-9 inhibition by TGF-β1. The inability of PP2 to prevent the inhibitory action of TGF-β1 excluded the participation of a PTK belonging to the Src family.

3.6. IFNs and TGF-β1 inhibit migration of monocytes through matrigel in a MMP-9 dependent manner

Assuming that the production of MMPs may facilitate cell migration across basal lamina, we investigated whether MMP-9 secretion correlates with the transmigration of monocytes across a reconstituted basement membrane using Transwells where the upper chamber was coated with Matrigel. Monocytes showed a significant percentage of 18 h-migration in the Matrigel-based assay (Fig. 6A). Neutralizing anti-MMP-9 antibody and the synthetic MMP-9 inhibitor (2R)-[4-biphenyl sulfonylamino]-*N*-hydroxy-3-phenyl propionamide significantly reduced the percentage of migration, respectively, to 39% and 50% of the control (Fig. 6A). We further examined the impact of IFN-α/β/-γ or TGF-β1 on the transmigration of monocytes. The percentage of migration of monocytes was significantly lower when the cells were incubated with IFN-β/-γ or

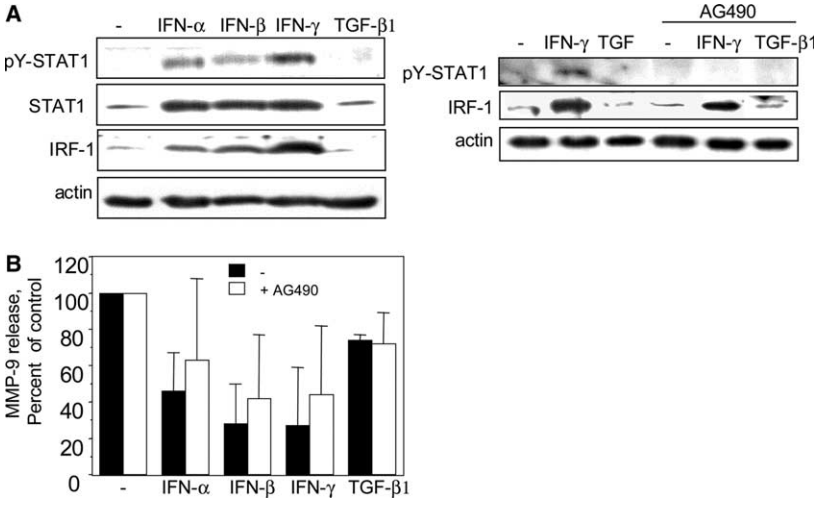

Fig. 4. *Western blot analysis of STAT1, pY-STAT1 and IRF-1 proteins in monocytes treated with IFNs, TGF-β1 and AG490*. Monocytes (1×10^6) were cultured in the absence or presence of IFNs (1000 U/mL) or TGF-β1 (10 ng/mL) for 24 h. Cells were pretreated with AG490 (5 μM) for 30 min before cytokine stimulation for 24 h. (A) Western blot analyses were performed as described in Section 2. Whole cell lysates were separated by 12% SDS–PAGE. (B) MMP-9 production in conditioned media of day-1 monocytes was assayed by ELISA. Data are calculated as percent of control (no stimulation or AG490 stimulation alone) and represent the mean of three separate experiments ± S.D.

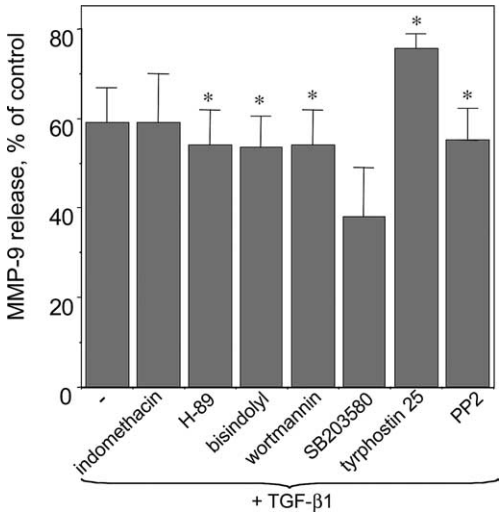

Fig. 5. *Effects of kinase inhibitors on TGF-β_1-mediated inhibition of MMP-9 production*. Monocytes (1×10^6) were cultured in the absence or presence of indomethacin (1 μM), H-89 (10 μM), bisindolylmaleimide (10 μM), wortmannin (1 μM), SB203580 (5 μM), tyrphostin 25 (5 μM), or PP2 (10 μM) and/or TGF-β1 (10 ng/mL) for 24 h. MMP-9 production in in conditioned media of day-1 monocytes was assayed by ELISA. Data are calculated as percent of control (no stimulation or pharmacological inhibitor stimulation alone) and represent the mean of n separate experiments. ($n = 3$, wortmannin; $n = 4$, indomethacin, H-89, bisindolylmaleimide, SB203580, PP2; $n = 7$, tyrphostin 25). Values are expressed as means ± S.D. $^*P < 0.010$ compared with control.

TGF-β1 during the overnight invasion assay (Fig. 6A). The lack of a significant inhibitory effect of IFN-α was related to the low rate of IFN-α-mediated MMP-9 secretion seen before 24 h (data not shown). Visualization of migrated cells indicated cell integrity for control and treated cells (Fig. 6B). This was further confirmed by the assessment that any treatment did not lead to cell necrosis (data not shown, <15% dead cells).

4. Discussion

In the present study, we determined the inhibitory actions of IFNs and TGF-β1 on MMP-9 expression and MMP-9-mediated invasiveness of human monocytes.

TNF-α stimulates MMP-9 production in human monocytes via NF-κB activation [14,19]. In this study, we showed that monocytes upon culture rapidly released MMP-9 via endogenous TNF-α, and such ability was maintained when cells differentiated into macrophages. IFN-γ and TGF-β1 which both accelerated spontaneous differentiation of monocytes, downregulated MMP-9 synthesis and release in differentiating monocytes. IFN-α/-β, which did not affect monocyte maturation, transcriptionally repressed MMP-9 as efficiently as IFN-γ. This inhibition was specific for MMP-9 since IFNs and TGF-β1 did not alter the production of MMP-2 under the same experimental conditions. Our data therefore suggest that MMP-9 production is not associated with the maturation process of monocytes.

We previously showed in a model of epithelial tumor, that IFN-mediated inhibition of MMP-9 expression occurred through STAT1 activation leading to the expression of IRF-1 which in turn inhibited NF-κB binding to the MMP-9 promoter [30]. Here, IFN-mediated MMP-9 downregulation in monocytes involved the IRF-1 pathway, since the JAK inhibitor AG490 blocked STAT1 and IRF-1 activation, and reverse the inhibitory action of IFN on MMP-9 release.

A recent study showed that the suppression of MMP-9 transcription by TGF-β1 was mediated by a region of MMP-9 promoter (−670/−591 bp) containing the NF-κB site (−600/−590 bp) [36] and the IFN enhancer core sequence (5′-GGAATTCC-3′, −600/−593 bp) previously shown by us to participate in the negative regulation of MMP-9 gene transcription [30]. However, our data clearly indicated that TGF-β1 did not affect the STAT1/IRF-1 pathway. TGF-β1 suppressed TNF-α-induced MMP-9 production by the myeloid MonoMac6 cell line via a PGE2- and PKA-dependent mechanism

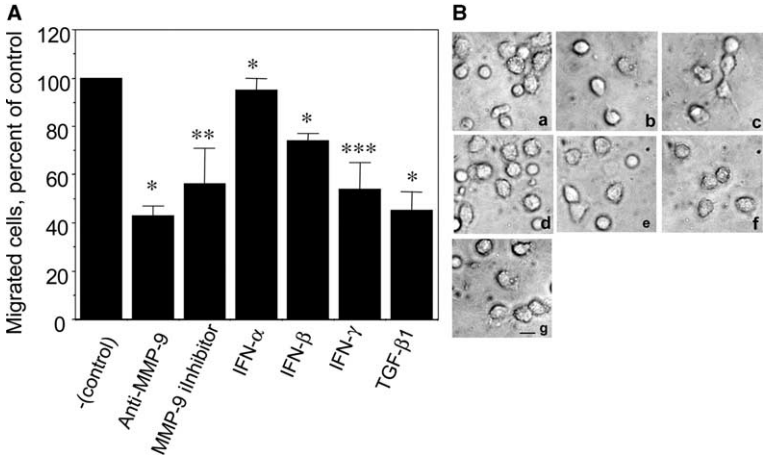

Fig. 6. *Impact of MMP-9 antibody and inhibitor, IFNs and TGF-β_1 on migration of monocytes through Matrigel*. (A) Freshly isolated monocytes plated (1×10^5 cells per chamber) in the Transwells coated with Matrigel as described in Section 2, were submitted to migration for 18 h at 37 °C in the absence or presence neutralizing MMP-9 antibody (10 μg/mL), MMP-9 inhibitor ((2R)-[4-biphenylsulfonylamino]-*N*-hydroxy-3-phenylpropionamide) (20 μg/mL), IFNs (1000 U/mL) or TGF-β1 (10 ng/mL). The control isotype (mIgG1) did not affect monocyte transmigration. 100% relative migration (control) represented migration of untreated monocytes (15%). Data represent the mean of three independent experiments. Values are expressed as means ± S.D. $^*P < 0.005$ for anti-MMP-9, IFN-α, FN-β and TGF-β1; $^{**}P < 0.020$ for MMP-9 inhibitor compared with control; $^{***}P < 0.008$ for IFN-γ compared with control. (B) Light microscopy of monocytes that migrated in the lower chamber. (a) minus, (b) +anti-MMP9, (c) +MMP-9 inhibitor, (d) +IFN-α, (e) +IFN-β, (f) +IFN-γ and (g) +TGF-β1. Magnification, 160×. Bar, 10 μm.

[37]. Here, the use of various inhibitors clearly excluded the cyclooxygenase and PKA pathways, as well as the PI-3K and PKC pathways. In contrast, a PTK activity appeared involved in TGF-β1-mediated MMP-9 inhibition because treatment with tyrphostin 25 resulted in a marked blockade of the inhibitory activity of TGF-β1. The picture emerges that TGF-β1 inhibits TNF-α-mediated MMP-9 induction in monocytes at least in a PTK-dependent manner. The elucidation of the PTK(s) involved requires further investigation.

We showed that blood monocytes migrate through reconstituted basement membrane (Matrigel) through a MMP-9-mediated transmigration process since MMP-9 antibody and MMP-9 inhibitor significantly reduced the migratory ability of monocytes. Importantly, IFN- and TGF-β1-mediated downregulation of MMP-9 elicited inhibition of monocyte invasion across the Matrigel. These data demonstrate the critical roles of IFNs and TGF-β1 in the MMP-9-mediated invasion of monocytes.

Collectively, our observations, by providing insights into the mechanisms of MMP-9 inhibition by IFNs and TGF-β1, may be useful as a strategy to attenuate MMP-9-mediated invasion of monocytes and, possibly, of MMP-9-producing tumoral cells in inflamed tissues.

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