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Th2 Cell Membrane Factors in Association with IL-4 Enhance Matrix Metalloproteinase-1 (MMP-1) While Decreasing MMP-9 Production by Granulocyte-Macrophage Colony-Stimulating Factor-Differentiated Human Monocytes

Carlo Chizzolini,2 Roger Rezzonico, Carmelina De Luca, Danielle Burger, and Jean-Michel Dayer

Monocytes/macrophages are directly involved in tissue remodeling and tissue destruction through the release of matrix metalloproteinases (MMP). In the present study, we examined the effect mediated by contact of polarized Th cells with mononuclear phagocytes on the production of MMP-1, MMP-9, and their inhibitor. Plasma cell membranes from Ag-activated Th1 and Th2 cells were potent inducers of MMP-1 production by THP-1 cells. Cell membrane-associated TNF was found to be only partially involved in MMP-1 induction by both Th1 and Th2 cells. In Th2 cells exclusively, membrane-associated IL-4 induced MMP-1 production by THP-1 cells. This membrane-associated IL-4 effect was additive to that of TNF and was specifically observed on MMP-1 as MMP-9 production was concomitantly inhibited. Similarly, soluble IL-4 induced THP-1 cells to produce MMP-1, its effect proving additive to that of soluble TNF and to that of cell membranes of mitogen-activated HUT-78 cells. Its activity was blocked by IL-4 neutralization, and was unaffected by the presence of indomethacin. These effects on THP-1 cells were observed at protein and mRNA levels. Although inhibitory on freshly isolated peripheral blood monocytes, soluble IL-4 enhanced T cell-induced MMP-1 and inhibited MMP-9 production both at protein and mRNA levels in monocytes cultured for 7 days in the presence of GM-CSF. Thus, in contrast with previously reported effects, Th2 and IL-4 specifically induce MMP-1 production by mononuclear phagocytes at various stages of differentiation. This IL-4 activity may be relevant to pathological conditions dominated by Th2 inflammatory responses, resulting in tissue remodeling and destruction. The Journal of Immunology, 2000, 164: 5952–5960.

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4 Abbreviations used in this paper: IL-1ra, IL-1 type II receptor (1, 2); IL-12; GM-CSF, IFN-α, monocyte chemoattractant protein-1, PGE2, and superoxide anions if induced by microbial products or inflammatory stimuli, and it enhances the production of IL-1 receptor antagonist (IL-1ra)3 and that of the decoy IL-1 type II receptor (1, 2). In addition, IL-4 decreases the expression of CD14, CD16, CD32, CD64, and CCR5 (3). However, although considered to be deactivating by some, monocytes exposed to IL-4 have enhanced expression of class II MHC Ags, of CD29, CD49e (VLA-5), CD13, CD23 (FceRII), β2 integrins, CXCR4, and an enhanced capacity to produce monocyte-derived chemokine (MDC) and alternative macrophage activation-associated CC chemokine (AMAC)-1 as well as tissue-type plasminogen activator (4–7). Furthermore, priming with IL-4 restores IL-12 production by mononuclear cells of HIV-1-infected patients (8), it induces the differentiation of monocytes into macrophages that have enhanced antitumor activity (9), and when used in conjunction with GM-CSF allows the differentiation of PB monocytes toward immature dendritic cells (10). Thus, in many circumstances, IL-4 may enhance rather than inhibit mononuclear phagocyte activities.

Mononuclear phagocytes play a central role in most inflammatory processes and may be involved in tissue remodeling and destruction by participating in the degradation of extracellular matrix (ECM). Degradation of ECM is handled by proteases, including a family of enzymes collectively named matrix metalloproteinases (MMP) or matrixins, secreted from the cells or expressed as plasma membrane-bound forms. The expression of many MMP is regulated transcriptionally. MMP require stepwise activation from inactive precursors (pro-MMP), and their activity depends further on interactions with ECM components and binding to endogenous inhibitors: tissue inhibitor of MMP (TIMP) (reviewed in Ref. 11). Interstitial collagenase (MMP-1) cleaves native fibrillar collagen type I-III, while 92-kDa gelatinase (MMP-9) attacks basement membrane collagen, elastin, fibronectin, in addition to denatured collagens. Mononuclear phagocytes produce MMP when stimulated by mitogens, ECM components, bacterial products, and by contact with activated T cells (12–16). MMP production in these cells is mediated in part by PGE2 and its effects on cAMP levels...

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PB monocytes spontaneously produce MMP-9, and its levels are increased upon exposure to LPS, TNF, IL-1, GM-CSF, and CD40 engagement (14, 15, 19–22). MMP-1, however, is not produced by freshly isolated PB monocytes, and low levels are induced in the presence of GM-CSF in conjunction with TNF or IL-1, or when stimulated by bacterial products (20). Alveolar macrophages respond more readily to inflammatory or bacterial stimuli producing MMP-1 or MMP-9 (23). IL-4 has been reported to inhibit MMP-1 and MMP-9 production (20, 24, 25), but inhibition has been linked to the capacity of IL-4 to inhibit PGE2 synthesis (1, 25).

In several inflammatory conditions, T cells are adjacent to mononuclear phagocytes and may affect their activities through contact-dependent mechanisms, inducing a variety of mediators including MMPs (15, 16). However, polarized T cells differ in their contact-dependent activating properties. Thus, Th1 cells powerfully induce proinflammatory IL-1β production, while Th2 clones are higher inducers of anti-inflammatory IL-1ra (26). We were therefore interested in investigating whether Th1 and Th2 cells behaved differently in inducing and regulating MMP production by mononuclear phagocytes. The results reported in this study indicate that both Th1 and Th2 cells induce the production of MMP-1. Unexpectedly, IL-4 in its soluble form or as Th2 cell membrane-associated molecule enhanced MMP-1 induced by cell-cell contact or by TNF, while inhibiting MMP-9 production. The IL-4 effect on MMP-1 was observed at both mRNA and protein levels, and differentiated the activities of Th1 and Th2 cells in MMP production.

Materials and Methods

Media, reagents, and Abs

Penicillin, streptomycin, RPMI 1640, FCS, and PBS were obtained from Life Technologies (Paisley, Scotland). Human AB serum was provided by the Blood Bank of the University Hospital (Geneva, Switzerland). Recombinant human IL-1, TNF, GM-CSF, and IFN-γ were obtained from Biogen (Cambridge, MA). Human rIL-4 (10 U/ml) was from Sandoz (Basel, Switzerland). IL-1α from Immunex (Seattle, WA), and IL-1α from Synergen (Boulder, CO). Ficol-Paque was from Pharmacia (Uppsala, Sweden); PHA from EY Laboratories (San Mateo, CA); and soluble TNF from American Type Culture Collection, Manassas, VA. Rabbit antihuman IFN-γ (1:1000) was from Tago (University of Texas, Dallas, TX); anti-IFN-γ was a gift from Dr. P. E. Lipsky (University of Texas, Dallas, TX); anti-IFN-γ was a gift from Dr. G. G. Garaotra (Human Genome Sciences, Rockville, MD); anti-IL-4, 25d12.11a mAb a gift from Dr. J. de Vries (DNAX, Palo Alto, CA); and soluble TNF receptor p55 (sFc-p55, anti-TNF) was from Hoffmann-LaRoche.

T cells

The human cutaneous T lymphoma cell line HUT-78 (27) was cultured in RPMI 1640 + 10% FCS at 1 x 10^5 cells/ml or activated by adding 1 μg/ml PHA and 5 ng/ml PMA for 16 h, then extensively washed before cell membrane preparation, as described below.

Ag-specific T cell clones were generated as previously described (26). Briefly, PBMC obtained from healthy donors were cultured in the presence of Ag (PPD or TT) in RPMI 1640 supplemented with 2 mM l-glutamine, 50 IU/ml penicillin, 50 μg/ml streptomycin, and 5% heat-inactivated pooled human AB serum. Cultures were supplemented with fresh medium containing 20 U/ml IL-2 at day 6–7, and T cells were cloned by limiting dilution at day 15 in 96-well plates (Costar, Cambridge, MA) in the presence of irradiated (5000 rad) allogeneic PBMC, PHA (1 μg/ml), and IL-2 (20 U/ml). Growing cells were further expanded in IL-2-containing medium and stimulated bimonthly in the presence of irradiated allogeneic PBMC and PHA (0.7 μg/ml). Cultured T cells were harvested 10–20 days after previous stimulation, washed extensively, and suspended in RPMI 1640 medium supplemented with 10% FCS. For Ag-dependent activation, macrocultures were performed in six-well trays, in which 8 x 10^5 T cells/well were cultured at 37°C in 5% CO2-air in the presence of 4 x 10^5 autologous irradiated APC (PB nonresetting forming cells) in 4 ml of medium with or without 10 μg/ml of TT or PPD. Controls included cultures of APC (4 x 10^5/ml) alone or T cells (8 x 10^5/ml) alone. In the majority of the experiments, T cells were activated in the absence of APC upon CD3 cross-linking on OKT3 (0.5 μg/ml)-coated plastic dishes (26). After 6 h of culture, the supernatants were collected and frozen for further cytokine determination. For membrane preparation, the cells were washed three times in PBS, then suspended in 1 ml of PBS containing 0.68 M sucrose, 20 μM PMSF, 0.2 μM pepstatin, and 5 mM EDTA. Briefly, cell suspensions were kept on ice and sonicated with five 5-s bursts of 40 W each; nuclei were pelleted by centrifugation at 4,000 x g for 15 min; and the supernatants were centrifuged at 100,000 x g for 45 min at 4°C. Membrane pellets were suspended in RPMI 1640 containing 10% FCS and 5 mM iodoacetamide, and frozen at −70°C until use.

Monocytes

The human monocytic cell lines THP-1 and U-937 (American Type Culture Collection, Manassas, VA) were maintained in RPMI 1640 supplemented with 10% heat-inactivated FCS (28). Fresh PB monocytes were obtained by aggregation in the cold, as described (29). Briefly, PBMC were incubated at 5 x 10^6/ml for 30 min at 4°C under rotation leading to monocyte aggregation, followed by 10-min incubation on ice. Pellets of aggregated monocytes were separated from nonaggregated PBMC by a gradient of FCS. Monocyte-enriched aggregates were further depleted in T and NK cells by rosetting with neuraminidase-treated SRBC. Polyoxymycin B (1 μg/ml) was present throughout the whole procedure performed in polypropylene tubes, unless otherwise stated (Falcon; Becton Dickinson, Heidelberg, Germany). Monocyte purity routinely consisted of >85% CD14+ cells, <1.5% CD3+ cells, and <1% CD19+ cells. Monocytes were then cultured for 7 days in the presence of GM-CSF (50 ng/ml) in RPMI 1640 + 10% FCS and polyoxymycin B, unless otherwise stated. At the end of the culture period, GM-CSF-treated monocytes (>95% CD14+) were extensively washed before use.

Cocultures of T cells with monocytes

THP-1 or U-937 cells were seeded at 5 x 10^6 cells/well in 96-well flat-bottom plates. Fresh monocytes or GM-CSF-treated monocytes were seeded at 2 x 10^6 cells/well. Various volumes of T cell membranes were added with either target cell at T cell equivalents/monocyte ratios ranging from 1/1 to 8/1 in 200-μl final volume of RPMI-FCS 10% with no polyoxymycin B, unless otherwise stated. After 48 h of culture in 5% CO2-midified air at 37°C, supernatants were harvested and stored at −20°C for further MMP or cytokine determination. In some assays, anti-CD40L, anti-IFN-γ, anti-IL-4 (IgG1), or irrelevant IgG1 was added at 10 μg/ml. IL-1α and sFc-p55 (anti-TNF) were used at 1 μg/ml (30). IL-4, TNF, and IL-1α were added at 5 ng/ml and IFN-γ at 300 U/ml, unless otherwise stated. Indomethacin was solubilized in ethanol and used at the indicated concentration with appropriate controls.

mRNA levels studies

For Northern blot experiments, 2–3.5 x 10^6 THP-1 cells or GM-CSF-treated CD14+ mononuclear cells were plated in 60-mm petri dishes. T cell membranes to the equivalent of 10^5 cells were then added to the cultures (typically 200 μl of cell membranes in 2.5 ml), and monocytes were further cultured for 6 or 14 h in 10% FCS medium. Total cellular RNA was extracted using TRIzol (Life Technologies) and analyzed by Northern hybridization with 32P-labeled cDNA probes specific for MMP-1, MMP-9, TIMP-1 (kind gift from Dr. H. G. Welgus, St. Louis, MO), and GAPDH (31, 32). Gels were autoradiographed, and signal intensity was determined by densitometry using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Cytokines, MMP, and TIMP-1 protein determination

Production of IFN-γ and IL-4 (Hoffmann-LaRoche), pro-MMP-1 (Binding Site, Birmingham, U.K.), total MMP-9 (R&D Systems, Minneapolis, MN), and TIMP-1 (Amersham, Little Chalfont, U.K.) was assessed by ELISA (33). The sensitivity threshold was 25 pg/ml for IFN-γ and IL-4, and 2 ng/ml for pro-MMP-1, MMP-9, and TIMP-1.
Statistical analysis

The nonparametric Mann-Whitney U test was used to compare means.

Results

Ag-activated Th1 and Th2 cell clones induce contact-dependent monocytic production of MMP

T cells and mononuclear phagocytes infiltrating inflamed tissues can be in close contact. We were interested in investigating the effect of T cell-to-monocyte contact in MMP production by mononuclear phagocytes. To minimize the effect of soluble products and to focus on the activities of cell membrane-associated molecules, we used cell membrane preparations as effectors rather than intact T cells. Pro-MMP-1 (thereafter MMP-1) production by THP-1 cells was observed when cell membranes were obtained from Ag-activated T cells or from T cells activated by CD3 cross-linking (Fig. 1, A and B). MMP-1 production was dependent on the amount of cell membranes added and was observed with both Th1 and Th2 cells. Resting T cells, Ag-pulsed APC, or T cells cultured with APC in the absence of Ag failed to induce significant levels of MMP-1. Under similar culture conditions, constitutive TIMP-1 production was not enhanced (not shown). Interestingly and unexpectedly, Th2 clones appeared to be as potent, if not more so, as Th1 clones in inducing MMP-1. Indeed, MMP-1 production by THP-1 cells induced by cell membranes of four Th1 clones and six

FIGURE 1. MMP-1 production by THP-1 cells cultured in the presence of membranes of polarized T cells. A, Cell membranes from Th1 cells. B, Cell membranes from Th2 cells. Th1 and Th2 cell clones were described previously (26). Cell membranes were prepared after 6 h of T cell activation and added to $5 \times 10^6$ THP-1 cells/well. A total of 20 μl was equivalent to $0.4 \times 10^6$ T cells. APC were autologous irradiated nonrosetting PBMC. Ag was PPD (10 μg/ml) for Th1 and TT (10 μg/ml) for Th2 cells. Th+a CD3 denotes T cell activation upon CD3 cross-linking. Th resting were T cell clones cultured without activators for 6 h. MMP-1 was measured by ELISA in 48-h culture supernatants. The results are from an experiment, representative of four, in which membranes were from four pooled Th1 cell clones and from five pooled Th2 cell clones. The ng/ml of IFN-γ and IL-4 in 6-h culture supernatant of Ag-activated T cells were 5.4 ± 1.2 and 0.6 ± 0.4 for Th1 clones, and 0.06 ± 0.06 and 9.3 ± 6.2 for Th2 clones, respectively.

FIGURE 2. Neutralization of membrane-associated IL-4 and TNF on Th2 cells inhibits their capacity to induce MMP-1 production by THP-1 cells. A, Representative results obtained with cell membranes (equivalent to 0.8 × $10^6$ cells/well) from a Th2 clone (224A4, activated by CD3 cross-linking (IFN-γ < 0.03 ng/ml; IL-4, 4.3 ng/ml). The inhibitory effect of IL-1ra was not observed in four additional experiments. B, Results obtained with cell membranes from seven distinct Th2 clones activated by CD3 cross-linking. THP-1 cells were $5 \times 10^3$/well; neutralizing Abs were added simultaneously to T cell membranes. MMP-1 was measured in 48-h supernatants by ELISA. Bars represent mean ± SD. The number of clones tested is shown in brackets.
Th2 clones activated by CD3 cross-linking, tested in parallel at identical cell/THP-1 ratios, was 144.8 ± (SD) 34.7 ng/ml for Th1 cells and 596.6 ± 571.4 ng/ml for Th2 cells (p = 0.033).

**Th2 cell membrane-associated IL-4 and TNF additively participate in inducing MMP-1 production by THP-1 cells**

To identify surface molecule(s) of activated T cells involved in contact-dependent MMP-1 production by THP-1 cells, the effect of several blocking or neutralizing agents was assessed. MMP-1 production induced by Th2 cells was substantially inhibited by neutralization of TNF, of IL-4, and inconsistently by the addition of IL-1ra (Fig. 2, A and B). When membrane-associated IL-4 and TNF were simultaneously neutralized, inhibition of MMP-1 production observed was higher than when either one of the cytokines was neutralized, indicating an additive effect of these two cytokines (Fig. 2B). In contrast, of the agents tested (anti-CD40, anti-CD40L, IL-1ra, anti-IFN-γ, anti-IL-4, anti-TNF), only anti-TNF inhibited MMP-1-inducing capacity of Th1 cells. The inhibition observed was inconsistent and varied between nil and 50% in several experiments with seven different clones (Fig. 3B, and not shown). Blockade of the CD40-CD40L engagement did not affect MMP-1 production induced by either Th1 or Th2 cell clones (Fig. 3, and not shown). To further investigate the specificity of such findings, experiments were performed in which MMP-1 and MMP-9 production was assessed in the same culture supernatants. Neutralization of membrane-associated TNF, of membrane-associated IL-4, or of both simultaneously inhibited MMP-1 induced by Th2 cells; however, only neutralization of TNF and not of IL-4 resulted in partial inhibition of MMP-9 induction by Th2 cells (Fig. 3B). When the same reagents were used on Th1 cell clones, only TNF neutralization resulted in inhibition of the induction of both MMP-1 and MMP-9, while anti-IL-4, as expected, did not.

**FIGURE 3.** Neutralization of membrane-associated IL-4 selectively inhibits MMP-1, but not MMP-9 production induced by Th2 cells on THP-1. A, Cell membranes (equivalent to 0.8 × 10⁶ cells/well) from a Th1 clone (900B7) activated by CD3 cross-linking (IFN-γ, 18.7; IL-4, 0.2 ng/ml). B, Cell membranes (equivalent to 0.8 × 10⁶ cells/well) from a Th2 clone (225C1) activated by CD3 cross-linking (IFN-γ < 0.03; IL-4, 19.8 ng/ml). a-TNF + a-IL-4 denotes anti-TNF + anti-IL-4 agents. Culture conditions as in the legend to Fig. 2. MMP-1 and MMP-9 were measured by ELISA. Bars are representative of the mean ± SD of duplicate cultures. Four other individual Th1 clones and six other individual Th2 clones were tested with similar results.

**FIGURE 4.** Soluble IL-4 alone and additively to TNF or to activated HUT cell membranes specifically stimulate MMP-1 production by THP-1 cells. A, Soluble cytokines IL-4, TNF, and IL-1α were used at 5 ng/ml, IFN-γ at 300 U/ml. B, HUT cell membranes equivalent to 0.4 × 10⁶ cells/well were obtained upon PHA + PMA activation and added to the cultures alone or together with the cytokines. C and D, Anti-IL-4 mAb was added to the culture 5 min earlier than IL-4. MMP-1 was measured in 48-h supernatants by ELISA. Similar results were obtained in three additional experiments. Note that in the presence of HUT-78 cell membranes (B and D), the MMP-1 production was at least one order of magnitude higher than that observed in the presence of soluble cytokines.
affect Th1 activities (Fig. 3A). Together, these data indicate that once activated, both Th1 and Th2 cell clones induce MMP-1 and MMP-9 production, and that for both MMP, membrane-associated TNF plays a role. In addition and surprisingly, membrane-associated IL-4 specifically participated in inducing MMP-1 production by THP-1 cells when Th2 cell clones were assayed.

Soluble IL-4 by itself and synergistically with TNF or with T cell membranes stimulates MMP-1 production by THP-1 cells

Since according to existing literature soluble IL-4 has an inhibitory effect on MMP-1 production by macrophages (24, 25), it was of interest to verify its effects in our assay system. When cultured in the presence of soluble IL-4, THP-1 cells produced small amounts of MMP-1 (Fig. 4A). The IL-4 effect was, however, greater than those of TNF, IL-1α, or IFN-γ. In addition, when IL-4 was used in conjunction with TNF, a synergistic effect was observed. Although MMP-1 levels were highest when IL-4 was added in more complex mixtures of cytokines, no synergism was observed when TNF was used in conjunction with IL-1α or IFN-γ (Fig. 4A). Similarly, the MMP-1-inducing effect of nonpolarized T cell membranes obtained from mitogen-activated HUT-78 cells was enhanced by IL-4, but not by the other cytokines tested (TNF, IL-1α, IFN-γ) (Fig. 4B), and the enhancing effect was abolished by IL-4 neutralization (Fig. 4, C and D). Similar data were obtained using the U-937 cell line instead of THP-1 cells (not shown). The IL-4 activity was dose-dependent (Fig. 5C) and unaffected by the presence of indomethacin (Fig. 5, A and B), thus indicating independence from PG synthesis. It was also unaffected by polymyxin B, ruling out LPS contamination (Fig. 5C). The IL-4 effect was observed both at the protein and at the mRNA levels (Fig. 6). Indeed, steady-state MMP-1 mRNA levels in THP-1 cells were slightly increased by soluble IL-4 alone, and IL-4 potentiated MMP-1 mRNA levels when used in conjunction with TNF or much more potently in conjunction with activated HUT-78 cell membranes. In the same experiments, TNF or activated HUT-78 cell membranes induced MMP-9 mRNA, while IL-4 specifically inhibited this effect. IL-4,
TNF, and IFN-γ did not substantially modify TIMP-1 mRNA levels in THP-1 cells, either at basal level or upon contact-dependent activation by HUT-78 cells (Fig. 6). Overall, these results indicate not only that soluble IL-4 is not an inhibitor, but on the contrary, that it is a potent inducer of MMP-1 production by THP-1 cells.

IL-4 enhances MMP-1 production when GM-CSF-pretreated peripheral CD14⁺ monocytes are activated by T cell membranes

THP-1 and U-937 are monocytoid cell lines maintained in vitro, and their capacity to produce MMP-1 in response to IL-4 may not be shared by mononuclear phagocytes obtained ex vivo. We addressed this question by assessing the effect of IL-4 on human monocytes derived from PB. When CD14⁺ cells were cultured for 1 wk in the presence of GM-CSF, they acquired the capacity to produce MMP-1. On these cells, soluble IL-4 (as well as soluble TNF) had no or minimal capacity to induce MMP-1 production. However, when GM-CSF-treated CD14⁺ mononuclear phagocytes were activated by membranes of HUT-78 cells, MMP-1 production was observed. In four distinct experiments, soluble IL-4 substantially enhanced HUT-78-induced MMP-1 production (p = 0.02), which was abrogated by IL-4 neutralization (Fig. 7). The IL-4 effect on MMP-1 production was specific, because in the same supernatants in which MMP-1 production was increased, MMP-9 production was inhibited (p = 0.02) (Fig. 7). Northern blot experiments showed that

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<th>Table 1. IL-4 regulation of T cell contact-dependent MMP-1 production by freshly isolated and GM-CSF-pretreated peripheral CD14⁺ monocytes</th>
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a Monocytes were plated immediately after purification at 1 × 10⁶ cells/well, or after 7 days of culture in GM-CSF (50 ng/ml) at 0.2 × 10⁶/well, then exposed or not to IL-4 (5 ng/ml) for 30 min before being cultured for further 48 h in the presence (or absence) of membranes of mitogen-activated HUT-78 T cells (equivalent to 0.8 × 10⁶ cells/well). MMP-1 was measured by ELISA. Data represent the mean ± SD of two distinct experiments in which the same monocytes were used before or after 7 days of culture in GM-CSF.

* p < 0.05 for difference in MMP-1 produced upon HUT-78 cell stimulation in cultures with and without IL-4. Numbers in parentheses represent the percentage of MMP-1 production induced by HUT-78 in the presence of IL-4 compared to production induced by HUT-78 alone.

FIGURE 7. IL-4 selectively enhances MMP-1 production by GM-CSF-treated CD14⁺ mononuclear phagocytes activated by contact with HUT cells. PB CD14⁺ cells were cultured for 7 days in the presence of GM-CSF, then washed and cultured (0.2 × 10⁶ cells/well) for an additional 48 h in the presence of membranes of mitogen-activated cells (equivalent to 0.8 × 10⁶ cells/well). IL-4 used at 5 ng/ml and anti-IL-4 at 10 μg/ml were added simultaneously with HUT-78 membranes. MMP-1 and MMP-9 were measured by ELISA. Bars represent the mean ± SD of four distinct experiments.

FIGURE 8. Steady-state mRNA levels of MMP-1, MMP-9, and TIMP-1 are induced by HUT-78 cell membranes or TNF, and are differentially modulated by soluble IL-4. A, Northern blot analysis. B, Densitometric scanning quantification of MMP-1 and MMP-9 mRNA signals normalized to GAPDH. CD14⁺ PB monocytes cultured for 7 days in GM-CSF were extensively washed, then plated at 5 × 10⁶ cells/well and cultured for additional 14 h under the different experimental conditions. Total RNA was extracted and analyzed (4 μg/lane) by Northern blot hybridization with specific cDNA probes. Membranes from activated HUT-78 cells were equivalent to 12 × 10⁶ cells/well. IL-4 and TNF were used at 5 ng/ml, and anti-IL-4 mAb at 10 μg/ml. Data are representative of two similar experiments in which the SD of normalized results were within 15% of the mean.
mRNA levels of MMP-1 induced by HUT-78 cell contact were enhanced in the presence of IL-4, while mRNA levels of MMP-9 were inhibited, and that IL-4 activities were abrogated by IL-4 neutralization (Fig. 8). In contrast with the experiments performed with THP-1 cells, the steady-state levels of TIMP-1 mRNA were greatly enhanced by HUT-78 cell membranes. The induced TIMP-1 levels were unaffected by IL-4 and TNF both at mRNA and protein levels.

**IL-4 differentially regulates contact-dependent MMP-1 production in freshly isolated and in GM-CSF-pretreated peripheral CD14⁺ monocytes**

Under our standard culture conditions, no MMP-1 production was detected on freshly isolated PB monocytes. However, when the number of monocytes was increased from 1 × 10⁶ cells/ml to 5 × 10⁶ cells/ml, MMP-1 production was induced by HUT-78 cell membranes (Table I). We thus compared the effect of IL-4 on MMP-1 production by freshly isolated and GM-CSF-pretreated monocytes. IL-4 alone did not induce significant MMP-1 production on either cells. However, when the cells were preincubated for 30 min with IL-4, their response to HUT-78 contact was dramatically different. Indeed, MMP-1 production was significantly (p < 0.05) decreased in freshly isolated monocytes, while it was significantly increased (p < 0.05) in monocytes from the same donor when previously cultured in GM-CSF for 7 days (Table I). These results indicate that the regulation of MMP-1 production by IL-4 is dependent on the differentiation status of monocytes.

**Discussion**

CD4⁺ T cells may regulate the responses of mononuclear phagocyte through the release of soluble mediators and by direct cell-to-cell contact, a powerful mechanism in inducing the production of MMP (15, 16). Different model systems have been used in the present study to assess the effect of T cell polarization in inducing MMP production by mononuclear phagocytes. The monocytic THP-1 and U-937 cell lines, as well as freshly separated and GM-CSF-pretreated PB CD14⁺ monocytes have been used as responders. Although differing in many respects, these model systems indicate that Th2 cells and IL-4 specifically induce or enhance MMP-1 production by mononuclear phagocytes at various stages of differentiation with the notable exception of freshly isolated PBMC, in which IL-4 has inhibitory activity. The MMP-1-inducing/enhancing capacity of Th2 cells and of IL-4 is due to their intrinsic biological activity and not to contaminants. First, Th2 cells needed to be activated to induce MMP-1 production by THP-1 cells in an IL-4-dependent manner. Indeed, resting Th2 cells kept under similar culture conditions did not have this capacity. Second, IL-4-neutralizing mAb partially inhibited the Th2 cell-inducing capacity, while not affecting Th1 cell activities. Third, soluble rIL-4 shared with Th2 cell membranes the capacity of inducing MMP-1 on THP-1 cells, its effect was abrogated by IL-4 neutralization, and it was unaffected by the presence of polymyxin B, which inactivates LPS. Fourth, GM-CSF-pretreated PB CD14⁺ monocytes showed enhanced MMP-1 production when activated by HUT-78 cell membranes in the presence of IL-4, an effect observed at both mRNA and protein levels. Fifth, MMP-1 induction by Th2 cells and IL-4 was specific as far as under identical culture conditions MMP-9 production was decreased and TIMP-1 was unaffected. The lack of TIMP-1 modulation and the inhibition of MMP-9 production by IL-4 reported in this work are in agreement with previous publications (24, 25, 34). Interestingly, IL-4 was inhibitory to MMP-1 production by freshly isolated PB CD14⁺ monocytes in response to T cell contact. An inhibitory activity of IL-4 in MMP-1 production by monocytes activated by mitogens, or by GM-CSF plus TNF, or GM-CSF plus IL-1 has also been observed by others (20, 25). Several hypotheses may be formulated to explain the differential effect of IL-4 on MMP-1 production by freshly isolated and GM-CSF-cultured monocytes. It has already been shown that culture of monocytes in GM-CSF results in the loss of γc expression (35). This loss is accompanied by the selective loss of the IL-4 capacity to suppress LPS-induced TNF production and by decreased STAT-6 activity (35). Thus, we can hypothesize that the capacity of IL-4 to inhibit MMP-1 production is dependent on γc expression and γc-dependent intracellular signaling. When γc is no longer expressed, IL-4 enhances rather than inhibits MMP-1 production. Alternatively, culture in GM-CSF may affect the phosphorylation pattern of IL-4-Rα in response to IL-4 and influence the signal transduction pathways directly linked to this chain. Indeed, IL-4-Rα has a distinct domain structure that results in the activation of a specific array of signaling pathways (36). Whatever the mechanism involved, it will be important to verify whether the effect on MMP-1 production by in vivo differentiated macrophages resembles those observed with GM-CSF-cultured or freshly isolated monocytes. Indeed, according to in situ hybridization, MMP-1 and MMP-9 mRNA were represented heterogeneously in alveolar macrophages and U-937 cells differentiated in the presence of PMA (37).

The use of T cell membranes allowed us to test the net biological effect of T cell contact on mononuclear phagocyte production of MMP, i.e., the algebraic sum of positive and negative signals. Although not part of the present study, previous experiments performed in our laboratory have documented that MMP production induced by T cell membranes is mimicked by activated, paraformaldehyde-fixed T cells, and that plasma cell membrane as opposed to nuclear or cytosolic preparations carried the biological activity (15). In the attempt to identify molecules involved in the contact-dependent inducing capacity, membrane-associated TNF was identified as being a relevant molecule to induce MMP-1 and MMP-9 production in both Th1 and Th2 cells. This is in agreement with the reported capacity of soluble TNF to induce MMP-9 production by mononuclear phagocytes when used alone, or to induce MMP-1 when used in conjunction with GM-CSF (19, 20). Although membrane-associated TNF was involved, TNF neutralization did not abrogate the capacity of Th1 cell membranes to induce MMP-1 and MMP-9 production, thus indicating that additional molecules could play an important role. Although the existence of a membrane-associated form of TNF has been documented, no firm data exist on the presence of membrane-associated form of IL-4. The absence of a transmembrane region in IL-4 sequence is not against this possibility, because other cytokines, such as IL-1α, IL-10, and IFN-γ, with no transmembrane domains have been identified associated with plasma cell membranes (38, 39). Upon T cell activation, IL-4 may thus, at least transiently, be retained within the cell membrane in a biologically active form (26). Previous reports have indicated that IL-4 decreases PG synthesis, therefore inhibiting PG-dependent MMP production (1, 25). In this respect, it is not surprising that in our experiments indomethacin did not inhibit MMP-1 production in THP-1 cells activated by IL-4 and indicate a PG-independent pathway underlying MMP-1 induction by IL-4 (Fig. 5).

When comparing the responses of THP-1 cells and GM-CSF-pretreated PB CD14⁺ monocytes with contact with membranes from HUT-78 cells, a major difference was observed in the regulation of TIMP-1 mRNA. THP-1 cells showed high constitutive expression of TIMP-1 mRNA, which was neither modulated by T cell membranes, nor by exogenous cytokines. Conversely, PB CD14⁺ monocytes had low basal expression of TIMP-1 mRNA.
that was highly induced by T cell membranes with no further modulation by IL-4 or TNF. Thus, in both THP-1 cells and GM-CSF-treated PB monocytes, IL-4 enhanced MMP-1 production with no effect on its specific inhibitor, suggesting a possible net increase in collagenolytic activity.

Th2 cells and their soluble products are currently considered to be preferentially anti-inflammatory (2), but proinflammatory IL-4 activities have been documented in some in vivo models (40, 41). The present data suggest that Th2 cells induce mononuclear phagocytes, at least in certain stages of their maturation and differentiation, to preferentially produce MMP-1, which is involved in native collagen cleavage, and therefore in tissue destruction and remodeling. Th2-dominated inflammatory disorders have been relatively poorly investigated in this aspect. However, as an example, severe allergic asthma, a Th2-linked disorder, is now recognized as a condition that involves pulmonary remodeling (42). Fibrillar collagen present in pulmonary parenchyma could be attacked, and Th2 cells might participate in this process by inducing mononuclear phagocytes to produce MMP-1. A possible scenario in which polarized T cells interact with mononuclear phagocytes in a Th2-dominated inflammatory reaction leading to some tissue destruction and remodeling could involve IL-4 released by Ag-activated effector Th2 cells or by mast cells upon specific IgE cross-linking. The effect of IL-4 could then be 2-fold. On the one hand, it could induce resident or newly recruited mononuclear phagocytes to produce MDC and AMAC-1 chemokines (6, 43). Preferential recruitment of activated Th2 cells will then be ensured by their preferential expression of CCR4 and CCR8 that bind MDC (44). On the other hand, soluble IL-4 and newly recruited activated Th2 cells by direct cell contact could induce mononuclear phagocytes to produce MMP-1, thus attacking fibrillar collagen and contributing to ECM degradation (45). Indeed, alveolar macrophages producing AMAC-1 have been described, particularly in asthmatic patients.

Stein and coworkers have first reported that in response to IL-4, inflammatory macrophages adopt an alternative activation phenotype, distinct from that induced by IFN-γ (46). This phenotype is characterized by high clearance of mannoseylated ligands, enhanced MHC class II Ag expression, and reduced secretion of proinflammatory cytokines (47). Based on our finding, the selective capacity to produce MMP-1 in response to IL-4 can thus be added to the repertoire of alternatively activated macrophages.

References


