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Bi-Directional Induction of Matrix Metalloproteinase-9 and Tissue Inhibitor of Matrix Metalloproteinase-1 During T Lymphoma/Endothelial Cell Contact: Implication of ICAM-1

Fawzi Aoudjit,[1] Edouard F. Potworowski, and Yves St-Pierre[2]

The mechanisms that lead to the expression of matrix metalloproteinases (MMP) and tissue inhibitors of MMP (TIMPs) during the invasive process of normal and transformed T cells remain largely unknown. Since vascular cells form a dynamic tissue capable of responding to local stimuli and activating cells through the expression of cytokine receptors and specific cell adhesion molecules, we hypothesized that the firm adhesion of T lymphoma cells to endothelial cells is a critical event in the local production of MMP and TIMP. In the present work, we show that adhesion of lymphoma cells to endothelial cells induced a transient and reciprocal de novo expression of MMP-9 mRNA and enzymatic activity by both cell types. Up-regulation of MMP-9 in T lymphoma cells was concomitant to that of TIMP-1, and required direct contact with endothelial cells. Induction of MMP-9, but not of TIMP-1, was blocked by anti-LFA-1 and anti-intercellular adhesion molecule-1 Abs, indicating that induction of MMP-9 and TIMP-1 in lymphoma cells required direct, yet distinct, intercellular contact. In contrast, the induction of MMP-9 in endothelial cells by T lymphoma cells did not necessitate direct contact and could be achieved by exposure to IL-1 and TNF, or to the supernatant of T lymphoma cell culture. Together, these results demonstrate that firm adhesion of T lymphoma cells to endothelial cells participates in the production of MMP-9 in both cell types through bi-directional signaling pathways, and identify intercellular adhesion molecule-1/LFA-1 as a key interaction in the up-regulation of MMP-9 in T lymphoma cells. *The Journal of Immunology,* 1998, 160: 2967–2973.

N ormal and cancer cells can invade tissues because they secrete matrix metalloproteinases (MMPs)[3], which degrade the extracellular matrix of basement membranes (1, 2). MMPs are secreted as proenzymes and acquire their active form outside the cells after proteolytic cleavage. Recent studies have shown that two MMP enzymes, MMP-2 (M_r 72,000 gelatinase A) and MMP-9 (M_r 92,000 gelatinase B) can play a crucial role during cancer cell invasion. Elevated levels of MMP-9 have been found in different types of tumors in vivo (3, 4), and up-regulation of MMP-9 in tumor cells conferred an invasive property to nonmetastatic cells (5). MMP activity is inhibited by specific tissue inhibitor of metalloproteinases (TIMPs) (6). TIMP-1 and TIMP-2 preferentially form complexes with the proenzymes of MMP-9 and MMP-2, respectively, although both can also inhibit the activity of other MMPs. Reduction of TIMP expression by antisense strategy has also been shown to confer both tumorigenic and metastatic properties to noninvasive 3T3 cells (7). It is therefore the equilibrium between MMPs and TIMPs that determines the degree of invasiveness and the metastatic potential of a given cancer cell.

The expression of MMPs is closely regulated by activating agents, including cytokines and cell adhesion molecules of the integrin family (8–10). In T cells, the expression of MMP-2 and MMP-9 has been extensively studied and shown to depend on the activation state following exposure to chemotactic factors or cytokines, such as IL-2 and IL-4 (11–13). Integrin-mediated adhesion of T cells to recombinant cell adhesion molecules showed that vascular cell adhesion molecule-1 (VCAM-1)/very late Ag-4 (VLA-4) interaction is sufficient to induce MMP-2 expression in T cells (14). Local concentration of MMP, however, is likely not to depend solely on the invading T cells, but also on stromal cells in close proximity. Indeed, we and others have shown that T cells could induce MMP-9 expression in a variety of cell lines, including monocytic and fibroblastic cells (15, 16).

There is an indication that MMP-9 is a key player in the dissemination of T lymphoma cells to peripheral tissues. Although TIMP-1 counterbalances MMP-9 bioactivity, clinically aggressive lymphomas have shown surprisingly elevated levels of both MMP-9 and TIMP-1 (17–19). This observation suggests that either the induction of MMP-9 and TIMP-1 are temporally separated, or that both genes are closely coregulated during the invasion process. It is indeed possible that the TIMP-1 inhibitory effect on MMP-9 is masked by its enhancing effect on cell growth, particularly in the case of T lymphoid cells (20). The transcriptional regulation of MMP-9 and TIMP-1 during the dissemination process of normal and transformed T cells is still, however, largely unknown. Their co-expression in lymphoma cells suggests that they could depend on a common induction mechanism. Alternatively, the observation that some lymphoma cell clones express

Table I. *Sequences of the oligoprimers used in the detection of TIMPs, MMPs, and cytokines expression*

Gene	Sense Sequence	Antisense Sequence	NT (bp)
MMP-2	CTTTGCAGGAGACAAGTTCTGG	TTAAGGTGGTGCAGGTATCTGG	705
MMP-9	CCATGAGTCCCTGGCAG	AGTATGGATGTTATGATG	380
TIMP-1	CTTTGCATCTCTGGCATCTGG	AAGTAGACAGTGTTCAGGC	655
TIMP-2	TGCAGCTGCTCCCCGGTGCAC	TTATGGGTCCTCGATGTCAAG	584
TNF-α	CAGCCTCTTCTCATTCCTGC	GACAGGTACAACCCATCCG	313
IFN-γ	CAAGTGGCATAGATGTGGAA	CTGGACCTGTGGGTTGTT	266

MMP-9 and TIMP-1 separately supports the idea that their induction could be mediated by temporally separated transcriptional activation involving unique or distinct stimuli (19).

As vascular cells form a dynamic tissue capable of responding to its environment and/or activating cells through the production of cytokines and cell surface expression of cell adhesion molecules, we hypothesized that contact between T lymphoma cells and endothelial cells (EC) is a key event that controls expression of MMP-9 and TIMP-1 at the site of extravasation. In the present work, we have examined the expression of MMP-2 and MMP-9 mRNA and enzymatic activity, as well as the expression of their natural inhibitors, TIMP-1 and TIMP-2, during adhesion of LFA-1-positive T lymphoma cells to EC in vitro. We showed that interaction between T lymphoma cells and EC leads to the induction of MMP-9 in both cell types. The inducing signals were, however, very distinct. Whereas the induction of MMP-9 in EC was due to lymphoma-derived soluble factors, increased expression of MMP-9 in lymphoma cells required intercellular adhesion molecule-1 (ICAM-1)-mediated adhesion. We further found that the expression of TIMP-1 paralleled that of MMP-9 in T lymphoma cells, but did not necessitate LFA-1/ICAM-1 interaction. Together, these results demonstrate that firm adhesion of T lymphoma cells to ECs participates in the production of MMP-9 and TIMP-1 in both cell types through tightly coordinated bi-directional signaling pathways, and identify ICAM-1/LFA-1 as a key interaction in the up-regulation of MMP-9 in T lymphoma cells upon contact with endothelium.

Material and Methods

Cell lines and reagents

The mouse T lymphoma cell line 164T2 was established in vitro from a radiation-induced T cell lymphoma in C57BL/Ka mice, as previously described (21). The 164T2 cells develop into massive lymphoid tumors in kidneys, liver, and spleen, following their i.v. injection to syngeneic mice (41). They express CD3, LFA-1, ICAM-1, and ICAM-2 constitutively, but not VLA-4. As an in vitro model to study the T lymphoma-EC interaction, we have used the endothelioma cell line b-end.3. In addition to expressing the von Willebrand factor, they express a repertoire of cell adhesion molecules indistinguishable from normal endothelium (such as E- and P-selectins, VCAM-1, ICAM-1, CD31, ICAM-2, etc.), and up-regulate their expression following stimulation with inflammatory cytokines, such as IL-1 and TNF, with kinetics similar to those reported for primary ECs. The similarity of this cell line to primary ECs has been well established in previous reports and has therefore been extensively used by many investigators as an in vitro model to study the ability of leukocytes to interact with vascular endothelium (22–26). All cells were maintained in culture using RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine, 10 mM HEPES, and antibiotics (complete medium). The inflammatory cytokines were purchased from Genzyme (Cambridge, MA).

Adhesion of T lymphoma cells to ECs

To study the effect of adhesion of T lymphoma cells to ECs, b-end.3 cells (5×10^5) were grown to confluency in 60-mm tissue culture wells by overnight culture. T lymphoma cells (5×10^6) were resuspended into 2 ml of complete serum RPMI medium for 6, 12, and 24 h at 37°C, 5% CO_2. Firm adhesion was evident at 6 h after addition of the T lymphoma cells. The cocultures were then washed twice with PBS and the adherent lym-

phoma cells were removed by washing with a warm solution containing trypsin (0.004% w/v) and EDTA (0.002% w/v) dissolved in PBS. This treatment allowed the removal of more than 95% of T lymphoma cells, leaving intact the EC monolayer, as assessed by flow cytometric analysis using mAbs to Thy1-2 Ag.

Coculture in transwells

Coculture experiments separating cells with polycarbonate membrane inserts with a 0.4-μm pore size (Trans-well; Costar, Cambridge, MA) were conducted after presoaking the inserts with 1 ml of complete medium and plating ECs in 60-mm tissue culture wells in 2 ml of complete RPMI medium. The ECs were grown to confluency, and 5×10^6 164T2 lymphoma cells were added in the upper chamber of the inserts. Cocultures were conducted for 6, 12, and 24 h. Total cellular RNA was then extracted from both lymphoma and ECs. In some experiments, blocking mAbs (50 μg/ml) were added 15 min before coculture experiments. The anti-ICAM-2 blocking mAb (IgG2a, clone 3C4) was kindly provided by Dr. T. A. Springer Center for Blood Research, Harvard Medical School, Cambridge, MA. Abs to LFA-1 (I21.7/7) and ICAM-1 (YN-1) were derived from hybridomas obtained from the American Type Culture Collection (Rockville, MD) and were purified by standard protein G chromatography.

RNA isolation and analysis

Total cellular RNA was isolated from lymphoma and ECs using the Trizol reagent (Life Technologies, Mississauga, Canada) according to the manufacturer's instructions. First strand cDNA was prepared from 3 μg of total cellular RNA in 20 μl of reaction volume using 40 U of M-MuLV reverse transcriptase (Boehringer Mannheim, Laval, Canada). After reverse transcription, the MMP-9, TIMP-1, MMP-2, and TIMP-2 were amplified using specific primers directed against the 5' end of the transcripts (Table I). Expression of β-actin (Stratagene, La Jolla, CA) and IL-1β (Clontech, Palo Alto, CA) mRNA was monitored using commercially available primers. TIMP-2-specific primers were carefully chosen to react with the different mRNA messages possibly resulting from alternative polyadenylation/termination site usage observed previously (27). Amplification was conducted using the PCR core kit (Boehringer Mannheim). Thirty cycles of amplification were performed in an MJ Research (Watertown, MA) Thermal cycler (model PTC-100TM) using the following programmed step cycle: 94°C for 1 min, 58°C for 2 min, and 72°C for 3 min. The amplification for each gene was in the linear curve. Five to 10 μl of the reaction mixture was size separated on a 1.5% agarose gel, and specifically amplified products were detected by ethidium bromide staining and UV transillumination. Semiquantitative analysis was conducted using a computerized densitometric imager (Model GS-670; Bio-Rad, Mississauga, Canada).

Zymography

MMP activity of cell culture supernatants was determined as previously described (16). Briefly, supernatants (1 ml) were centrifuged at 1200 rpm for 10 min to remove contaminating cells and debris, and were lyophilized and resuspended in 500 μl of PBS. Aliquots of 20 μl were subjected to electrophoresis on a 7.5% SDS-PAGE containing 1 mg/ml of denatured collagen. After electrophoresis, the gel was washed to remove SDS and incubated in a renaturating buffer (50 mM Tris, 5 mM $CaCl_2$, 0.02% NaN_3, 1% Triton X-100) for 18 h at 37°C. The gels were stained with Coomassie brilliant blue and destained in 30% (v/v) methanol/10% (v/v) acetic acid. The proteolytic activity was identified as a clear band on a blue background. Quantitative analysis of activity was conducted using a computerized densitometric imager.
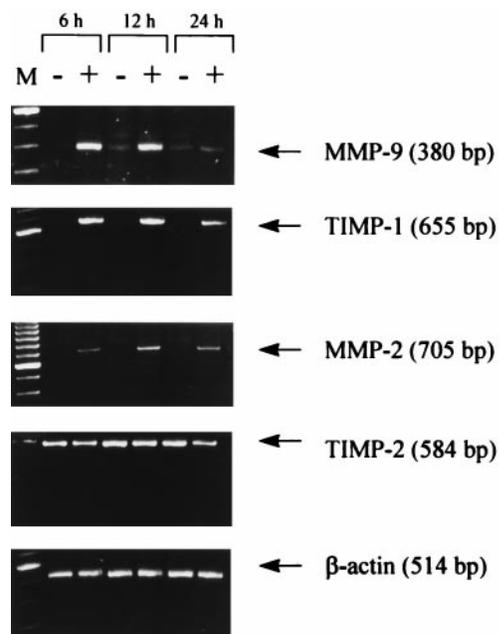
FIGURE 1. Detection of MMP and TIMP transcripts in lymphoma cells. Detection of transcripts was measured by RT-PCR in lymphoma cells cultures alone (−) or in contact (+) with ECs. Cocultures of lymphoma and ECs were conducted for the indicated intervals. M, m.w. standards (100-bp DNA ladder). The thick band represents the 600-bp marker. Results are representative of at least three independent experiments.

FIGURE 2. Detection of MMP and TIMP transcripts in ECs. Detection of transcripts was measured by RT-PCR in EC cultures alone (−) or in contact (+) with ECs. Cocultures of lymphoma and ECs were conducted for the indicated intervals. M, m.w. standards (100-bp DNA ladder). The thick band represents the 600-bp marker. Results are representative of three independent experiments.

Results

Induction of MMP-9 and TIMP genes in T lymphoma cells and EC upon cell-cell contact

To study the expression of MMPs and TIMPs upon adhesion of T lymphoma cells to EC, the cells were cocultured using a confluent monolayer of EC. In this coculture, most, if not all, T lymphoma firmly adhered to EC, as evidenced by the necessity of using trypsin to detach T lymphoma cells from EC. So the expression of MMP and TIMP gene expression in both cells could be assayed separately, lymphoma cells were detached at the indicated time after initiation of the coculture (6 to 24 h) using a warm solution (37°C) of PBS containing a low concentration of trypsin and EDTA, as previously described (14). Using mAbs to Thy-1.2, we showed that more than 95% of T cells were removed while less than 2% of EC were detached by this procedure (data not shown). Following removal of T lymphoma cells from EC coculture, total RNA extraction from EC cells was conducted by adding directly the Trizol reagent to the intact monolayer. Total RNA was then extracted from both cell preparations and subjected to semiquantitative reverse transcriptase (RT)-PCR. The primers used for RT-PCR are listed in Table I (28, 29), and had all been pretested for their functional integrity. PCR control reactions were conducted using β-actin-specific primers to standardize the relative amount of cDNA templates in each reaction. Our results showed that coculture of T lymphoma cells with EC induced time-dependent de novo expression of MMP-9 and TIMP-1 genes in lymphoma cells (Fig. 1). The detection of de novo synthesis of MMP-9 mRNA observed at 6 h postcontact was consistent with the results obtained in T lymphoma cells following exposure with PMA (16, 30). A reproducible but low copy number of MMP-2 transcripts were also detected in lymphoma cells in contact with EC, but necessitated twofold more cDNA templates compared with MMP-9, TIMP-1, and TIMP-2 genes. The induction of MMP-9
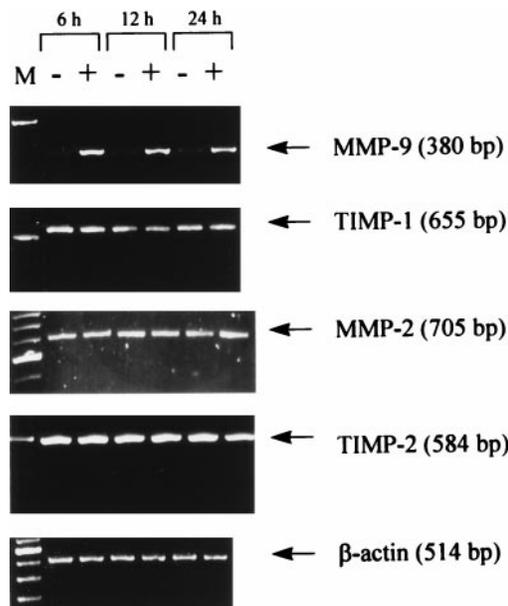
and of TIMP-1 genes appeared to be transient, as indicated by the lower intensity of the PCR products obtained from RNA of lymphoma cells in contact with EC for 24 h. The expression of TIMP-2 in lymphoma cells was constitutive and was not modulated following coculture with EC. These results suggested that MMP-9 and TIMP-1 were concomitantly induced in T lymphoma cells following contact with EC.

We next tested the influence of contact with lymphoma cells on regulation of MMP and TIMP in EC. We indeed found that MMP-9 gene expression was induced in EC following contact with the tumor cells (Fig. 2). As observed with lymphoma cells, MMP-9 was induced in EC early after contact (6–12 h) with lymphoma cells. In contrast to lymphoma cells, however, TIMP-1, TIMP-2, and MMP-2 were expressed constitutively in EC, and did not vary significantly upon coculture with lymphoma cells. The different repertoire of MMP and TIMP gene expression in both cell types confirmed the successful separation of T cells from EC by the mild trypsin treatment. Together, these results demonstrate that binding of lymphoma cells to EC induced bi-directional signaling that regulates MMP and TIMP gene expression.

Production and secretion of MMP-9 and MMP-2 following coculture of lymphoma and ECs

To assess the production of MMP by lymphoma and EC, the presence of the bioactive proteases was assessed by zymography in culture supernatant obtained from both cells cultured separately with or without prior contact. We found that constitutive production of MMP-9 (97 kDa) and MMP-2 (62 kDa) by T lymphoma cells cultured alone was barely detectable (Fig. 3). Very low levels of MMP-9 were detected in EC cultured alone, while they secreted significant amounts of MMP-2 constitutively. The patterns of MMP secretion by both cells before contact thus reflects the expression observed by RT-PCR. The two bands observed of approximately 92 to 97 kDa likely correspond to the pro-MMP-9
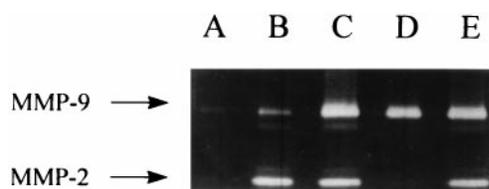
FIGURE 3. MMP production and secretion. Gelatin-zymography of serum-free supernatants obtained from 164T2 lymphoma cells (*lane A*) and EC (*lane B*) when cultured alone, or from supernatants of overnight coculture (*lane C*). To determine the origin of the MMP-9 secretion in cocultures, cells were separated after an initial contact (6 h) as described in *Material and Methods*, washed in PBS, and lymphoma cells (*lane D*) and EC (lane D) were cultured again in serum-free medium overnight to determine the origin of MMP-9 secretion. The MMP-9 (*upper band*) and MMP-2 (*lower band*) were located at approximately 97 kDa and 62 kDa, respectively. Results are representative of three independent experiments.

(major band) and active forms of MMP-9 (minor band), while the 62-kDa band represents the active forms of MMP-2. When supernatants from overnight coculture of T lymphoma cells and EC were tested, we found, as expected by the RT-PCR results described above, a significant increase in MMP-9 production, while that of MMP-2 did not change significantly. To establish whether the increased concentration of MMP-9 was derived from both EC and lymphoma cells, we separated both cell types after an initial contact and cultured them separately for an overnight period at 37°C. Zymographic analysis of the supernatants confirmed that production of MMP-9 upon coculture originated from both cell types. No MMP-2 was detected in the supernatant of T lymphoma cells.

Induction of MMP-9 and TIMP-1 in lymphoma cells, but not in ECs, necessitates direct contact during coculture

To assess whether MMP-9 induction in both cells necessitated direct contact, or was mediated by cytokine production, we compared the induction of the MMP-9 gene in both cell types following direct contact with the induction obtained in transwell cocultures. In the transwell design, lymphoma cells (upper chamber) were separated from EC (lower chamber) by a porous 0.4-μM membrane. After 6, 12, and 24 h of coculture, cells were harvested and examined for MMP-9 expression. Controls included cells cultured alone and cells cultured in direct contact. Our results showed that the physical barrier of transwells completely abrogated the ability of EC to induce MMP-9 in T lymphoma cells (Fig. 4 *upper panel*), suggesting that induction of MMP-9 in lymphoma cells required direct contact with EC. In contrast, we still observed an up-regulation of MMP-9 in EC following coculture with T lymphoma cells using the transwells (Fig. 4 *lower panel*), although the kinetic of induction was slower, likely due to the loss of the local effect normally provided by cell proximity during intercellular contact. These results demonstrate that up-regulation of MMP-9 in T lymphoma cells and EC following contact is mediated in response to distinct stimuli. The results obtained in coculture chambers were confirmed by stimulation with conditioned medium as we found that culture supernatant from the lymphoma cell culture was able to induce MMP-9 expression in EC (data not shown), indicating that the ability of lymphoma cells to induce MMP-9 in EC was constitutive, and was not secondary to an initial contact with EC. As expected, supernatants from EC culture had no effect on MMP-9 and TIMP-1 expression in T lymphoma cells (data not shown). Since TIMP-1 gene expression paralleled that of MMP-9 in lymphoma cells, we next tested whether up-regulation of the TIMP-1 gene in lymphoma cells also required contact. Using the
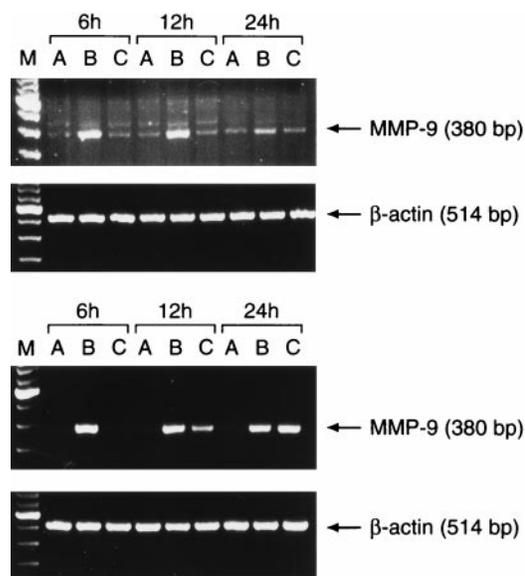

FIGURE 4. Induction of MMP-9 in transwell cocultures. Expression of MMP-9 was measured by RT-PCR in lymphoma (*upper panel*) and ECs (*lower panel*) cultured alone (*lanes A*), cocultured with direct contact (*lanes B*), and cocultured in transwells using a porous (0.4-μm pore size) polycarbonate membrane (*lanes C*). Cultures were conducted for the indicated time intervals. M, m.w. standards (100-bp DNA ladder). The thick band represents the 600-bp marker. Results are representative of three independent experiments.

same transwell design, we found that induction of TIMP-1 in T lymphoma cells by EC was also abolished when both cells were physically separated (Fig. 5). These results raised the possibility that expression of both MMP-9 and TIMP-1 in lymphoma cells following contact with EC was regulated by a common stimulus.

The role of ICAM-1 and LFA-1 interaction in MMP-9 gene expression by lymphoma cells

VLA-4/VCAM-1-mediated intercellular adhesion is necessary for the induction of MMP-2 in T cells (14). We have previously shown that the 164T2 T lymphoma cells do not express VLA-4, but constitutively express high levels of LFA-1 (Y. St-Pierre, unpublished observations). On the other hand, the b-end-3 cells express a large number of cell adhesion molecules, including ICAM-1, ICAM-2, and VCAM-1 (24, 25). To determine whether LFA-1-mediated adhesion played a role in the expression of MMP-9 and TIMP-1 in
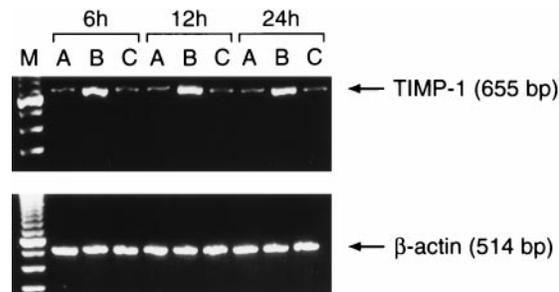

FIGURE 5. Induction TIMP-1 in lymphoma cells in transwell cocultures. Expression of TIMP-1 in lymphoma cells cultured alone (*lanes A*), cocultured with direct contact (*lanes B*), and cocultured cells in transwells using a porous (0.4-μm pore size) polycarbonate membrane (*lanes C*). Cultures were conducted for the indicated time intervals. M, m.w. standards (100-bp DNA ladder). The thick band represents the 600-bp marker. Results are representative of three independent experiments.
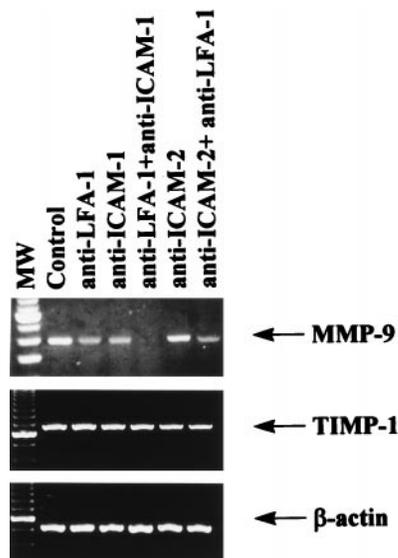
FIGURE 6. Blocking of MMP-9 induction but not of TIMP-1 by mAbs. mAbs specific for LFA-1, ICAM-1, and ICAM-2 (50 μg/ml) were added to cells 15 min before coculture of lymphoma and ECs. Cultures were conducted for 6 h and processed for RT-PCR as described. Specificity of the blocking effect of anti-LFA-1 and anti-ICAM-1 was demonstrated by the inability of anti-ICAM-2, alone or with anti-LFA-1, to inhibit expression of MMP-9. M, m.w. The thick band represents the 600-bp marker. Results are representative of three independent experiments.

FIGURE 7. Expression of inflammatory cytokines in T lymphoma cells and induction of MMP-9 in EC following exposure to TNF and IL-1. A, Detection of cytokine expression was measured by RT-PCR in 164T2 lymphoma cells cultured alone. IFN-γ-specific primers were tested on the RAW murine cell line (data not shown). B, MMP-9 expression following overnight culture of EC at different doses of IL-1 and TNF. M, m.w. standards (100-bp DNA ladder). The thick band represents the 600-bp marker. Results are representative of two independent experiments.

T lymphoma cells following contact with EC, we conducted a series of in vitro experiments using blocking Abs to LFA-1, ICAM-1, and ICAM-2. We found that addition of anti-ICAM-1 and anti-LFA-1 added separately significantly inhibited the ability of EC to induce MMP-9 expression in lymphoma cells (Fig. 6). Addition of anti-LFA-1 and anti-ICAM-1 together completely abolished the induction of MMP-9. In contrast, the presence of the Abs did not inhibit the ability of EC to induce TIMP-1 expression in lymphoma cells, indicating that the concomitant expression of MMP-9 and TIMP-1 in lymphoma cells following contact with EC involves distinct stimuli. The functional relationship between LFA-1/ICAM-1 interaction and MMP-9 expression was specific, as demonstrated first by the inability of mAb to ICAM-2 to block MMP-9 expression, and second by the inability of mAb to LFA-1 and ICAM-1 to block induction of TIMP-1.

The role of inflammatory cytokines in MMP-9 expression in EC

It is well known that vascular ECs are highly susceptible to activation by exposure to inflammatory cytokines, such as IL-1 and TNF. To determine whether these cytokines could be involved in the ability of T cells to increase MMP-9 expression in EC, we examined the expression of these cytokines in T lymphoma cells. Our results showed that T lymphoma cells constitutively expressed mRNA messages for both IL-1β and TNF-α, but not those encoding IFN-γ (Fig. 7A). To determine whether these cytokines can induce MMP-9 expression in EC, we tested their ability to stimulate MMP-9 expression and we found that exposure of EC to these inflammatory cytokines does indeed induce the expression of MMP-9, in a dose-response pattern (Fig. 7B).

Discussion

The production of MMP-2 and MMP-9 has been proposed as a mechanism by which normal and transformed T cells penetrate the subendothelial basal lamina to reach target tissues. In the present work, we demonstrate that adhesion of T lymphoma cells to EC
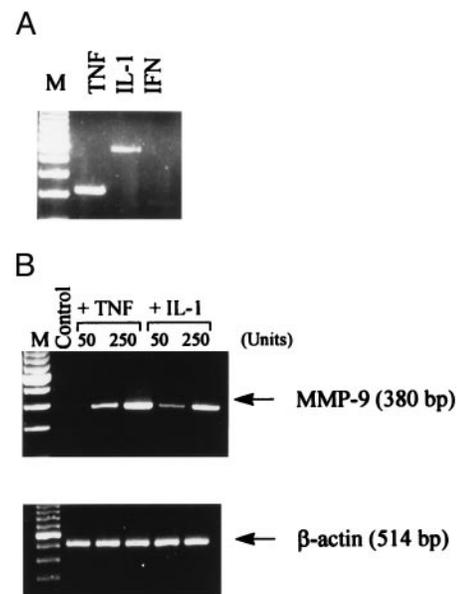
participates in the production of MMP-9 and TIMP-1 through bidirectional signaling pathways, and identify for the first time ICAM-1/LFA-1-mediated adhesion as a necessary interaction in the induction of MMP-9 in T lymphoma cells.

Our work further supports the previous observations that a functional relationship exists between MMP production by circulating T cells and intercellular adhesion to EC. In normal CD4-positive Th1 cell clones, up-regulation of MMP-2 protein, activity, and transient mRNA expression at the surface of murine CD4-positive Th1 cells is dependent upon adhesion to ECs through VLA-4/VCAM-1 interaction (14). The lack of VLA-4 expression on the lymphoma cell lines used in our study may be the reason for the weak induction of MMP-2 in lymphomas following contact with EC. Similarly, the functional relationship between LFA-1/ICAM-1 and MMP-9 may also explain the previously reported inability of purified ICAM-1 to induce MMP-2, and of purified VCAM-1 to induce MMP-9 expression in T cells (14). Thus, MMP-2 and MMP-9, which have similar substrate specificity, can both be induced in T cells following firm adhesion to EC, but through distinct adhesion mechanisms. In our system, however, stimulation of T lymphoma cells with anti-LFA-1 mAbs immobilized on plastic failed to induce MMP-9 expression (F. Aoudjit, unpublished observations), suggesting that while LFA-1/ICAM-1 interaction is essential to induce MMP-9 in T lymphoma cells, other as yet unidentified signals are necessary as well. This observation is consistent with the inability of purified ICAM-1 alone to induce any detectable gelatinolytic activity in T cells (14).

The repertoire of expression of TIMP that we observed was in agreement with previous studies that showed that TIMP-2, but not TIMP-1, is constitutively expressed in lymphoma cell lines of the T cell lineage (31). The observation that clinically aggressive lymphoma express high levels of TIMP-1 (17–19) may be indicative of an in vivo positive selection of clones with sustained expression of TIMP-1 following contact with EC. At first glance, one might

conclude that the concomitant up-regulation of TIMP-1 and MMP-9 by T lymphoma cells is paradoxical, since increased expression of TIMP-1 may not favor tissue invasion despite increased MMP-9 production and secretion. This might not be the case, however, since recent results using transgenic models suggest that TIMP-1 expression in vivo can either increase or decrease tumor invasion in a tumor cell-specific manner (32, 33). In fact, there are indications that TIMPs can have growth stimulatory activity on certain cell types, including on lymphoma cells and tumors of various origins (20, 34). From the transcriptional point of view, our results are consistent with those of others showing that the TIMP-1 gene is highly inducible following exposure to inducing agents (35–37). Our data are also consistent with preferential coregulation of MMP-9 and TIMP-1 in lymphoma cells observed in malignant forms of human non-Hodgkin's lymphoma, and that are thought to play an important role in controlling their biologic aggressiveness (38). The coregulation of these genes, however, is only temporal, since their induction upon contact with EC requires distinct signals, as evidenced by the requirement for LFA-1/ICAM-1 interaction in the case of MMP-9, but not TIMP-1. The fact that these genes are sometimes expressed independently of each other in some non-Hodgkin's lymphoma clones or in different T cell clones is thus likely to reflect prior exposure to only one stimulus (19).

Our results may also have a significant impact on the understanding of dissemination of lymphoma cells to peripheral tissues. Previous studies have shown that specific high endothelial venules recognition processes involving cell adhesion molecules are operative during lymphoid neoplasms and seem to influence tumor dissemination (39, 40). The T lymphoma cell line that we have used in this study has an invasive phenotype, as these cells injected i.v. give rise to massive tumors in peripheral tissues (41). It is thus possible that the expression of MMP-9 by lymphoma cells is implicated in the invasion of peripheral tissues by lymphoma cells. Interestingly, we have recently observed that ICAM-1-deficient mice were completely resistant to T cell lymphoma invasiveness,[4] indicating that the functional relationship between MMP-9 and ICAM-1 is essential for the establishment of peripheral T lymphoid tumors. This conclusion is further supported by our results showing that the invasive phenotype of T lymphoma cells is manifested after homing, and that overexpression of MMP-9 in lymphoma cells increases the frequency of lymphoma development.[5] Previous studies had also shown that mAbs to LFA-1 could at least partially inhibit lymphoma dissemination in mice (42, 43). The observed effect of anti-LFA-1 could thus very well be due to its ability to interfere with MMP-9 expression by lymphoma cells. This possibility is further supported by the study of Hauzenberger et al. (44), who reported that cross-linking by immobilized LFA-1 mAbs can trigger a general invasive phenotype on T cells.

Our results have shown that bi-directional signaling during contact between lymphoma cells and ECs leads to high expression of MMP-9 in both cell types. We established clearly, using transwells and conditioned medium, that up-regulation of MMP-9 in EC was mediated by soluble factors. The constitutive gene expression and production of inflammatory cytokines, such as IL-1 and TNF, by lymphoma cells, and the induction of MMP-9 in EC following exposure to these same cytokines, strongly indicate that TNF-α

and IL-1, which previously have been shown to regulate MMP expression, are among the soluble factors that could trigger MMP-9 production in EC. Work is currently in progress to identify the soluble factors present in the supernatant of T lymphoma cells that are responsible for the induction of MMP-9 in EC.

In conclusion, we have shown that bi-directional signaling upon adhesion of T lymphoma cells to ECs is a determinant in the local equilibrium between MMPs and TIMPs, and identified ICAM-1/LFA-1 as a key interaction in the up-regulation of MMP-9 in T lymphoma cells. Further elucidation of the underlying signaling mechanisms is likely to provide new insights in the design of therapeutic targets for controlling lymphoma metastasis.

Acknowledgments

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