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Matrix Metalloproteinase-19 Is Expressed in Myeloid Cells in an Adhesion-Dependent Manner and Associates with the Cell Surface

Simon Mauch,* Cornelia Kolb,* Birgit Kolb,* Thorsten Sadowski,*† and Radislav Sedlacek**†

We have previously reported the isolation of the human matrix metalloproteinase (MMP)-19 (also referred to as RASI) from a synovium of a patient suffering from rheumatoid arthritis and its expression at the cell surface of activated PBMC. In this study, we have analyzed the regulation and cell surface expression of human MMP-19 in several human cell lines and blood-derived cells. Among the cell lines analyzed, MMP-19 is largely expressed by lung fibroblasts as well as by myeloid cell lines THP-1 and HL-60. After fractionating PBMC into CD14<sup>-</sup> and CD14<sup>+</sup> populations we found that only the latter one expresses MMP-19. Although the myeloid cell lines as well as CD14<sup>+</sup> cells express MMP-19 without stimulation, its production can be up-regulated by phorbol esters (PMA) or by adhesion. The adhesion-dependent expression was down-regulated or even abrogated by blockade of adhesion or interfering with adhesion-controlling signaling using α-tocopherol. We have shown that MMP-19 associates with the cell surface of myeloid cells. This cell surface association was not affected by phospholipase C. However, acidic treatment of the THP-1-derived cell membranes abolished the immunoprecipitation of MMP-19 thereof. Moreover, a high salt treatment of THP-1 cells diminished the MMP-19 detection on the cell surface. This implicates a noncovalent attachment of MMP-19 to the cell surface. Because a truncated form of the MMP-19, in which the hemopexin-like domain was deleted (Δ<sub>hp</sub>MMP-19), does not associate with the surface, the hemopexin-like domain appears to be critical for the cell surface attachment of human MMP-19. The Journal of Immunology, 2002, 168: 1244–1251.

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The capability of MMP-19 to associate with the cell surface is unexpected, because it has no obvious transmembrane domain such as membrane-type MMP. A similar phenomenon has been previously described for gelatinase A (MMP-2) and its hemopexin fragment, PEX. For example, both the MMP-2 and the PEX fragment bind to the α<sub>5</sub>β<sub>1</sub> integrin (14, 15).

Recently, we have reported that activated PBMCs express hMMP-19 on their cell surface. Because MMP-19 can, for instance, cleave collagen type IV, tenasin C, fibrin, and fibrinogen, its activity may be important for the physiology of PBMC, especially for their transmigration through blood vessel walls (16). In the work reported in this study, we have focused on the analysis of the MMP-19 expression in PBMC, especially in myeloid cells. MMP-19 is expressed by myeloid cells in an adhesion-dependent manner and associates with the cell surface by an interaction with the hemopexin-like domain.

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Materials and Methods
RNA preparation and semiquantitative RT-PCR
The isolation and analysis of mRNA coding for MMP-19 was performed using an RT-PCR assay based on the primer dropping method (17). This semiquantitative PCR was performed in 100-μl (100 ng RNA) reaction volumes on a PREM thermal cycler (LEP Scientific, Serva Feinbiochemica, Heidelberg, Germany). All preparations were free of genomic DNA. Each reaction contained 3 μl of cDNA from the previously described reverse transcription reaction (corresponding to cDNA synthesized from 100 ng of total RNA) or 3 μl of negative control, 10 mmol/L Tris-HCl (pH 9), 50 mmol/L KCl, 1.5 mmol/L MgCl2, 0.1% Triton X-100, 0.2 mg/ml BSA, 0.2 mmol/l of all four dNTPs, 2.5 U of Taq-Polymerase (Appligene Oncor, Pleasanton, CA) and 0.5 μmol/L MMP-19-specific primers. Primer and template cDNA were separated from the other reaction components by a layer of paraffin wax until the first denaturation step. Each PCR was performed in a single thermal cycle at 94°C for 45 s, an annealing step at 62°C for 45 s, and an elongation step at 72°C for 45 s. A total of 5 μl of the GAPDH-specific primer pair (0.5 μmol/l each) were added after 15 cycles and PCR was continued for another 15 cycles. The number of cycles for the MMP-19 and the GAPDH-specific primer pairs were chosen to be in the exponential range of the amplification reaction to obtain semiquantitative results (data not shown). All RT-PCR experiments were repeated 3 times. The sequence of primers as follows MMP-19, 5'-TGGGTTCTGTG-3' and 5'-ACGGTTGAGCGAGGAC-3' (product length, 626 bp); GAPDH, 5'-H11032GAACCCAGTCC-3' and 5'-H11032GGTGATCTGGG-3' (product length, 498 bp).

Primary cells and cell lines
Blood-derived cells were fractionated by centrifugation using an LSM medium (ICN Pharmaceuticals, Costa Mesa, CA) according to the manufacturer’s recommendations. The isolated cells, consisting of monocytes and macrophages as well as T and B lymphocytes were resuspended in RPMI 1640 medium supplemented with 0.5–50% autologous serum. For the adhesion assay, freshly isolated PBMC were cultured in ultra-low attachment plates (Sigma, Deisenhofen, Germany) in presence of autologous serum (20%).

3% CD14+ cell fraction of PBMC was isolated using MACS columns according to the manufacturer’s recommendations (Miltenyi Biotec, Gießebisch Gladbach, Germany).

Human peritoneal macrophages, obtained from a patient with chronic renal failure undergoing peritoneal dialysis, were washed with PBS and directly subjected to flow cytometry analysis.

The myeloid cell lines (THP-1 and HL-60) and the lung fibroblasts (SV90), as well as the other cells (Hela, Hep G2, Jurkat, and ECV304), were cultured in RPMI 1640 medium supplemented with 5% FCS, 25 mM HEPES, and 2 mM glutamine. For the induction of gene expression, 20 ng/ml PMA was used.

Anti-MMP-19 Abs
Two specific anti-MMP-19 Abs were used. First, the immunoaffinity-purified, polyclonal, rabbit anti-MMP-19 Abs designated anti-Pep21 that recognizes the hinge region of hMMP-19, was raised by immunization with the keyhole limpet hemocyanin-coupled peptide and by immunoadsorption purification using immobilized peptide Pep21 (4). Second, the mAb CK8/4 was prepared by immunization with the keyhole limpet hemocyanin-coupled peptide and by immunoadsorption purification using immobilized peptide Pep21 (4). The CD14+/H9262 fraction of PBMC was isolated using MACS columns according to the manufacturer’s recommendations (Miltenyi Biotec, Gießebisch Gladbach, Germany).

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Labeling of the cell surface and immunoprecipitation
A modification of the cell surface biotinylation method according to the Pierce’s protocol was used. Briefly, a solution of 1 mg/ml EZ-Link Sulfo-NHS-LC-Biotin (Pierce, Sankt Augustin, Germany) was used to label 4 × 10⁸ cells. Labeling was performed in the dark at 4°C for 45 min. After labeling, cells were washed four times with PBS/0.15% glycine at 4°C and then used for immunoprecipitation.

For immunoprecipitation, the purified mAb CK8/4 (2 μg) was coupled to Dynabeads M-450 rat anti-mouse IgG1 (Deutsche Dynal, Hamburg, Germany) according to the manufacturer’s recommendations. To precipitate MMP-19, 5 × 10⁶ THP-1 cells were washed twice with PBS followed by lysis with RIPA buffer (1% Nonidet P-40, 0.1% SDS, 1% Na-deoxycholate, 10 mM Tris-HCl, pH 8, 140 mM NaCl, and Complete (Boehringer Mannheim, Mannheim, Germany) protease inhibitor without EDTA) for 45 min on ice. The cell remnants were removed by centrifugation. A 100-μl aliquot of Dynabead Ab solution was added to the cell lysates and this suspension was gently mixed at 4°C for 2 h. After polyacrylamide electrophoresis, the precipitated proteins were identified with both specific Abs (CK8/4 and anti-Pep21) and by using streptavidin-peroxidase.

To test the type of cell surface association of MMP-19, THP-1 cells were pretreated with a high-salt buffer (500 mM NaCl, 10 mM Na₂HPO₄, pH 11) and then lysed with TSA buffer (10 mM Tris-HCl, pH 8, 140 mM NaCl, Complete inhibitor without EDTA) containing 1% Nonidet P-40. Cell membranes were then precipitated using ultracentrifugation using a two-step sucrose gradient as described (18).

Construction and expression of deletion mutants of MMP-19
In addition to the full-length MMP-19, two truncated forms of MMP-19 were constructed in which either the hemopexin-like domain (ΔhMMP-19) or the catalytic domain (ΔcMMP-19) was deleted. All three constructs were tagged with an HA tag (YPYDVPDYA), a nonapeptide derived from human influenza hemagglutinin protein. The HA tag was added to the hinge region. The constructs were produced by PCR using TurboPfu (Stratagene, La Jolla, CA) and then transduced into 293T cells using the following primer pairs: 1) for ΔcMMP-19 (HA tag between the catalytic domain and the hinge region), proHA-r(5'-AGGGGTTAATGGGCACTGTTAGGTTAATAGGCTTTGGTGAAAG-3') and hemo-f(5'-GACCCTTCTAGACTGTAAGGATCTTGCTGCTG-3') for ΔhMMP-19 (C-terminal HA tag in the hinge region), reverseHA-r(5'-TTCAACATGCTAGCTCGGAGGACCTGTGCAATCTTGCTGCTG-3') and proHA-f(5'-GACCCTTCTAGACTGTAAGGATCTTGCTGCTG-3').

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Flow cytometry and analysis of MMP-19 cell surface expression
The flow-cytometry analysis was performed using a FACScan device (BD Immunocytometry Systems, San Jose, CA) using LysII II systems. The expression of MMP-19 was followed by incorporation of [3H]thymidine (1 μCi/ml) in 90 μl of PBS with 2% newborn calf serum, 0.1% NaN₃, and immunopurification, polyclonal, rabbit anti-MMP-19 Abs. The incubation with primary Abs was followed by incubation with C3-conjugated goat anti-rabbit IgG (Di-anova, Hamburg, Germany). To test the type of the cell surface association of MMP-19, THP-1 cells were also pretreated with an acidic buffer (50 mM Na-acetate, 85 mM NaCl, 5 mM KCl, 1% FCS) to release the molecules that were covalently attached to the cell surface as previously described (19). Phospholipase C (PLC) treatment of THP-1 cells was done using PLC from Bacillus cereus (Roche, Basel, Switzerland) in Earle’s medium according to the manufacturer’s recommendations. Briefly, 1.5 × 10⁶ cells were washed twice and then resuspended in 100 μl of Earle’s medium and 0.5 U of PLC was added. After incubation at 37°C for 1 h, cells were washed twice and analyzed by flow cytometry as described above.

Results
Expression profile of MMP-19
To investigate the regulation of MMP-19 gene expression and to establish a model for further studies of MMP-19 function, we first analyzed the expression of MMP-19 mRNA in various cell types using a semiquantitative RT-PCR assay (17). Among the cells line studied, the lung fibroblast cell line SV80 as well as two myeloid cell lines, THP-1 and HL-60, expressed MMP-19 at the mRNA level. However, the unstimulated promyelocytic cell line HL-60 exhibits a weaker signal than monocyteic THP-1 cells (Fig. 1).

In contrast, the hepatocyte cell line HepG2 does not express MMP-19, which correlates with the lack of MMP-19 mRNA message in human liver (7, 8). The cervix carcinoma line HeLa, the cell line ECV304 (previously reported as an endothelial line), and
the lymphoblastoid T cell line Molt 4 expressed no MMP-19 mRNA (Fig. 1).

Expression of MMP-19 in PBMCs

The above analysis of MMP-19 expression in various cell lines suggests that myeloid cells are the major producers of MMP-19 in blood-derived cells. In contrast, based on Northern analysis (7, 8) as well as flow cytometry analysis (4), it has been reported that unstimulated PBMC do not express MMP-19. To address whether this observation points to a specific endogenous stimulus driving the expression of MMP-19, we isolated PBMC using a Ficoll (Sigma) gradient and cultured them in a medium supplemented with autologous serum. Freshly isolated PBMC appear to be negative for the MMP-19 mRNA while the cultured PBMC show significant induction of MMP-19 mRNA (Fig. 2A). This up-regulation can be seen after 16 h and correlates positively with the concentration of the autologous serum present in medium such that the higher the content of serum used the stronger the MMP-19 expression observed.

CD14+ fraction of PBMC expresses MMP-19

Based on the results described above, we focused further on studying the MMP-19 regulation in myeloid cells. First, we compared the expression of MMP-19 mRNA among unsorted PBMC, and PMBC which were separated into CD14+ (predominantly monocytes and macrophages) and the CD14- (lymphocytes) populations. The analysis was performed immediately after the sorting of freshly isolated PBMC (Fig. 2B). In all three populations, no mRNA message of MMP-19 could be identified under these conditions. In the second set of experiments, the above cells were sorted into CD14+ and CD14- populations, and then cultured for 2 days in RPMI 1640 medium supplemented with an autologous serum (Fig. 2C). Under these conditions it was evident that MMP-19 mRNA expression was induced in the CD14+ fraction without additional stimulation. The weak band in the CD14- fraction probably originates from cross-contamination with myeloid cells because the magnetic sorting achieved only 95% purity of the CD14- population according to FACScan analysis (data not shown). Third, similar results were obtained when PBMC were cultured for 2 days under identical conditions and then sorted into the CD14+ and CD14- populations. As shown in Fig. 2D, the entire MMP-19 mRNA message in PBMC originated from CD14+ cells.
The expression of MMP-19 is up-regulated during differentiation of myeloid cells

As can be seen in Fig. 1, THP-1 and HL-60 cells differ in the level of MMP-19 expression. This difference could mirror their distinct differentiation potential. While the THP-1 cells develop into macrophages, HL-60 can differentiate into several cell types. Upon stimulation with PMA, HL-60 cells differentiate into monocytes, which proceed further into macrophages (20, 21). To gain insight into how MMP-19 is regulated during differentiation of myeloid cell types both myeloid cell lines were stimulated with PMA. This treatment induces differentiation with concurrent inhibition of proliferation in HL-60 cells (20). Expression of MMP-19 between these myeloid cell lines was then compared using a semiquantitative RT-PCR (Fig. 2E). Although the expression of MMP-19 is barely visible in the unstimulated HL-60 cells, both cell lines show strong and comparable expression after the PMA stimulation, indicating that macrophages are major producers of MMP-19.

Regulation of MMP-19 expression through adhesion

Induction of the MMP-19 expression due to addition of serum cannot be explained by a simple stimulatory effect of the serum because an autologous serum was used. Various factors that are present in a serum such as cytokines, growth factors and adhesion molecules could be possible stimuli leading to up-regulation of MMP-19 in the culture. Although adhesion mechanisms (e.g., between blood-derived cells and endothelium) are very complex in vivo, a simplified interaction among serum adhesion factors, cells, and the surface of the cell culture vessel can also induce gene expression. If the adhesion molecules are involved in regulation of the MMP-19 expression, a weakening of the adhesion to the vessel surface should inhibit the up-regulation of MMP-19.

Although PBMC cultured in the medium supplemented with the autologous serum do up-regulate MMP-19 mRNA, the expression level was minimal when the ultra-low attachment cell culture vessels were used (Fig. 3A). Moreover, we treated PBMC with α-tocopherol (Sigma), which is known to inhibit adhesion in cells such as monocytes and macrophages. Blocking of cell adhesion by α-tocopherol involves a protein kinase C pathway and is independent of its action as an antioxidant, because β-tocopherol as well as other chemically similar substances do not exhibit such antiadhesion effect (22–24).

A clear down-regulation of expression was achieved using 10 μM α-tocopherol while full inhibition of the MMP-19 induction is reached at 50 μM α-tocopherol. β-tocopherol did not influence the expression of MMP-19. The same inhibition could be observed when cultured PBMC were stimulated with 20 ng/ml PMA (Fig. 3B). HL-60 cells, stimulated with 20 ng/ml PMA, also showed no MMP-19 mRNA induction when they were cultured and treated in the same way as PBMC (Fig. 3C).

Both reduction of the adhesiveness by the low-attachment surface and the blockade of adhesion by α-tocopherol indicate that the MMP-19 expression in myeloid cells is regulated by cell adhesion.

MMP-19 associates with cell surface of myeloid cells

Previously, we have shown that hMMP-19 associates with the cell surface of activated PBMC and Chinese hamster ovary cells transfected with MMP-19 (4). Based on these data and the expression analysis above, we have further analyzed the cell surface association of MMP-19 in blood-derived cells.

PBMC were isolated from human fresh blood using a Ficoll gradient and then cultured for 2 days in the presence of 20% autologous serum. The association of MMP-19 with the cell surface was studied using flow cytometry (FACScan) and by immunoprecipitation of biotin-labeled cell surface molecules. In accordance with our previous studies, MMP-19 could not be found in freshly isolated PBMC, whereas a clear cell surface expression was seen in cultured cells (Fig. 4, A and B).

Because we predominantly detected the mRNA of MMP-19 in the CD14+ cell, i.e., myeloid cells, we examined whether these cells carry MMP-19 on their cell surface by staining the cells with both anti-MMP-19 and anti-CD14 Abs. Corresponding to the MMP-19 mRNA expression, the MMP-19 protein could not be found at the surface of CD14- cells (Fig. 4C). However, it was present on the cell surface of CD14+ cells, i.e., monocytes and macrophages (Fig. 4D). Peritoneal macrophages also exhibit an apparent cell surface expression of MMP-19 (Fig. 4F). Similar results were obtained upon analysis of the myeloid cell lines. Whereas MMP-19 can be detected on the cell surface of both THP-1 and HL-60 cells (Fig. 4, F and G), it is not expressed at the surface of Jurkat cells, even after stimulation with PMA (Fig. 4, H and I).

To confirm the results obtained with FACSscan analysis we used a new high-affinity mAb CK8/4 recognizing the propeptide domain of MMP-19. The Ab was used to precipitate MMP-19 from a whole cell lysate and also from the cell surface labeled with biotin. The precipitated proteins were analyzed by immunoblotting analysis. A small portion of the expressed MMP-19 could be detected using immunoblotting after 10 times concentration of the THP-1

FIGURE 3. Adhesion-dependent regulation of MMP-19. PBMC were cultured for 2 days with 20% autologous serum without additional stimulation. A, Induction of MMP-19 expression was compared among cells cultured in normal cell plates, cells grown on ultra-low attachment plates, and cells treated either with α-tocopherol or β-tocopherol. B, PBMC were cultured under same conditions as in the above experiment, except for the treatment with 20 ng/ml PMA. C, HL-60 cells, cultured and analyzed under comparable conditions as the PBMC, do not up-regulate MMP-19 in the case of inhibition of the cell adhesion.
cell culture medium obtained after a 3-day culture. MMP-19 could not be detected in the supernatant without concentration using acetone precipitation (data not shown). A specific MMP-19 band was detected with both the CK8/4 mAb and the polyclonal monospecific anti-MMP-19 (anti-Pep21) Abs (Fig. 5A). The anti-Pep21 Abs, recognizing the hinge region of MMP-19, verified the specificity of the precipitation by CK8/4. Moreover, the cell surface THP-1 cells were biotinylated and then lysed with RIPA buffer. The mAb CK8/4 was used to precipitate MMP-19, which in the case of its cell surface localization has to be biotinylated. Indeed, the biotinylated MMP-19 was clearly detected after incubation of the Western blot with streptavidin-coupled peroxidase (Fig. 5B).

MMP-19 associates with cell surface via its hemopexin-like domain

Based on both the flow cytometry analysis and the precipitation of biotin-labeled cell surface molecules, we conclude that MMP-19 associates with the surface of human monocytes and macrophages as well as THP-1 and HL-60 cell lines, i.e., human cells of myeloid origin.

Because MMP-19 belongs to the “classical” MMPs, which do not have a transmembrane region, the nature of its cell surface association is unclear. Identification of the mode of association with the cell surface could help to delineate the role of MMP-19 in myeloid cells. There are several theoretical possibilities of how MMP-19 could associate to the cell surface. First, MMP-19 could be anchored to the cell membrane with its signal sequence as a type II transmembrane protein. This seems to be unlikely because MMPs are usually secreted and the signal sequences are cleaved off during the transport through the endoplasmatic reticulum. In addition, MMP-19 has a predicted cleavage site of its signal sequence between the amino acid residues 18 and 19. Second, MMP-19 has an unusual C-terminal tail of 37 amino acids that is rich in threonine residues and has a partially hydrophobic character (4, 7, 8). Such a sequence could represent a hypothetical signal for the addition of a GPI anchor. Third, MMP-19 could be bound to the cell surface via interaction with a cell surface receptor, for
instance, as in the case of MMP-2, through interaction with the integrin αβ3 (14).

To examine the possible cell surface association of MMP-19 via a GPI anchor, THP-1 cells were treated with PLC. This enzyme exhibits specificity for phosphatidylinositol and lysophosphatidylinositol and should thus liberate most of the GPI-anchored molecules from the cell surface. As can be seen in the Fig. 6, A–D, addition of PLC to THP-1 cells did not affect the MMP-19 cell surface association, although CD14, a GPI-anchored molecule that is specifically expressed on monocytes and macrophages, was released. In addition to the PLC treatment we isolated cell membranes of THP-1 cell using two-step centrifugation over sucrose gradient. The membranes were then treated with an alkaline, high-salt solution to dissociate cell surface molecules that are not covalently bound. It can be seen in Fig. 6E that such a treatment prevented precipitation of the membrane-bound MMP-19 with the mAb CK8/4. A similar result was achieved by washing of THP-1 cells with an acetate buffer which also diminished the cell surface association of MMP-19 (Fig. 6, F–G). The above experiments indicate that MMP-19 is not covalently bound to the cell surface.

Thus, MMP-19 should presumably associate with the cell surface via an interaction with a receptor-like molecule. Such an association has been already described for MMP-2 that binds to the integrin αβ3 via its hemopexin-like domain (14, 15).

To test this possibility, we prepared MMP-19 constructs lacking either the catalytic (ΔcatMMP-19) or hemopexin-like (ΔhpMMP-19) domains. To distinguish the recombinant MMP-19 from an endogenous one we placed a hemagglutinin protein tag (HA tag) into the hinge region of MMP-19. THP-1 cells that naturally express MMP-19 at the cell surface were then transfected with recombinant MMP-19 cDNA. The transcription of the constructs was monitored by RT-PCR (data not shown). A FACScan analysis was used to analyze the cell surface association (Fig. 7, A–D). We used a full-length MMP-19 that associates with the cell surface as a control (Fig. 7B). The deletion of the hemopexin-like domain had a profound effect on the MMP-19 localization. As can be seen in the Fig. 7C, the ΔhpMMP-19 is not able to associate with the surface. The second construct, the ΔcatMMP-19, still associates with the surface although with a slightly diminished abundance (Fig. 7D).

Based on these experiments, we conclude that MMP-19 in myeloid cells associates with the cell surface noncovalently and that the hemopexin-like domain plays a critical role in this interaction.

**Discussion**

We have previously reported that MMP-19 is expressed on the surface of activated PBMC. In this study we have analyzed the expression of hMMP-19 at the mRNA and protein levels in various human cell lines as well as in blood-derived cells. Based on this analysis, we have focused on the characterization of hMMP-19 cell surface association in cells of myeloid origin.

First, the expression of hMMP-19 at the mRNA level was studied. Among the analyzed cell lines, the hepatocytic cell line HepG2, the cervix carcinoma line HeLa, the epithelial cell line ECV304 (previously reported as an endothelial cell line), and T cell lines Molt 4, and Jurkat (data not shown) did not produce a detectable amount of MMP-19 mRNA. The absence of MMP-19 mRNA in the HepG2 cells corresponds with the lack of mRNA of MMP-19 in liver (7, 8). The expression of MMP-19 in the lung fibroblastic cell line SV80 correlates with its expression in lung (7, 8).

The two cell lines of myeloid origin, THP-1 and HL-60, produce MMP-19, although the expression in HL-60 cells is only weak under normal conditions. These myeloid lines vary in their differentiation capacity. However, they can be stimulated to differentiate along the monocytes/macrophages cell lineage after their stimulation with PMA. While THP-1 cells differentiate into macrophages, HL-60 cells stimulated with PMA possess the capacity to differentiate into monocytes and then macrophages. Moreover, a treatment of HL-60 cell with PMA induces their differentiation into monocytes and macrophages (not to neutrophils and granulocytes) and inhibits their proliferation at the same time (20, 21).

After stimulation with PMA, both cell lines up-regulate mRNA for MMP-19. Because the up-regulation of MMP-19 is first observable after a 6-h delay and is stable for at least 2 days, this is

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**FIGURE 6.** MMP-19 associates with the cell surface via a noncovalent interaction. THP-1 cells were treated with PLC and then stained for CD14 and MMP-19. Whereas CD14, a GPI-anchored molecule, decreased its cell surface abundance after this treatment (B and D), cell surface-associated MMP-19 remained unaffected (A and C). Open histogram, specific Abs (anti-MMP-19 or anti-CD14); gray histogram, control Abs; dotted line, histograms without the treatment with PLC (A and B). The cell membranes of THP-1 cells separated via centrifugation using the sucrose gradient. The membranes were analyzed using immunoblotting either directly or after treatment with alkaline high-salt solution. E. The mAb CK 8/4 was used for detection of precipitated MMP-19. THP-1 cells were washed with an acetate buffer and then stained with the anti-Pep21 Abs. This acidic treatment diminished the cell surface abundance of MMP-19 as well (F and G).
MMP-19 among them macrophages, also decreased the expression of certain adhesion molecules, such as ICAM-1, VCAM-1, and CD11b/CD18 in several cell types. It is known that antioxidants, such as \( \alpha \)-tocopherol, which inhibits cellular adhesion in certain cell types, among them macrophages, also decreased the expression of MMP-19 in a concentration-dependent manner. This compound is known to suppress expression of several adhesion molecules, including ICAM-1, VCAM-1, endothelial leukocyte adhesion molecule-1 (E-selectin), and CD11b/CD18 in several cell types. Indeed, it has been reported that \( \alpha \)-tocopherol inhibits several processes, including cell proliferation, platelet aggregation, and monocyte adhesion (22–24, 31).

The effect of \( \alpha \)-tocopherol on the MMP-19 expression is not due to its antioxidative properties because \( \beta \)-tocopherol, another antioxidant compound, does not affect the regulation of MMP-19. \( \beta \)-tocopherol has identical antioxidative properties to \( \alpha \)-tocopherol and has the same biological availability (22–24).

The phenomenon of adhesion-coupled induction of expression is also known for other MMPs. For instance, blocking of the interaction between \( \alpha_5 \beta_1 \) integrin and fibronectin inhibits the expression of gelatinase B and, moreover, the ligation of the fibronectin receptor Mac-1 (\( \alpha_m \beta_2 \)) increases the production of this MMP (32, 33).

Regarding the MMP-19 expression in PBMC, we have analyzed which cell types exhibit a surface expression of this enzyme. Freshly isolated, nonactivated PBMC do not have cell surface MMP-19. In contrast, MMP-19 is also found in freshly isolated peritoneal macrophages and in myeloid cell lines HL-60 and THP-1.

Because MMP-19 has no obvious transmembrane domain, an alternative mechanism for its cell surface association must exist. The 37-aa-long C-terminal domain of MMP-19 exhibits hydrophobic characteristics and could, at least theoretically, contain a signal for the addition of a GPI anchor. However, PLC treatment did not affect its surface association despite the fact that CD14 was released from THP-1 cells.

Moreover, any covalent binding of MMP-19 to the cell membrane appears impossible because treatment of the cell membranes with an alkaline high-salt buffer abolished the membrane association of MMP-19. The cell surface association of MMP-19 was also reduced after washing with acidic acetate buffer.

\( \text{hMMP-19} \) appears to be bound via association with a cell surface receptor-like molecule. Such an association has already been described for several other MMP. For instance, gelatinase A (MMP-2) binds to the vitronectin receptor integrin \( \alpha_v \beta_3 \) via its hemopexin-like domain (14).

To determine which structural domain of MMP-19 is involved in the cell surface association, we prepared two mutant constructs of MMP-19. The construct without the catalytic domain was still detectable on the cell surface. There was only a slight decrease in MMP-19 expression. In addition, MMP-19 is also found in freshly isolated peritoneal macrophages and in myeloid cell lines HL-60 and THP-1.

**FIGURE 7.** MMP-19 associates with the cell surface via its hemopexin-like domain. To analyze which of the structural domains play a critical role in the cell surface binding, the following constructs were made: whole-MMP-19 construct encoded for whole MMP-19 (B) and the HA tag was inserted into hinge region; \( \Delta_p \text{-MMP-19} \) construct in which the catalytic domain was deleted and the HA tag inserted between the propeptide domain and the hinge region (C); and \( \Delta_{\text{hinge}} \text{-MMP-19} \) in which the hemopexin-like domain was deleted and HA tag was inserted C-terminally (D), i.e., behind the hinge region. After transfection, the cell surface localization of MMP-19 was detected with anti-HA tag Abs. Open histogram, staining with anti-HA tag Abs; gray histogram, control staining.
MMP-19 abundance on the surface. The second construct was made by deletion of the whole hemopexin-like domain. This deletion had a dramatic effect on binding to the cell surface. hMMP-19 lacking the hemopexin-like domain could not be detected on the cell surface. This kind of association is reminiscent of that of MMP-2. Nevertheless, the receptor or the cell surface association partner remains to be identified. Its identification could help to understand the distinct migration capabilities of blood-derived cells.

Taken together the results presented in this study show that MMP-19 is expressed in myeloid cells and that its up-regulation occurs in an adhesion-dependent manner. The MMP-19 expressed by myeloid cells associates with their cell surface via the hemopexin-like domain. Nevertheless, the association of MMP-19 with the cell surface is not a general phenomenon, because fibroblasts and endothelial cells do not possess surface MMP-19 (our unpublished observation). Endothelial cells isolated from umbilical arteries accumulate MMP-19 in cellular protrusions (10). However, the association of MMP-19 with cell surface may be critical for the spatial and temporal control of the activity of MMP-19, which in turn could affect the migratory behavior of myeloid cells, especially monocytes and macrophages.

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References