EMMPRIN (CD 147) is a central activator of extracellular matrix degradation by *Chlamydia pneumoniae*-infected monocytes. Implications for plaque rupture

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Summary

*Chlamydia (C.) pneumoniae* are thought to infect monocytes and use them as vectors into the vessel wall, where they may accelerate atherosclerosis. We investigated the effects of *C. pneumoniae* on monocytic matrix metalloproteinase (MMP) activation with focus on the role of the extracellular matrix metalloproteinase inducer EMMPRIN. Human monocytes or monocytic MonoMac6 cells were infected with *C. pneumoniae*. Infection enhanced mRNA- and surface expression of EMMPRIN and Membrane-type-1 Matrix Metalloproteinase (MT-MMP), plus the secretion of MMP-7, MMP-9 and the urokinase receptor (uPAR). Chlamydial heat shock protein 60 was identified to be partially responsible for EMMPRIN and MMP-9 induction, while *C. trachomatis*-infection had no stimulatory effect, indicating a *C. pneumoniae*-specific activation pathway. Suppression of EMMPRIN by gene silencing almost completely hindered the induction of MT1-MMP and MMP-9 by *C. pneumoniae*, suggesting a predominant regulatory role for EMMPRIN. Moreover, *C. pneumoniae*-infected monocytes exhibited increased MMP- and plasmin-dependent migration through “matrigel”. Additionally, incubation of SMCs with supernatants of *C. pneumoniae*-infected monocytes induced MMP-2 activation, which was inhibited by IL1-Receptor antagonist or anti-IL-6-mAb, indicating paracrine intercellular activation pathways. In conclusion, *C. pneumoniae* induce MMP activity directly in monocytes through an EMMPRIN-dependent pathway and indirectly in SMCs via monocytederived cytokines.

Keywords

EMMPRIN; MMPs, *C. pneumoniae*; monocytes , plaque rupture

Introduction

Inflammation of the vascular wall is a key process in the induction and progression of atherosclerosis (1, 2). Within the plaque, interaction between activated monocytes/macrophages and adjacent smooth muscle cells (SMCs) may contribute to plaque progression (3). This interaction appears to be driven by extrinsic conditions such as hypercholesterolemia, diabetes, smoking, hypertension, and – potentially – infection with *C. pneumoniae* (4, 5). As an obligate intracellular bacterium, *C. pneumoniae* has been detected in circulating monocytes and in atherosclerotic lesions (6, 7). Recent studies suggest that monocytes systemically disseminate *C. pneumoniae* and thereby may act as vectors for the bacteria (8, 9). We recently demonstrated that *C. pneumoniae*-infected monocytes preferably adhere to vascular walls at predilection sites for atherosclerosis under conditions of high shear stress in vivo (10). Furthermore, it was shown that *C. pneumoniae* induce gelatinase secretion in macrophages (11) and histomorphological studies have linked an unstable plaque phenotype to chlamydial infection (12, 13).

Destabilization and rupture of the atherosclerotic plaque are thought to be triggered by stepwise activation of proteases including the plasminogen and matrix metalloproteinase (MMP) activation systems (1). The proforms of MMPs become activated by membrane type-1 matrix metalloproteinases (MT-MMPs) or by soluble proteases such as plasmin or MMP-7 (matrilysin) (14). Cell-associated proteolysis is mediated by protease receptors such as the urokinase receptor (uPAR) (15) or membrane...
type-1 matrix metalloproteinase (MT1-MMP, MMP-14) (16), which bind and activate urokinase-type plasminogen activator (uPA) and MMP-2, respectively.

The regulation of MMP synthesis is not yet fully understood: The recently discovered extracellular matrix metalloproteinase inducer (EMMPRIN) (CD 147), a 58-kDa cell surface glycoprotein of the immunoglobulin superfamily was originally described on tumor cells (17). On tumor cells, EMMPRIN can induce the synthesis of MT1-MMP and MMP-9. In addition, EMMPRIN is expressed in human atheroma and is induced upon monocyte differentiation in vitro (18). However, no functional relevance of EMMPRIN for MMP activation in cardiovascular cells has yet been described.

In this study, we provide evidence that C. pneumoniae-infection of monocytes induces proteolytic, matrix-degrading activity via a complex activation cascade involving EMMPRIN as a key regulatory receptor. Moreover, we provide evidence for the functional relevance of C. pneumoniae-induced, monocyte-derived cytokines leading to paracrine MMP activation in adjacent vascular smooth muscle cells.

Materials and methods

Reagents
E. coli-lipopolysaccharide (LPS) and polymyxin B were obtained from Gibco. Recombinant MCP-1, IL-1 receptor antagonist, blocking mAbs anti-human TNF-α (clone 28401) and anti-human IL-6 (clone 6708) were from R&D Systems. Recombinant TNF-α and IL-1β were from Sigma, recombinant IL-6 was from Calbiochem. IL-6 and FITC-conjugated mAb mouse anti-Chlamydia pneumoniae (ACI) were from Progen, mouse anti-human EMMPRIN (clone 1G6.2) from Chemicon, mouse anti-human MT1-MMP (clone 114–1F2) from Chemicon, mouse anti-human MT1-MMP (clone 114–1F2) was from Oncogene. The MMP-inhibitor galardin (GM6001) and its non-inhibiting control peptide were from Chemicon, the plasmin inhibitor aprotinin was from Dako. Chlamydial HSP60 was manufactured and purified as described (19).

Cells

Human monocytes were isolated as described (10). The cell line MonoMac6 represents human monocytes with a closely related pattern of surface receptors and monocyte-like behaviour (20). Human coronary artery smooth muscle cells (SMCs) and SMC basal medium were from Clonetics. Cells were used after 2 to 5 passages.

Infection protocol

C. pneumoniae CM-1 (ATCC; VR-1360) and C. trachomatis (L2, ATCC, VR-902B) were cultured and purified as described. Monocytic cells were infected as described (9, 10). Briefly, isolated monocytes or MonoMac6 cells were washed twice, resuspended at a density of 250 000 cells/ml and inoculated with 5 inclusion forming units (IFU) of C. pneumoniae or C. trachomatis per cell for 24 hours. Successful infection was routinely confirmed by fluorescence microscopy and flow cytometry. On average, 25–35% of cells were infected. In some experiments (see Fig. 2B) isolated human monocytes were infected with increasing concentrations (IFU: 5, 20, 50) of C. pneumoniae or C. trachomatis followed by quantification of infected monocytes as well as of the respective MMP activity.

Purification of HSP60 from C. pneumoniae

The prokaryotic expression vector pTrcHis (Invitrogen) coding for a N-terminally His-tagged HSP60 from C. neumoniae was kindly provided by Dr. B. Kaltenboeck (Auburn, USA). The corresponding protein was expressed in E. coli (BL-21, Invitrogen). Supernatants of bacterial lysates were incubated with Ni-NTA agarose beads (Qiagen) for 2h at 4°C. Beads were washed three times (PBS pH 8.0, 20 mM imidazol) and applied to a CR10 column (Amersharm Pharmacabio Biotech). The protein was eluted and subsequently loaded onto a Superdex 200HR size exclusion column (Amersharm Pharmacabio Biotech). Elution fractions were
analyzed by SDS-PAGE (10%) and silver staining, presence of chlamydial HSP60 was confirmed by western blotting. The endotoxin content of the pooled fractions was 0.17 IU/µg protein or less, as determined by the LAL assay performed according to FDA standards (ACILA GMN mbH). Recombinant chlamydial HSP60 was further purified using polymyxin B-agarose columns (Detoxi-gel, Pierce).

Flow cytometry, ELISA, and gelatin zymography
Flow cytometry and Gelatin zymography were performed as described (2, 12, 21). ELISAs for soluble uPAR (Loxo), MMP-7 and MMP-9 were from Amersham

Western blot
Cells were lysed in RIPA buffer (20mM Tris-HCL, pH 8.0, 150mM NaCl, 1%NP-40, 0.5 deoxycholate, 0.1% SDS, 1mM EDTA, 10µg/mL leupeptin, 2µg/mL aprotinin, 1mM PMSF). Protein was quantified using BioRad protein assay reagents. Cell lysates were subjected to precasted 10%-SDS-Page-gels under reducing conditions. Pre-stained molecular markers (Invitrogen) were used to estimate the molecular weight of samples. Proteins were transferred to Hybond-ECL membrane (Amershan-Pharmacia) in running buffer with 20% methanol. After blocking non specific sites, blots were incubated with mouse mAbs anti-human EMMPRIN (HIM6, Becton Dickinson, 10µg/ml), anti-human MT1-MMP (clone 114–1F2, Oncogene, 10µg/ml) or anti-human β-actin (AC 15, Sigma-Aldrich, 640ng/ml). After washing, blots were incubated with biotinylated secondary anti-mouse antibody (Santa Cruz, 200ng/ml), and finally chemiluminescence staining was performed by western Blotting Luminol Reagent (Santa Cruz), according to the manufacturer’s instructions.

Real-time reverse transcriptase-polymerase chain reaction
After extraction (RNeasy Mini Kit, Qiagen), RNA was reverse-transcribed using omniscript reverse transcriptase (Qiagen) and oligo d(T) primer (Gibco). PCR (annealing 57°C) was performed with the HotStarTaqTM DNA Polymerase (Qiagen) with following primers (see Table 1).

Small interfering (siRNA) mediated gene silencing of EMMPRIN
SiRNA for EMMPRIN exon sequence and nonsilencing control siRNA were purchased from Qiagen (2-For-Silencing). The following EMMPRIN-Duplex was used: sense: 5’- (CGG CCA UGC UGG UCU GCA A)TdT-3’, antisense: 5’- (UUG CAG ACC AGC AUG GCC G) TdT-3’. Successful suppression of EMMPRIN was routinely confirmed by FACS analysis.

Table 1: Primers.

<table>
<thead>
<tr>
<th>Product</th>
<th>Forward primer (5’-3’)</th>
<th>Reverse primer (5’-3’)</th>
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<tr>
<td>EMMPRIN</td>
<td>GGCCGTGAAGTGCGTCAAGAC</td>
<td>GCCACGATGCCCAGGAA-3’</td>
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<tr>
<td>MT1-MMP</td>
<td>GCCTGCGTCCATCAACACT</td>
<td>CCCCCTCTGCCTCCACCTCAA</td>
</tr>
<tr>
<td>β-actin</td>
<td>CACTCTTCAGGCCTTCTCTCC</td>
<td>CGGACTCGTCATCTCCTGCTT</td>
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Monocyte migration through “matrigel”
Migration of monocyctic cells through a mass of ECM (“matrigel”) was measured using a Cell Invasion Assay Kit from Chemicon, which contains a tissue plate with 12 well culture inserts.
The inserts contain a porous (8µm) polycarbonate membrane covered by a viscous layer of matrigel, which consists of a mixture of matrix components which occlude the membrane pores. Therefore, successful cell migration through matrigel requires proteolytic, matrix degrading activity. Medium containing MCP-1 (10µg/mL) was placed into the lower compartment. Non-infected or C. pneumoniae-infected monocyctic cells (0.5 x 10⁹/mL) were placed onto the filters in the absence or presence of the MMP-inhibitor galardin (GM6001, 20µg/mL), its non-inhibiting control peptide (20µg/mL) or of the plasmin-inhibitor aprotinin (50 kIU/mL). After 12 hours, migrated cells were harvested from the lower compartment, stained with crystal-violet and densitometrically quantified.

Statistical analysis
Comparisons between group means were performed using ANOVA. Data represent mean±SEM. A value of p < 0.05 was regarded as significant.

Results
C. pneumoniae upregulates EMMPRIN and MT1-MMP
We hypothesized that C. pneumoniae infection can stimulate the stepwise expression and activation of proteolysis. Monocyctic MonoMac6 cells were infected with C. pneumoniae for 48 hours, washed, and fluorescently stained. Confocal microscopy and flow cytometry using a fluorescence-conjugated anti-chlamydial mAb revealed infection of 25 to 35% of cells (not shown). Figure 1A demonstrates an enhanced receptor surface expression on C. pneumoniae-infected cells of both EMMPRIN (mean fluorescence 82.2±53.7 vs. 42.4±29.5; n=10; p<0.01) and MT1-MMP (mean fluorescence vs. 78.9±51.7 vs. 40.3±30.6; n=11; p<0.01) by using FACS analysis. The enhanced expression of EMMPRIN and MT1-MMP on C. pneumoniae-infected cells was confirmed by western blotting (Fig. 1B). Figure 1C demonstrates an increased expression of EMMPRIN- and MT1-MMP-mRNA by C. pneumoniae-infected monocytes as compared to non-infected cells.

C. pneumoniae induces monocyctic release of uPAR
uPAR serves both as an adhesion receptor, which is essential for integrin-mediated monocyte recruitment (22), but also as a receptor for uPA and, thereby, as an activator of the plasminogen activation cascade. Previously, we demonstrated that the surface and gene expression of uPAR is elevated in C. pneumoniae-infected monocytes (10) as well as in vivo in circulating monocytes from patients with acute myocardial infarction (23). Consistent with that data, we are now able to show that C. pneumoniae infection of monocyctic cells leads to enhanced release of soluble uPAR (Fig. 2A).

C. pneumoniae induces monocyctic release of MMP-7 and MMP-9
EMMPRIN induces novel synthesis of various MMPs including MT1-MMP or MMP-9 (17). MT1-MMP or soluble matrilysin (MMP-7) can bind and activate soluble pro-MMP-2 (24, 25), while uPAR/uPA-mediated plasmin generation can transfer various pro-MMPs into their respective enzymatically active form (26). Therefore, enhanced expression of these three cell surface receptors prompted us to study expression and activity of the monocyte-derived proteases MMP-7 and MMP-9 in monocyctic MonoMac6 cells. In fact, enhanced secretion of both MMP-7 and the enzymatically inactive proMMP-9 were detected by ELISA (Fig. 2A). In addition, gelatin zymography confirmed enhanced secretion of the 98 kDa proMMP-9 in isolated human monocytes upon C. pneumoniae-infection (Fig. 2B).

Moreover, we compared the effects of C. pneumoniae to C. trachomatis: Isolated human monocytes were infected with increasing concentrations of C. pneumoniae or C. trachomatis. Figure 2B demonstrates that C. trachomatis did not induce MMP activation at infection rates of 30% of the cells, a rate that allow-

Figure 3: siRNA-mediated suppression of EMMPRIN hinders the induction of MT1-MMP and MMP-9 by C. pneumoniae. Human monocyctic MonoMac6 cells were left untreated or were transfected with non-inhibiting control-siRNA or specific EMMPRIN-blocking siRNA. After 48 hours, cellular infection with C. pneumoniae was performed as described in Methods. After additional 24 hours, the A) EMMPRIN protein expression was analyzed by western blot and B) cell surface expression of EMMPRIN and MT1-MMP was analysed by flow cytometric analysis as described in Methods. Columns represent the mean±SD of the mean fluorescence of four independent experiments. *p<0.05; (C) SDS-Page gelatin zymography of cell culture supernatants shows a hinderance of MMP-9 induction in EMMPRIN-suppressed cells in comparison to medium-treated or control-siRNA-treated cells. The figure shows one representative of four independent experiments.
ed strong induction of MMP-9 by *C. pneumoniae*. Even at 50% infection rate, *C. trachomatis* induced MMP-9 only slightly (if any). Thus, *C. pneumoniae* appears to be specifically equipped to stimulate MMP activity in human monocytes. However, as shown in Figure 2C, stimulation of isolated monocytes with heat inactivated (30min, 65°C) *C. pneumoniae* also caused an enhanced EMMPRIN protein expression, albeit to a minor extent.

**EMMPRIN is involved in the induction of MT1-MMP and MMP-9 in *C. pneumoniae*-infected monocytes**

In order to study the potential functional relevance of EMMPRIN in *C. pneumoniae*-induced monocyctic MMP-activation, siRNA-technology was used to specifically suppress EMMPRIN expression. *C. pneumoniae*-induced surface expression of EMMPRIN was successfully reduced by EMMPRIN specific siRNA-duplexes by 50% (Fig. 3A). A nonsilencing RNA duplex was used as a negative control and showed no inhibitory effect. Further analysis revealed that EMMPRIN suppression hindered the enhancement of MT1-MMP upon cell infection by 55% (Fig. 3A). Consistently, EMMPRIN blockade effectively hindered the induction of MMP-9 upon *C. pneumoniae*-infection, whereas the nonsilencing control-siRNA had no inhibitory effect (Fig. 3B). Overall, these data indicate a predominant role of EMMPRIN as a central stimulator of MMP induction in monocytes.

**Chlamydial heat shock protein (chHSP60) stimulates monocyctic EMMPRIN and MT1-MMP surface expression**

During chronic persistent infections in monocytes/macrophages, *C. pneumoniae* produce substantial amounts of heat shock protein 60, which was shown to be present within human atherosclerotic plaques. We found an increased surface expression of EMMPRIN and MT1-MMP on chHSP60-stimulated monocytes (Fig. 4A). In accordance with the results of Kol et al. (27), monocyte incubation with chHSP60 revealed a dose-dependent increase of MMP-9 activity (Fig. 4B). These data indicate that chHSP60 at least partially is involved in the *C. pneumoniae*-specific activation pathway.

**C. pneumoniae induces monocyctic cell migration through matrigel by MMP and plasmin activity**

In order to study the functional relevance of *C. pneumoniae*-induced proteolytic activity, we investigated monocyctic migration through a layer of ECM-components, called “matrigel”. Successful migration through “matrigel” requires matrix degrading, proteolytic activity. Figure 5 demonstrates that *C. pneumoniae* strongly increased cell migration through matrigel, which was abolished by the plasmin-inhibitor aprotinin or the MMP-inhibitor galardin (GM-6001), but not by its non-inhibiting control peptide. These data indicate that both the plasminogen and the MMP activation pathway act in concert to effectively achieve matrix degradation by *C. pneumoniae*-infected monocytes.
C. pneumoniae-infected monocytes induce MMP-2-secretion by smooth muscle cells via cytokines

Within the atherosclerotic plaque, interaction between activated monocytes/macrophages and SMCs leads to increased cellular activation and inflammation, promoting a circulus vitiosus that may cause plaque progression and rupture. C. pneumoniae are known to stimulate secretion of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), or IL-6 by mononuclear cells (27, 28). Consistently, C. pneumoniae-infected monocytes secreted inflammatory cytokines such as IL-18 and IL-6 (data not shown). Therefore, we investigated if and by what means C. pneumoniae-infected monocytes might stimulate protease activity in adjacent vascular SMCs. First human vascular SMCs were stimulated with recombinant cytokines which had previously been found to be secreted by C. pneumoniae-infected monocytes. In fact, gelatin zymography revealed an increased MMP-2 activity of IL1-β and IL-6 stimulated SMCs (Fig. 6A). Next, SMCs were incubated with supernatants of human monocytes which had previously been infected with C. pneumoniae for 24 hours and with appropriate controls.

After one hour incubation, monocyte supernatants were removed from SMCs by gentle washing, and SMCs were further incubated with medium for additional 12 hours prior to harvesting their supernatants for gelatin zymography. Figure 6B shows that supernatants of cultivated, resting, non-infected monocytes only slightly induce SMC-secretion of proMMP-2 in comparison to medium treatment. In contrast, supernatants of C. pneumoniae-infected monocytes strongly induced secretion of proMMP-2 by SMCs, which was effectively inhibited in the presence of blocking mAbs anti-IL-6 or IL-1-receptor antagonist (IL1-RA) (Fig. 6C). Only marginal (if any) inhibitory effects were achieved by blockade of anti-TNFα. Together, C. pneumoniae-infected monocytes enhance proteolytic activity not only by direct secretion of MMPs, but also by stimulating MMP-activity in adjacent vascular cells via inflammatory cytokines such as IL-1β and IL-6.

The observed effect of C. pneumoniae-infected monocytes to stimulate adjacent SMCs by IL-1 and IL-6 release prompted us to study the influence of these cytokines to excite EMMPRIN and MMP activation within monocytes in a potential paracrine activation pathway. As shown in Figure 6D, we found no substantial EMMPRIN inhibiting effect of anti-IL-6, anti-TNFα or IL1-receptor antagonist on C. pneumoniae-infected monocytes.

Discussion

Matrix metalloproteinases make an essential contribution to the progression and rupture of atherosclerotic plaques. In specific, the presence of MT1-MMP, MMP-2, MMP-7 and MMP-9 within vascular walls has been linked to an unstable plaque phenotype (1, 3). In this study, we demonstrate that C. pneumoniae-infected monocytes may trigger MMP activation i) directly by increasing the expression, secretion and activation of MMPs through upregulation of EMMPRIN and ii) indirectly by inducing MMP-activation in adjacent vascular smooth muscle cells via the release of inflammatory cytokines (IL-6, IL-18).

Many studies have reported that C. pneumoniae are present within atherosclerotic plaques (6, 7), where they are thought to stimulate atherogenic inflammatory reactions (11, 28). We have previously shown that C. pneumoniae can induce the recruitment of infected monocytes to the vascular walls in vivo even in non-atherosclerotic mice. This process appears to be mediated by activation of the integrin adhesion receptor system (10). The present study describes potential atherogenic activities of C. pneumoniae-infected monocytes within the vascular wall. C. pneumoniae-infected monocytes stimulated proteolytic activity and breakdown of ECM. These processes seem to be mediated by a stepwise activation of cell-associated proteases such as MMP-7, -9 and plasmin. Enhanced protease activity was associated with increased expression of the receptors EMMPRIN, MT1-MMP and uPAR. EMMPRIN-suppression via siRNA-technology hindered the induction of MT1-MMP and MMP-9. Therefore, the presence of EMMPRIN appears to be required for an adequate MMP induction in monocytes indicating a key regulatory role for EMMPRIN.

Recently, EMMPRIN has been described to be shed from coronary smooth muscle cells upon stimulation with oxidized low-density lipoproteins (29). In our setting, western blot analy-
sis of cell culture supernatants did show soluble EMMPRIN, however there were no differences between non-infected or C. pneumoniae-infected monocytes (data not shown).

Previously, it was suggested that Chlamydia can be transmitted from monocytes to SMCs and, thereby, may stimulate atherogenic activities in neighboring cells within the vascular wall (30, 31). We found that C. pneumoniae-infected monocytes can stimulate SMCs without directly infecting them. Consistent with previous data (27, 28), C. pneumoniae-infected monocytes secreted inflammatory cytokines such as IL-1β and IL-6 (data not shown). Thereby, infected monocytes can stimulate SMCs to release proMMP-2, since this pathway can be inhibited by IL-1-receptor antagonist or by blocking mAb anti-IL-6. Interestingly, enhanced cytokine production by infected monocytes may also induce MMPs and related receptors in adjacent, non-infected monocytes: Flow cytometric analysis revealed that the entire population within the group of infected monocytes showed upregulation of protease receptors (see Fig. 1), while only 25–30% of those cells positively stained for C. pneumoniae and thus could be considered “infected”. Notably, both EMMPRIN and MMP-9 induction of infected monocytes were not affected by additional blockade of TNF-α or of IL-6 or IL-1β (see Fig. 6D), indicating that these cytokines are not directly involved in monocytic EMMPRIN and MMP induction by C. pneumoniae.

Thus, C. pneumoniae not only directly activate the respective infected cell type but also induce pericellular inflammatory processes involving surrounding vascular cells. These processes act in concert to accumulate MMP activity within the vascular wall. These data may provide a concept of how monocytes/macrophages and SMCs co-work on the rupture of the fibrous cap by the respective secretion of cell-type specific MMPs.

Experimental, animal, and clinical studies suggest the existence of a unique C. pneumoniae-specific activation pathway (32, 33). In this study, we detected hardly any MMP activation by C. trachomatis-infected monocytes, even with high infection rates (Fig. 2B). These observations may provide a pathophysiological basis for the observation that C. trachomatis (in contrast to C. pneumoniae) did not accelerate atherosclerosis in C. trachomatis-infected animals, even when the bacterium was present in the vessel wall (33). These data are consistent with our previous work, which showed C. pneumoniae-specific stimulation of monocyte adhesion (10). Moreover, chlamydial HSP60 was identified to be the antigen which may account at least in part for the upregulation of EMMPRIN as well as of MT1-MMP and MMP-9. Recently, we and others demonstrated that cHSP activates NFκB via Toll-Like-Receptor-2 and –4 in endothelial cells and in macrophages followed by inflammatory cytokine secretion (19, 34, 35). Although not tested in our setting in detail, chSP and the two TLRs may be involved in the observed IL-1β and IL-6 release of monocytes, which stimulate the proMMP-2 secretion of adjacent SMCs.

Consistent with previous reports (36), even heat-inactivated bacteria were able to stimulate monocytes in our setting, albeit to a minor extent as compared to viable bacteria (Fig. 2 C). Extensive work has been performed to fully elucidate the exact mechanism of how C. pneumoniae infection specifically activates monocytes (37). However, the unique C pneumoniae-specific activation pathway has not been identified yet.

In the present study, chlamydial LPS did not play a predominant role for monocyte activation, since polymyxin B treatment had no inhibitory effect (data not shown).

Recent clinical trials revealed that a therapeutic intervention using currently available antibiotics in patients with coronary heart disease does not yield clinical success for secondary prevention (38, 39). Future work is needed to clearly evaluate the potential role of the fact that C. pneumoniae is only one potential player among others.

In conclusion, C. pneumoniae-infection of monocytes cells induces an activation cascade leading to matrix degradation via increased synthesis, expression, and/or activation of various protease receptors and MMPs, which may yield an unstable plaque phenotype. EMMPRIN appears to play a key role for MMP activation in monocytes. Additionally, intercellular activation pathways involving inflammatory cytokines appear to contribute to these processes. Our data support the concept that C. pneumoniae can actively contribute to inflammatory, atherogenic processes within the atherosclerotic plaque and, thereby, may promote plaque progression and rupture.

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Abbreviations

C. pneumoniae: Chlamydia pneumoniae; ECM: extracellular matrix; EMMPRIN: extracellular matrix metalloprotease-inducer; IL: interleukin; LPS: lipopolysaccharide; MCP-1: monocyte chemoattractant protein-1; MMP: matrix metalloproteinase; mAb: monoclonal antibody; MT-MMP: membrane-type matrix metalloproteinase; SMC: smooth muscle cell; TNF-: tumor necrosis factor-; uPA: urokinase-type plasminogen activator; uPAR: urokinase receptor.

References


