Chlamydia pneumoniae Proteins Induce Secretion of the 92-kDa Gelatinase by Human Monocyte-Derived Macrophages

Pirjo Vehmaan-Kreula, Mirja Puolakkainen, Matti Sarvas, Howard G. Welgus, Petri T. Kovanen

Abstract—Chlamydia pneumoniae, an intracellular Gram-negative respiratory bacterium, and macrophages are present in inflammatory tissue sites such as atherosclerotic lesions, where abnormal degradation of the extracellular matrix takes place. To evaluate the potential of C pneumoniae for participation in matrix destruction, we studied the effect of this bacterium on the production of 3 matrix-degrading metalloproteinases, 92-kDa gelatinase, interstitial collagenase-1, and stromelysin-1, and their natural inhibitor TIMP-1 (tissue inhibitor of metalloproteinases-1) by human monocyte–derived macrophages differentiated in vitro. Spontaneous production of collagenase and stromelysin by these cells was minimal and was not influenced by C pneumoniae. In contrast, the cells secreted substantial basal quantities of 92-kDa gelatinase, the secretion of which was stimulated (on average, 2.5-fold) by C pneumoniae. C pneumoniae regulated the expression of 92-kDa gelatinase by macrophages at the pretranslational level. Macrophages secreted only small quantities of TIMP-1. The chlamydial proteins Omp2, MOMP, and HSP60 were also found to participate in the induction of 92-kDa gelatinase by C pneumoniae. Denaturation of chlamydial proteins by boiling reduced 92-kDa gelatinase secretion only partially (by 35%), suggesting that the heat-stable lipopolysaccharide molecules also stimulate secretion of the enzyme. The results show that production of 92-kDa gelatinase by human macrophages is selectively upregulated by C pneumoniae, which suggests that these bacteria, when present in a macrophage-containing inflammatory environment, actively participate in the destruction of the extracellular matrix. (Arterioscler Thromb Vasc Biol. 2001;21:e1-e8.)

Key Words: humans ■ monocytes ■ macrophages ■ cellular activation ■ inflammatory mediators

Matrix metalloproteinases (MMPs) are structurally related and participate in the degradation of extracellular matrix components.1 At present, the MMP gene family consists of at least 17 zinc-dependent endopeptidases, which can be divided into subgroups of collagenases, gelatinases, stromelysins, and membrane-type MMPs.1 Besides participating in normal homeodynamics and developmental remodeling of connective tissues, the MMPs appear to contribute significantly, by their proteolytic activity, to the tissue damage seen in chronic inflammatory diseases such as rheumatoid arthritis,2 osteoarthritis,3 and atherosclerosis.4 MMPs, in most instances, are not produced constitutively, but their expression is induced in a cell type–specific manner by inflammatory mediators such as cytokines,5 cellular transformation,6 hormones,7 growth factors,8 and bacterial lipopolysaccharide (LPS).9

The MMPs secreted by macrophages include interstitial collagenase (MMP-1),10 72-kDa gelatinase (MMP-2),6 stromelysin-1 (MMP-3),9 matrilysin (MMP-7),11 92-kDa gelatinase (MMP-9),12 and a unique elastase designated macrophage metalloelastase (MMP-12).13 Macrophages also produce 2 specific inhibitors of MMPs, known as tissue inhibitors of metalloproteinases-1 and -2 (TIMP-1 and TIMP-2).9,14 Of these, TIMP-1 interacts with the active forms of interstitial collagenase and stromelysin as well as with the active and latent forms of 92-kDa gelatinase.15 C pneumoniae is an obligate, intracellular, Gram-negative bacterium that is distinguished from other bacteria by a unique growth cycle.16 In this growth cycle there are 2 morphologically and functionally distinct cell types: the infectious elementary body (EB) and the reproductive reticulate body (RB).16 The outer envelope of the elementary body is composed of cysteine-rich structural proteins with molecular masses of 98, 60-doublet, 39.5, and 15.5 kDa.17 The 39.5-kDa major outer membrane protein (MOMP) is considered the primary target of immune response in C trachomatis, but in C pneumoniae infection, MOMP is less immunogenic.18 Outer membrane protein 2 (Omp2) is a second constituent of the chlamydial outer membrane complex; it has a molecular mass of ≈60 kDa.19 Omp2 has been found to be a major immunogen of C pneumoniae.19 Yet another 60-kDa protein has been identified in C pneumoniae; it is a homolog of C trachomatis GroEL and a member of the heat shock protein family, HSP60.20 C pneumoniae are able to multiply within macrophages, where they persist for long periods without causing any damage until they are reactivated by
immunosuppression\(^1\) or by coincidental infection with other organisms. *C pneumonias* has been implicated as a causative agent of several common respiratory infections, especially pneumonias.\(^1\) Importantly, a serological association was also found between *C pneumonias* infection and coronary heart disease.\(^2\) Interestingly, *C pneumonias* has been identified within macrophages and smooth muscle cells of atherosclerotic coronary artery specimens at necropsy.\(^2\) However, the mechanisms by which *C pneumonias* affects the natural history of atherogenesis remain poorly understood. Very recently, Kol et al\(^2\) reported the presence of HSP60 of *C pneumonias* in human carotid atheromas. These investigators also reported that chlamydial HSP60 derived from *C trachomatis* stimulates the expression of tumor necrosis factor-α (TNF-α) and MMP-9 by mouse peritoneal macrophages.\(^2\)

In our study, we have examined the capacity of live *C pneumonias* bacteria and some of its protein components to influence the production of interstitial collagenase, stromelysin, 92-kDa gelatinase, and TIMP-1 by human monocyte-derived macrophages. *C pneumonias* induced 92-kDa gelatinase expression by macrophages but had no influence on the production of interstitial collagenase or stromelysin. Stimulation of monocyte-derived macrophage 92-kDa gelatinase production by *C pneumonias* occurred via the chlamydial-derived proteins Omp2, MOMP, and HSP60.\(^2\)

**Methods**

**Reagents**

Ficoll-Paque was purchased from Pharmacia LKB Biotechnology. Macrophage serum-free medium, RPMI 1640, Dulbecco’s modified Eagle’s medium, Dulbecco’s PBS without Ca\(^{2+}\) and Mg\(^{2+}\), fetal bovine serum, and penicillin/streptomycin (10 000 IU/mL and 10 000 μg/mL, respectively) were obtained from Gibco. Human recombinant granulocyte macrophage colony-stimulating factor (GM-CSF) was generously provided by Novartis Oy (Espoo, Finland). LPS, derived from *Escherichia coli* serotype O111:B4, ethidium bromide, Coomassie brilliant blue R-250, calf thymus DNA, and Triton X-100 were purchased from Sigma Chemical Co. Gelatin was obtained from Merck KGaA. Meglumine diatrizoate (Urografin) was from Schering AG. Co. Gelatin was obtained from Merck KGaA. Meglumine diatrizoate (Urografin) was from Schering AG. *C trachomatis* and L\(_2\) were obtained from purchased from Biological Materials Distribution Center, Washington Research Foundation. All other chemicals used were of reagent grade.

**Isolation and Culture of Macrophages**

Human monocytes from healthy control subjects were isolated fromuffy coat cells (obtained from the Finnish Red Cross Blood Transfusion Service, Helsinki, Finland) by Ficoll-Paque as described earlier.\(^3\) The culture medium was replaced with fresh serum-free medium, and supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10 ng/mL GM-CSF (medium A) after 24 hours and every 48 hours thereafter. Experiments were started either with freshly isolated monocytes (day 0 of culture) or with monocytes that had been cultured for 7 days in vitro. These monocytes that had differentiated in vitro are hereafter referred to macrophages.

**Growth and Purification of *C pneumonias***

A Finnish Mycoplasma-free (no Mycoplasma DNA detected by polymerase chain reaction [PCR]) *C pneumonias* isolate K-6 was propagated in HL cells. Infected cells were collected and sonicated, and the bacteria were purified through a gradient of meglumine diatrizoate.\(^4\) Purified organisms were resuspended in sucrose/phosphate/glutamic acid and stored in aliquots at −70°C until used. The infectivity, measured as inclusion-forming units (IFUs) of the bacteriolar preparation, was determined in cycloheximide-treated HL cells.\(^5\)

**Recombinant *C pneumonias* Proteins**

*C pneumonias* proteins MOMP, Omp2, and HSP60 were produced in the heterologous host, *Bacillus subtilis*, strain 168, which is apathogenic and secretes no toxins. As a Gram-positive bacterium it is devoid of endotoxin (LPS). Protein production and purification have been described in detail by U. Airaksinen et al (unpublished data, 1998). Briefly, genes encoding the above proteins were amplified by PCR and inserted into an expression vector for intracellular production. HSP60 was purified with affinity chromatography based on His-tag in native state. Both MOMP and Omp2 were found in *B subtilis* as inclusion bodies, and they were purified after denaturation with urea; there was no renaturation step.

**Treatment of Macrophages With *C pneumonias* and Its Proteins**

Before experiments, each well of macrophages was washed 3 times with PBS, and 1 mL of fresh medium A was added. The cells were then incubated for 48 hours in either the absence or presence of *C pneumonias*. In a third series of wells, the macrophages were exposed to the indicated doses of *C pneumonias* for either 48 or 1.5 hours, after which the medium was replaced by fresh medium that did not contain *C pneumonias*. In other experiments, macrophages were incubated with the indicated doses of chlamydial proteins for 48 hours. After incubation, the conditioned media were collected, centrifuged at 400g for 5 minutes at 20°C, and kept frozen at −20°C until their MMP or TIMP contents were quantified by ELISA. For zymography, the macrophages were incubated for 48 hours in a protein-free medium consisting of RPMI supplemented with 10 ng/mL GM-CSF, 100 U/mL penicillin, and 100 μg/mL streptomycin (medium B).

**Gelatin Zymography**

Gelatinolytic activity was assessed by SDS–polyacrylamide gel electrophoresis of conditioned medium (10 μL) under nonreducing conditions in gels containing 10% polyacrylamide and 1 mg/mL gelatin. To renature the proteins after electrophoresis, SDS was removed from the gels by washing them twice for 15 minutes at room temperature in 2.5% Triton-X-100. Gels were then washed once with buffer containing 50 mmol/L Tris (pH 8.2), 5 mmol/L CaCl\(_2\), and 0.5 μmol/L ZnCl\(_2\), after which the gels were incubated in this buffer overnight at 37°C. To detect bands with gelatinolytic activity, gels were stained with 0.5% Coomassie brilliant blue R-250. For molecular-weight standardization, we used a low-molecular-weight standard from Pharmacia.

**Immunological Assays**

Competitive-binding ELISAs for interstitial collagenase, stromelysin, 92-kDa gelatinase, and TIMP-1 were performed on cell supernatants, as described previously.\(^2\) These assays are sensitive in the nanogram range and use polyclonal antibodies that recognize the free enzymes with avidities equal to those of the enzymes complexed with their substrates or with the TIMPs.

**Reverse Transcription (RT)–PCR Analysis of 92-kDa Gelatinase Expression**

Total RNA was isolated from macrophages by using an ultra pure TRIZol reagent (Gibco). The RNA was transcribed into cDNA with a Superscript preamplification system (Gibco BRL). The obtained cDNA was further amplified by PCR with specific oligonucleotides for human 92-kDa gelatinase (sense, 5'-CAGTGGCACCC-CTCAGAGC-3'; antisense 5'-GCCACTTGTGCGGCAATAGG-3'). In a control experiment, the expression level of a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH; sense, 5'-ACCACGGTCTTCAGCTAGC-3'; antisense, 5'-TCCACAC-CCTGTTGGCTGTA-3'), generating a 452-bp DNA fragment, was found to be comparable both in the control and stimulated macrophages. PCR was performed under the following conditions: for 92-kDa gelatinase, 94°C (1 minute) for 1 cycle; 94°C (30 seconds),
63°C (1 minute), and 72°C (45 seconds) for 40 cycles; and the final incubation at 72°C for 10 minutes; for GAPDH, 94°C (1 minute) for 1 cycle; 94°C (30 seconds), 65°C (30 seconds), and 72°C (30 seconds) for 30 cycles; and the final incubation at 72°C for 10 minutes. Aliquots (7 μL) of the PCR product were electrophoresed through 1.2% agarose gels containing 0.4 μg/mL ethidium bromide. Gels were illuminated with UV light and photographed with Polaroid film (Polaroid Ltd, UK).

Measurement of Cellular DNA

After incubations, the cells in each well were washed and dissolved in 0.5 mL of 0.2% Triton X-100, 1 mmol/L NaOH, for assay of their DNA content.28 Calf thymus DNA was used as a standard.

Statistical Analysis

Results were analyzed by using a random-effects model for repeated measurements.29 These models take into account that repeated measurement from the same individual are correlated. For all models, the random intercept for each individual was fitted to adjust for between-individual variability.29 Differences were considered to be statistically significant at a P value <0.05.

Results

Effect of *C pneumoniae* Bacterium on Production of 92-kDa Gelatinase, Interstitial Collagenase, Stromelysin-1, and TIMP-1

Blood monocytes obtained from healthy individuals were allowed to differentiate into macrophages, and then the effect of *C pneumoniae* on their ability to secrete 3 different MMPs was studied: 92-kDa gelatinase (MMP-9), interstitial collagenase (MMP-1), and stromelysin (MMP-3) (Figure I). The macrophages spontaneously released substantial amounts of 92-kDa gelatinase (first panel), a finding consistent with a previous report.6 Among these donors, some variability was noted in the basal production of the enzyme (0.14 to 0.35 μg 92-kDa gelatinase per μg cellular DNA/48 hours). *C pneumoniae* induced dose-dependent stimulation of 92-kDa gelatinase secretion. Notably, the cells from each donor (n=5) were stimulated by *C pneumoniae*, the stimulatory effect varying from 1.3- to 3.6-fold. At the largest amount of *C pneumoniae* used (3×10⁴ IFU), secretion of the enzyme was augmented, on average, 2.5-fold. For comparison, the expected 243-bp product.27 The RT-PCR signal for 92-kDa gelatinase was significantly increased after *C pneumoniae* stimulation (Figure III).

Effects of Different Exposure Times to *C pneumoniae* on 92-kDa Gelatinase Secretion

In the above experiments, the human macrophages had been exposed to *C pneumoniae* for 48 hours. We also tested whether shorter contacts of the macrophages with *C pneumoniae* would be sufficient to induce 92-kDa gelatinase secretion. In this experiment, either the macrophages were incubated for 48 hours in the absence of the above agents (A). The contents of 92-kDa gelatinase, interstitial collagenase, stromelysin, and TIMP-1 in the culture media were quantified by ELISA. The results are expressed as μg MMP or TIMP-1 secreted per μg cellular DNA per 48 hours (note the different scales). Symbols indicate values for cells derived from a single buffy coat (n=5 for panel 1, n=3 for panels 2–4), and horizontal lines indicate median of the values. *P<0.0001 for comparison with control (A).
Macrophages for 1.5 hours, the stimulatory effect on 92-kDa gelatinase secretion by the cells was almost the same as when the bacteria were incubated with the cells for 48 hours.

**Effects of Chlamydial Proteins Omp2, MOMP, and HSP60 on 92-kDa Gelatinase Secretion**

Macrophages were incubated with the indicated doses of the chlamydial proteins Omp2, MOMP, and HSP60 for 48 hours (Figure V). Omp2 increased a dose-dependent stimulation of 92-kDa gelatinase production, maximally ≈5-fold. Chlamydial MOMP also induced 92-kDa gelatinase secretion by the cells, but in sharp contrast to Omp2, the maximal effect was found at the lowest concentration used (0.1 μg/mL), which increased 92-kDa gelatinase by ≈5-fold. When higher concentrations were used (up to 2 μg/mL), a dose-dependent inhibition of stimulated 92-kDa gelatinase secretion was observed back to basal levels. If higher concentrations were used, MOMP was cytotoxic to the cells. HSP60 of *C pneumoniae* induced 92-kDa gelatinase secretion by the near-maximal effect obtained at the lowest concentration used (0.1 μg/mL, Figure V). Induction of 92-kDa gelatinase secretion by HSP60 was maximally 2- to 3-fold, which equaled that induced by *E coli* LPS (data not shown). No induction of 92-kDa gelatinase was noted when the macrophages were incubated with the corresponding buffers without proteins.

**Figure II.** Gelatin zymography for detection of 92-kDa gelatinase from human monocyte-derived macrophages. Monocytes were allowed to differentiate for 7 days in vitro, and the culture medium was then replaced by 1 mL of medium B (in each well). The cells were then exposed to the indicated quantities of live *C pneumoniae* for 48 hours. As controls, monocyte-derived macrophages were incubated for 48 hours in the absence of the above agents (negative control) or with *E coli* LPS as a positive control. Aliquots (10 μL) of the culture media were run in a 10% polyacrylamide gel containing 1 mg/mL gelatin and stained with 0.5% Coomassie brilliant blue R-250. Three independent experiments yielded similar results.

**Figure III.** RT-PCR analysis of 92-kDa gelatinase mRNA expression in monocyte-derived macrophages. Cultured (7 days) monocyte-derived macrophages were exposed to the indicated quantities of live *C pneumoniae* or *E coli* LPS for 48 hours. As controls, monocyte-derived macrophages were incubated for 48 hours in the absence of the above agents. RNA extraction and RT-PCR analysis were performed as described in Methods. The method was standardized by the expression of a housekeeping gene, GAPDH.

**Figure IV.** Effect of different times of exposure to *C pneumoniae* on 92-kDa gelatinase secretion by monocyte-derived macrophages. Monocytes were obtained from 4 subjects and cultured for 7 days in vitro to allow the cells to differentiate into macrophages. The cells were then incubated for 48 hours in either the absence (A) or presence (C) of *C pneumoniae*. In a third series of wells (B), the macrophages were exposed to the indicated quantities of live *C pneumoniae* for 1.5 hours, after which the culture media were replaced by fresh media that did not contain *C pneumoniae*. The 92-kDa gelatinase content of the culture supernatants was quantified by ELISA. The results are expressed as μg of 92-kDa gelatinase secreted per μg cellular DNA per 48 hours. Symbols indicate values for cells derived from a single buffy coat (n = 4), and horizontal lines indicate median of the values. *P < 0.002 for comparison with control (A).

**Figure V.** Effects of chlamydial proteins Omp2, MOMP, and HSP60 on 92-kDa Gelatinase Secretion

The above experiment with the 3 chlamydial proteins Omp2, MOMP, and HSP60 was then repeated with freshly prepared monocyte-derived macrophages derived from 3 different subjects (Figure VI). In this experiment, the macrophages were exposed to 3 different concentrations of the proteins for either 1.5 or 48 hours and using the same protocol as

**Figure VI.** Effects of chlamydial proteins Omp2, MOMP, and HSP60 on 92-kDa gelatinase secretion by monocyte-derived macrophages. Monocytes were obtained from 3 subjects, allowed to differentiate into macrophages for 7 days in vitro, and then exposed to the indicated quantities of chlamydial proteins Omp2, MOMP, or HSP60 for 48 hours. The 92-kDa gelatinase content of the culture supernatants was quantified by ELISA. The results are expressed as fold stimulation compared with the control macrophages, which were incubated in the absence of the above chlamydial proteins. The absolute values for 92-kDa gelatinase secretion by control macrophages derived from 3 individuals were 0.07, 0.09, and 0.15 (0.10 ± 0.02; mean ± SEM) μg/μg DNA per 48 hours. Error bars indicate SEM (n = 3). *P < 0.001, †P < 0.001, and ‡P < 0.002 concentration used as continuous variable.
Effect of Heat Treatment on C pneumoniae–Induced 92-kDa Gelatinase Secretion, µg/µg Cellular DNA per 48 h

<table>
<thead>
<tr>
<th>Incubation</th>
<th>92-kDa Gelatinase Secretion, µg/µg Cellular DNA per 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (no addition)</td>
<td>0.40±0.05 (background)</td>
</tr>
<tr>
<td>Positive control (E coli LPS, 5 µg/mL)</td>
<td>0.95±0.11*</td>
</tr>
<tr>
<td>C pneumoniae (3×10^6 IFU)</td>
<td>0.82±0.07*</td>
</tr>
<tr>
<td>Boiled C pneumoniae (3×10^6 IFU)</td>
<td>0.67±0.08*</td>
</tr>
</tbody>
</table>

IFU indicates inclusion-forming unit.

Monocytes were isolated from 3 different subjects and allowed to differentiate into monocyte-derived macrophages for 7 days. The cells were then exposed for 48 hours to E coli LPS (5 µg/mL), to live C pneumoniae (3×10^6 IFU), or to C pneumoniae (3×10^6 IFU) that had been boiled for 20 minutes. Control cells (negative controls) were incubated for 48 hours in the absence of the above agents. After incubation, the 92-kDa gelatinase content of each culture supernatant was quantified by ELISA. The results are expressed as µg of 92-kDa gelatinase secreted per µg cellular DNA per 48 hours (mean±SEM, n=6, duplicate wells for each of the 3 buffy coats). *P<0.004 for comparison with negative controls.

Effect of Monocyte Differentiation on Basal and C pneumoniae–Induced 92-kDa Gelatinase Secretion

Production of 92-kDa gelatinase was studied as a function of cellular differentiation by comparing freshly harvested human monocytes with cells from the same source differentiated in vitro for 7 days. As shown in Figure VIIA, the unstimulated control monocytes secreted detectable quantities of 92-kDa gelatinase, and this basal production was increased (by ~6-fold) when the cells were allowed to differentiate into macrophages (Figure VIIIB). In both monocytes and monocyte-derived macrophages, constitutive secretion was described for the experiment shown in Figure IV. Comparison of panels A and B in Figure V reveals similar patterns of upregulation of the 92-kDa gelatinase after short and prolonged antigenic stimulation of the macrophages. Moreover, the results were similar to those seen in Figure V, with MOMP possessing the strongest stimulatory effect at the lowest and Omp2 at the highest concentration used.

Effect of Heat Treatment on C pneumoniae–Induced 92-kDa Gelatinase Secretion

To further evaluate which C pneumoniae components participated in the induction of 92-kDa gelatinase by macrophages, the bacteria were boiled for 20 minutes before addition to the cell cultures (Table). Heat treatment of C pneumoniae reduced the effect on 92-kDa gelatinase secretion by these bacteria moderately, on average, by 35% (from a value of 0.42 to a value of 0.27 µg/µg cellular DNA per 48 hours, considering negative control as the background activity to be subtracted). This result was observed in each of the studied (n=3) buffy coats and strongly suggests that chlamydial LPS, which is thermostable (in contrast to the proteins), also participates in the induction of 92-kDa gelatinase production.
bacteria did induce both secretion and transcription of 92-kDa gelatinase. We did not investigate the mechanism of gene activation, but some information on the conditions required for activation is available. The promoter region of the gene for 92-kDa gelatinase contains nuclear factor (NF)-κB binding sites, which play a critical role in the expression of this gene.30 Interestingly, oxidized LDL induces 92-kDa gelatinase expression in human monocyte–derived macrophages, and an increase in 92-kDa gelatinase expression was associated with nuclear binding of NF-κB to specific sequences in the promoter region of the 92-kDa gelatinase gene.31 Therefore, it is possible that the C pneumoniae–mediated effect on 92-kDa gelatinase expression was due to activation of NF-κB. This possibility is supported by a recent observation demonstrating that C pneumoniae infection of vascular smooth muscle and endothelial cells activates NF-κB.32

The other intriguing question relates to the finding that C pneumoniae stimulated secretion of 92-kDa gelatinase but not secretion of interstitial collagenase or of stromelysin. In vitro, stimulation of all these 3 MMPs by an extracellular mediator depends on activation and binding of activator protein (AP)-1 to specific sequences in their promoters.33 However, induction of the 92-kDa gelatinase gene transcription also requires activation of NF-κB.34 If C pneumoniae activated NF-κB without activating other factors essential in regulation of the other MMPs, infection of macrophages would result in selective induction of 92-kDa gelatinase. Further studies are required to resolve this issue.

We found that at least 3 proteins, Omp2, MOMP, and HSP60, induced 92-kDa gelatinase secretion by macrophages, Omp2 being the primary antigen responsible for the upregulation of 92-kDa gelatinase. Of special interest is the chlamydial HSP60, since it has been found to colocalize with its homolog, human HPS60, within plaque macrophages.23 However, since denaturation of the chlamydial proteins by boiling the bacteria reduced 92-kDa gelatinase secretion by only ~35%, the residual 65% of remaining inducing capacity is likely to be due to the heat-stable LPS molecules that are present in the bacteria. Our study parallels the study performed in murine macrophages, in which HSP60 was found to upregulate 92-kDa gelatinase.23 In this respect, the responses of human and murine macrophages are similar. However, the relative potencies of the various antigens and the specificity of their effect on MMP secretion remain to be studied in the mouse system.

Incubation with the bacteria for as little as 1.5 hours, which is enough to allow their ingestion,34 fully induced 92-kDa gelatinase production by the macrophages. Also, the various chlamydial proteins were able to induce secretion of 92-kDa gelatinase by macrophages even after incubation for 1.5 hours. Unfortunately, we were unable to create conditions in which phagocytosis of the bacteria had been blocked. Therefore, we do not know whether the various chlamydial proteins and LPS can induce the 92-kDa gelatinase production by the macrophages. Also, the various chlamydial proteins were able to induce secretion of 92-kDa gelatinase by macrophages even after incubation for 1.5 hours. Unfortunately, we were unable to create conditions in which phagocytosis of the bacteria had been blocked. Therefore, we do not know whether the various chlamydial proteins and LPS can induce the 92-kDa gelatinase production by the macrophages. Also, the various chlamydial proteins were able to induce secretion of 92-kDa gelatinase by macrophages even after incubation for 1.5 hours. Unfortunately, we were unable to create conditions in which phagocytosis of the bacteria had been blocked. Therefore, we do not know whether the various chlamydial proteins and LPS can induce the 92-kDa gelatinase production by the macrophages. Also, the various chlamydial proteins were able to induce secretion of 92-kDa gelatinase by macrophages even after incubation for 1.5 hours. Unfortunately, we were unable to create conditions in which phagocytosis of the bacteria had been blocked.

The image shows a graph with the title “Comparison of the Effects of C trachomatis Serotypes C and L2 and of C pneumoniae on 92-kDa Gelatinase Production.” The x-axis is labeled “Inclusion-forming units,” and the y-axis is labeled “92-kDa gelatinase secretion (μg/mg DNA/48h).” The graph compares the effects of different organisms on 92-kDa gelatinase secretion. The organisms studied include C pneumoniae, C trachomatis serotypes C and L2, and C trachomatis L2. The results show that C pneumoniae stimulates 92-kDa gelatinase secretion to a greater extent than the other organisms. The graph also includes a bar chart with the label “Discussion.”

Discussion
The data presented demonstrate that C pneumoniae can increase the capacity of macrophages to produce 92-kDa gelatinase by stimulating the expression of this enzyme. Such increase in the expression of 92-kDa gelatinase could result in increased proteolytic activity in the extracellular microenvironment of the stimulated macrophages, leading to enhanced extracellular remodeling. In present work, C pneumoniae...
mononuclear cells.\textsuperscript{35} These observations suggest that the 92-kDa gelatinase induction in monocyte-derived macrophages is a specific response of those chlamydial types capable of being phagocytosed by mononuclear cells and capable of causing infection (\textit{C. pneumoniae}, \textit{C. trachomatis L\textsubscript{2}}). Importantly, addition of latex beads did not induce 92-kDa gelatinase production, demonstrating that phagocytosis alone is not sufficient for this response. In atherosclerotic plaques, \textit{C. pneumoniae} normally reside inside macrophages,\textsuperscript{22} and in this respect, the in vitro conditions used were similar to those in the plaques. After exposure to the live bacteria, fewer than 1\% of the macrophages contained \textit{C. pneumoniae} inclusions (indicative of replicating bacteria) even after incubation for 48 hours. We do not know whether this small fraction of cells was responsible for the entire secretion of 92-kDa gelatinase. However, since even killed \textit{C. pneumoniae} can induce the production of 92-kDa gelatinase,\textsuperscript{23} mere ingestion of \textit{C. pneumoniae} without replication appears to be sufficient for induction of the enzyme. Since the phagocytosed bacteria are at various developmental stages and, accordingly, some of them may not form inclusions, the fraction of macrophages that contained \textit{C. pneumoniae} and contributed to the production of 92-kDa gelatinase is likely to be higher than 1\%. Finally, \textit{Haemophilus influenzae}, which, like \textit{C. pneumoniae} and \textit{E. coli}, is also a Gram-negative LPS-containing bacterium, failed to induce production of 92-kDa gelatinase (R. Pastila et al, unpublished observations, 1977). Thus, not any infective agent may provoke 92-kDa gelatinase induction in monocyte-derived macrophages in humans.

The activity of metalloproteinases on substrates of the extracellular matrix depends on the balance existing between these enzymes and their endogenous inhibitors, the TIMPs. The activity of metalloproteinases on substrates of the extracellular matrix depends on the balance existing between these enzymes and their endogenous inhibitors, the TIMPs.

The earlier finding that TNF-\alpha and interleukin-1\beta\textsuperscript{3}\textsuperscript{3} and the present finding that \textit{C. pneumoniae} and its proteins can increase 92-kDa gelatinase production by human monocyte-derived macrophages in vitro, but not that of interstitial collagenase or stromelysin, reveals differences in the mechanisms regulating the production of these 3 metalloproteinases. Provided that \textit{C. pneumoniae} actually leads to activation of macrophages in atherosclerotic plaques and induces secretion of active 92-kDa gelatinase by these cells, the ensuing matrix degradation would increase the susceptibility of the plaques to rupture. This suggests a mechanism by which \textit{C. pneumoniae} could contribute to the pathology of atherothrombosis.

An association has been found between infection with \textit{C. pneumoniae} and myocardial infarction. Interestingly, recent clinical findings showed that acute respiratory infection is associated with an increased risk of acute myocardial infarction.\textsuperscript{42} In a case-control study, previous use of tetracyclines or quinolones, antibiotics that are active against \textit{C. pneumoniae}, was associated with a lower incidence of first-time acute myocardial infarction.\textsuperscript{43} Both of these findings include the possibility but do not prove that \textit{C. pneumoniae} is associated with myocardial infarction. Besides antimicrobial activity, some other pharmacological mechanisms, ie, suppression of metalloproteinase secretion by tetracyclines,\textsuperscript{44} could contribute to the reduced risk of acute myocardial infarction. Experience from other antibiotic intervention trials suggests that treatment with azithromycin\textsuperscript{45} or roxithromycin\textsuperscript{46} may also improve the prognosis of chronic and acute coronary heart disease. Unfortunately, the mere association of chlamydial infection with myocardial infarction and the results of these antibiotic trials cannot provide us with an answer as to whether the present in vitro observation is of clinical significance. Notably, chlamydial infection without seeding of the organisms in the coronary arteries, by causing systemic inflammation and elevation of C-reactive protein, also increases the risk of acute myocardial infarction.\textsuperscript{47} Moreover, the current modes of antibiotic treatment are not chlamydia-specific and may also have direct anti-inflammatory effects. However, eradication of \textit{C. pneumoniae} from macrophages in atherosclerotic plaques by treatment with antibiotics appears to be a means of preventing macrophages from secreting matrix-degrading enzymes, and in this way the therapy could also lower the risk of plaque rupture.

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References


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