# *Chlamydia pneumoniae* Enhances Cytokine-Stimulated Human Monocyte Matrix Metalloproteinases through a Prostaglandin E$_2$-Dependent Mechanism

Min P. Kim,[1] Charlotte A. Gaydos,[2] Billie Jo Wood,[2] Justin P. Hardick,[2] Yahong Zhang,[1] and Larry M. Wahl[1]*

*National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda,[1] and Johns Hopkins University School of Medicine, Baltimore,[2] Maryland*

**Exposure of human monocytes to *Chlamydia pneumoniae* resulted in a significant enhancement of matrix metalloproteinase (MMP) 1 and 9 production following stimulation with tumor necrosis factor alpha and granulocyte monocyte-colony stimulating factor. The effect of *C. pneumoniae* on monocyte MMPs was mediated through the induction of prostaglandin E$_2$. These findings may have implications for atherosclerotic plaque rupture.**

The role of *Chlamydia pneumoniae* in atherosclerosis is still uncertain. Evidence implicating this organism in atherosclerosis is based on the presence of *C. pneumoniae* in the atheroma (9–11) and seroepidemiology studies that link immunoglobulin A (IgA) to *C. pneumoniae* and coronary artery disease (13, 14). However, recent prospective controlled trials have shown that there is no correlation between the seropositivity to *C. pneumoniae* and coronary events (2, 15). Thus, there is still debate as to the role of *C. pneumoniae* in coronary artery disease, and present findings are not strong enough to support or dispute whether *C. pneumoniae* plays an active role in the development of atherosclerosis or if this organism contributes to plaque rupture.

Present understanding of the mechanism of *C. pneumoniae* infection of vessels is that the organism is transported by the monocytes. Because the pathogen is a common cause of respiratory infection, the alveolar monocytes exposed to or infected with the organism may travel in the bloodstream and enter the atherosclerotic plaque, where they undergo differentiation into macrophages and cause active *C. pneumoniae* infection. This explanation has been supported by a study involving experimental infection of mice with *C. pneumoniae* that showed that the bacteria were spread by peripheral blood monocytes (12).

Monocytes may play an important role in acute coronary syndrome through their production of matrix metalloproteinases (MMP) 1 and 9. MMP-1 is an interstitial collagenase that can cleave fibrillar collagens (types I, II, and III), and MMP-9 is a gelatinase that can degrade denatured or cleaved fibrillar collagens and basement membrane components, including type IV collagen. These enzymes have been colocalized in atherosclerotic plaques (4) and are capable of degrading the extracellular matrix in the thin fibrous cap of a vulnerable plaque, leading to the rupture of the atherosclerotic plaque.

Production of MMP-1 and -9 by monocytes is stimulated by endogenous inflammatory stimulants, tumor necrosis factor alpha (TNF-α), and granulocyte monocyte-colony stimulating factor (GM-CSF) (17). Thus, monocytes exposed to *C. pneumoniae* may produce larger amounts of MMPs in the presence of TNF-α and GM-CSF. Murine monocytes in the presence of *C. pneumoniae* have been shown to produce MMP-9 (8); similarly, human monocyte-derived macrophages exposed to *C. pneumoniae* had increased levels of MMP-9 but failed to show an increase in MMP-1, MMP-3, and a tissue inhibitor of MMP-1 (16).

To explore the effect of *C. pneumoniae* on monocyte MMP production, we compared the production of MMP-1 and MMP-9 by monocytes that were exposed to live or heat-killed *C. pneumoniae* followed by stimulation with TNF-α and GM-CSF, cytokines present in atheroma. Purified human peripheral blood cells were obtained by elutriation as previously described (17). Purified monocytes were plated at $5 \times 10^6$/ml of serum-free Dulbecco's modified Eagle medium (Bio Whittaker) in 12-well plates (Costar) and were adhered for at least 30 min prior to the addition of reagents. Some of the monocyte cultures were treated with live or heat-killed *C. pneumoniae* (strain AR39) as previously described (5). Six hours later the cultures were washed, and TNF-α (50 ng/ml; PeproTech) and GM-CSF (50 ng/ml; PeproTech) were added to some of the cultures. Proteins in the medium conditioned for 48 h were analyzed by Western blotting for MMP-1 with peptide-specific antibodies (generously provided by Henning Birkedal-Hansen, National Institute of Dental and Craniofacial Research, Bethesda, Md.) and for MMP-9 (Chemicon International, Temecula, Calif.) as previously described (17). All experiments were repeated at least three times with different donors. Monocytes exposed to live *C. pneumoniae* induced detectable MMP-1 at $10^6$ infectious units (IFU)/ml, whereas MMP-9 was detected at $10^5$ to $10^6$ IFU (Fig. 1A). However, when *C. pneumoniae*-treated monocytes were stimulated with TNF-α and GM-CSF, a slight enhancement of MMP-1 production by *C. pneumoniae* occurred at $10^2$ to $10^4$ IFU, with a greater increase

* Corresponding author. Mailing address: Immunopathology Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, 30 Convent Dr., Building 30, Room 325, Bethesda, MD 20892-4352. Phone: (301) 496-9219. Fax: (301) 402-1064. E-mail: lwahl@dir.nidcr.nih.gov.
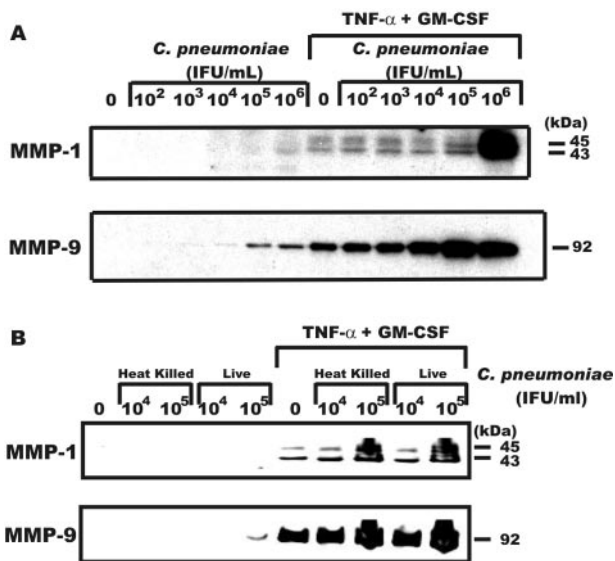
FIG. 1. *C. pneumoniae* increases the sensitivity of monocytes to MMP-1 and MMP-9 induction by TNF-α and GM-CSF. Monocytes were incubated with the IFU for 6 h, washed, and cultured in the presence or absence of TNF-α (50 ng/ml) and GM-CSF (50 ng/ml) for an additional 48 h. Supernatants were harvested at 48 h and were assayed for MMP-1 and MMP-9 by Western blotting. Panel A represents a dose response of IFU of live *C. pneumoniae*, whereas panel B compares IFU of live *C. pneumoniae* to the equivalent IFU of *C. pneumoniae* following heating at 90°C for 15 min.

at $10^5$ IFU and a substantial increase at $10^6$ IFU. Monocyte MMP-9 levels were also affected by *C. pneumoniae*, with a slight increase detected at $10^5$ and $10^6$ IFU and substantial increases occurring at $10^2$ to $10^6$ IFU when challenged with cytokines (Fig. 1A). We next compared heat-killed versus live *C. pneumoniae* to determine if contact and/or infection mediated the increase in MMPs. As shown in Fig. 1B, heat-killed and live *C. pneumoniae* caused a similar synergistic increase in cytokine-induced MMP production, indicating that contact with *C. pneumoniae* was sufficient to prime monocytes for enhanced MMP production.

Stimulation of monocyte MMPs by cytokines involves, at least in part, a prostaglandin $E_2$ ($PGE_2$)-dependent mechanism (17). To examine if *C. pneumoniae* increased MMP production in a $PGE_2$-dependent manner, the cyclooxygenase inhibitors indomethacin and aspirin (Sigma) were added to monocytes. Both cyclooxygenase inhibitors suppressed the synergistic increase in MMP-1 and MMP-9 production by the addition of cytokines to *C. pneumoniae*-treated monocytes (Fig. 2). To determine the $PGE_2$ levels in the cultures, the media conditioned for 24 h were analyzed for $PGE_2$ production by enzyme-linked immunosorbent assay (ELISA) (Cayman Chemicals, Ann Arbor, Mich., or Peninsula Laboratories, Inc., San Carlos, Calif.) according to the manufacturer's suggestions. Measurement of $PGE_2$ levels in the media revealed that *C. pneumoniae* alone caused a substantial increase in $PGE_2$ at $10^5$ and $10^6$ IFU, which was further enhanced when monocytes were stimulated with cytokines (Fig. 3A). The increase in the production of $PGE_2$ following exposure of monocytes to *C. pneumoniae* was inhibited by indomethacin (Fig. 3B). These findings demonstrate that the interaction of mono-
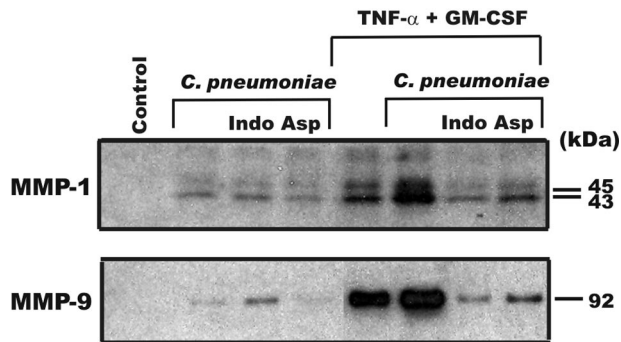
cytes with *C. pneumoniae* results in a substantial increase in the production of $PGE_2$ that, when coupled with additional signaling components induced by cytokines, results in a synergistic increase in MMP production.

The striking increase in MMP production by *C. pneumoniae*-primed monocytes by TNF-α and GM-CSF indicates that *C.*



FIG. 2. Indomethacin and aspirin inhibit *C. pneumonia* enhancement of MMP-1 and MMP-9. Monocytes were cultured for 6 h with *C. pneumoniae* ($10^5$/ml), washed, and cultured in the presence or absence of TNF-α plus GM-CSF and indomethacin (Indo) (1 μM) or aspirin (Asp) (1 μM) for 48 h. The levels of MMP-1 and -9 in the 48-h supernatants were determined by Western blotting.
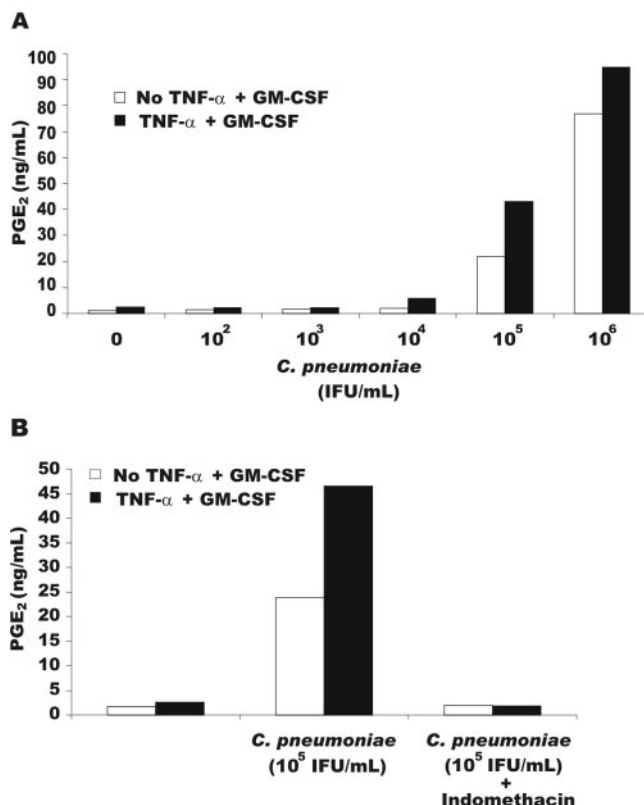


FIG. 3. Induction of $PGE_2$ by *C. pneumoniae*. Monocytes were incubated with different concentrations of *C. pneumoniae* IFU for 6 h, washed, and cultured with or without TNF-α plus GM-CSF for 24 h. The 24-h supernatants were assayed for $PGE_2$ by ELISA (A) and from cultures treated with 1 μM indomethacin (B). The data are representative of three separate experiments from different donors.

*pneumoniae* may play a role in the development of acute coronary syndrome by increasing the sensitivity of monocytes to cytokine stimulation when they enter a vulnerable plaque. These findings support results of a clinical trial that demonstrated that individuals taking macrolide antibiotics had significantly smaller numbers of ruptured plaque than the placebo group (6). Thus, treatment leading to *C. pneumoniae* clearance may be important in decreasing the events leading to acute coronary syndrome.

Monocytes may also contribute to the levels of *C. pneumoniae*, because infected monocytes express *C. pneumoniae* RNA for 3 days after infection (1) and are also resistant to the development of infectious progeny of the bacteria (1), which prevents apoptosis of monocytes (3). Moreover, the monocytes containing *C. pneumoniae* have altered functions, such as increased adherence to human aortic endothelial cells (7). These observations provide evidence for a role of *C. pneumoniae* in acute coronary syndrome and possible approaches that include the combination of antibiotics and cyclooxygenase inhibitors for treatment of acute coronary syndrome.

## REFERENCES

1. **Airenne, S., H. M. Surcel, H. Alakarppa, K. Laitinen, J. Paavonen, P. Saikku, and A. Laurila.** 1999. *Chlamydia pneumoniae* infection in human monocytes. Infect. Immun. **67:**1445–1449.
2. **Danesh, J., P. Whincup, S. Lewington, M. Walker, L. Lennon, A. Thomson, Y. K. Wong, X. Zhou, and M. Ward.** 2002. Chlamydia pneumoniae IgA titres and coronary heart disease; prospective study and meta-analysis. Eur. Heart J. **23:**371–375.
3. **Fan, T., H. Lu, H. Hu, L. Shi, G. A. McClarty, D. M. Nance, A. H. Greenberg, and G. Zhong.** 1998. Inhibition of apoptosis in chlamydia-infected cells: blockade of mitochondrial cytochrome c release and caspase activation. J. Exp. Med. **187:**487–496.
4. **Galis, Z. S., G. K. Sukhova, M. W. Lark, and P. Libby.** 1994. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J. Clin. Investig. **94:**2493–2503.
5. **Gaydos, C. A., J. T. Summersgill, N. N. Sahney, J. A. Ramirez, and T. C. Quinn.** 1996. Replication of *Chlamydia pneumoniae* in vitro in human macrophages, endothelial cells, and aortic artery smooth muscle cells. Infect. Immun. **64:**1614–1620.
6. **Gurfinkel, E.** 1998. Link between intracellular pathogens and cardiovascular diseases. Clin. Microbiol. Infect. **4**(Suppl. 4)**:**S33–S36.
7. **Kalayoglu, M. V., B. N. Perkins, and G. I. Byrne.** 2001. Chlamydia pneumoniae-infected monocytes exhibit increased adherence to human aortic endothelial cells. Microbes Infect. **3:**963–969.
8. **Kol, A., G. K. Sukhova, A. H. Lichtman, and P. Libby.** 1998. Chlamydial heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis factor-alpha and matrix metalloproteinase expression. Circulation **98:**300–307.
9. **Kuo, C. C., A. M. Gown, E. P. Benditt, and J. T. Grayston.** 1993. Detection of Chlamydia pneumoniae in aortic lesions of atherosclerosis by immunocytochemical stain. Arterioscler. Thromb. **13:**1501–1504.
10. **Kuo, C. C., J. T. Grayston, L. A. Campbell, Y. A. Goo, R. W. Wissler, and E. P. Benditt.** 1995. Chlamydia pneumoniae (TWAR) in coronary arteries of young adults (15–34 years old). Proc. Natl. Acad. Sci. USA **92:**6911–6914.
11. **Kuo, C. C., A. Shor, L. A. Campbell, H. Fukushi, D. L. Patton, and J. T. Grayston.** 1993. Demonstration of Chlamydia pneumoniae in atherosclerotic lesions of coronary arteries. J. Infect. Dis. **167:**841–849.
12. **Moazed, T. C., C. C. Kuo, J. T. Grayston, and L. A. Campbell.** 1998. Evidence of systemic dissemination of Chlamydia pneumoniae via macrophages in the mouse. J. Infect. Dis. **177:**1322–1325.
13. **Saikku, P., M. Leinonen, K. Mattila, M. R. Ekman, M. S. Nieminen, P. H. Makela, J. K. Huttunen, and V. Valtonen.** 1988. Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet **ii:**983–986.
14. **Saikku, P., M. Leinonen, L. Tenkanen, E. Linnanmaki, M. R. Ekman, V. Manninen, M. Manttari, M. H. Frick, and J. K. Huttunen.** 1992. Chronic Chlamydia pneumoniae infection as a risk factor for coronary heart disease in the Helsinki Heart Study. Ann. Intern. Med. **116:**273–278.
15. **Tavendale, R., D. Parratt, S. D. Pringle, R. A'Brook, and H. Tunstall-Pedoe.** 2002. Serological markers of Chlamydia pneumoniae infection in men and women and subsequent coronary events; the Scottish Heart Health Study Cohort. Eur. Heart J. **23:**301–307.
16. **Vehmaan-Kreula, P., M. Puolakkainen, M. Sarvas, H. G. Welgus, and P. T. Kovanen.** 2001. Chlamydia pneumoniae proteins induce secretion of the 92-kDa gelatinase by human monocyte-derived macrophages. Arterioscler. Thromb. Vasc. Biol. **21:**E1–E8.
17. **Zhang, Y., K. McCluskey, K. Fujii, and L. M. Wahl.** 1998. Differential regulation of monocyte matrix metalloproteinase and TIMP-1 production by TNF-alpha, granulocyte-macrophage CSF, and IL-1 beta through prostaglandin-dependent and -independent mechanisms. J. Immunol. **161:**3071–3076.

*Editor:* J. B. Bliska