

Polymorphic Membrane Protein (PMP) 20 and PMP 21 of *Chlamydia pneumoniae* Induce Proinflammatory Mediators in Human Endothelial Cells In Vitro by Activation of the Nuclear Factor- κ B Pathway

Alexander Niessner,¹ Christoph Kaun,¹ Gerlinde Zorn,¹ Walter Speidl,¹ Zeynep Türel,¹ Gunna Christiansen,⁴ Anna-Sofie Pedersen,⁴ Svend Birkelund,⁴ Susan Simon,² Apostolos Georgopoulos,² Wolfgang Graninger,² Rainer de Martin,³ Joachim Lipp,³ Bernd R. Binder,³ Gerald Maurer,¹ Kurt Huber,¹ and Johann Wojta¹

Departments of ¹Internal Medicine II, ²Internal Medicine I, and ³Vascular Biology and Thrombosis Research, University of Vienna, Vienna, Austria; ⁴Department of Medical Microbiology, University of Århus, Århus, Denmark

We tested whether polymorphic membrane proteins (PMPs) of *Chlamydia pneumoniae* might play a role in triggering an inflammatory response in human endothelial cells. Of 15 purified, recombinant chlamydial PMPs tested, 2 (PMP 20 and PMP 21) dose-dependently increased the production of the inflammatory mediators interleukin (IL)-6 and monocyte chemoattractant protein-1 (MCP-1), in cultured human endothelial cells; production of IL-8 was also increased. When endothelial cells were infected by live *C. pneumoniae*, an increase in the production of IL-6, IL-8, and MCP-1 was seen. We used adenovirus-induced overexpression of I κ B α —an inhibitor of nuclear factor (NF)- κ B—to demonstrate that PMP 20 and PMP 21 increase the production of IL-6 and MCP-1 in human endothelial cells by activation of the NF- κ B pathway, because, in cells overexpressing I κ B α , treatment with the respective PMP did not result in increased production of IL-6 and MCP-1. Thus, *C. pneumoniae* could, by interactions of its PMPs with the endothelium, contribute to the process of vascular injury during the development and progression of atherosclerotic lesions.

Chlamydia pneumoniae, an obligate intracellular bacterium, has been implicated in the initiation and progression of atherosclerosis [1]. An association between anti-*C. pneumoniae* antibodies and myocardial infarction, coronary heart disease, carotid atherosclerosis, and stroke has been described in several seroepidemiological studies [2–5]. Viable strains of *C. pneumoniae* have been both isolated and successfully cultured from athero-

sclerotic plaques, and the bacterium has been detected in atherosclerotic lesions in various vessels, by immunohistochemistry, in situ hybridization, identification of genomic material by polymerase chain reaction, or electron microscopy [6, 7].

Inflammation seems to be a key event in the development and progression of atherosclerotic lesions [8]. It is believed that infections by certain pathogens, such as *C. pneumoniae*, by initiation of an inflammatory response, might contribute to this disease process. In fact, several in vitro investigations have shown that *C. pneumoniae* is capable of infecting vascular endothelial cells, smooth muscle cells, and macrophages, thereby leading to initiation of inflammatory activation of these cells via the nuclear factor (NF)- κ B pathway, a process that results in increased expression of adhesion molecules, tissue factor, plasminogen activation inhibitor-1 (PAI-1), and inflammatory cytokines [9–12]. In endothelial

Received 22 October 2002; accepted 12 February 2003; electronically published 12 June 2003.

Financial support: Austrian Heart Foundation; Ludwig Boltzmann Foundation for Cardiovascular Research; Association for the Promotion of Research in Arteriosclerosis, Thrombosis, and Vascular Biology.

Reprints or correspondence: Dr. Johann Wojta, Div. of Cardiology, Dept. of Internal Medicine II, Währinger Gürtel 18-20, A-1090 Vienna, Austria (johann.wojta@univie.ac.at).

The Journal of Infectious Diseases 2003;188:108–13

© 2003 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/2003/18801-0015\$15.00

Table 1. Effect of polymorphic membrane proteins (PMPs) on interleukin (IL)-6 and monocyte chemoattractant protein-1 (MCP-1), in human umbilical vein endothelial cells (HUVECs).

Treatment	IL-6	MCP-1
Control	25.5 ± 4.2	291.4 ± 39.8
PMP 1	25.4 ± 1.9	326.5 ± 10.2
PMP 2	26.1 ± 2.4	287.1 ± 11.8
PMP 6	28.8 ± 3.6	276.1 ± 26.6
PMP 7	26.9 ± 2.6	276.3 ± 41.9
PMP 8	28.1 ± 2.5	291.8 ± 12.1
PMP 9	29.7 ± 3.9	325.9 ± 17.6
PMP 11	24.5 ± 1.3	329.6 ± 51.1
PMP 13	26.0 ± 4.4	326.8 ± 26.7
PMP 14	26.5 ± 0.8	324.8 ± 26.0
PMP 15	31.7 ± 0.8	328.7 ± 43.2
PMP 16	30.1 ± 2.8	317.1 ± 45.3
PMP 18	24.8 ± 1.9	312.7 ± 34.3
PMP 19	32.4 ± 2.9	301.7 ± 47.3
PMP 20	55.0 ± 11.2 ^a	944.6 ± 78.8 ^b
PMP 21	49.3 ± 10.9 ^a	819.0 ± 62.8 ^b
PMP 20 and PMP 21	56.9 ± 2.7 ^a	936.9 ± 28.9 ^b

NOTE. Data are mean ± SD pg/10⁴ cells/24 h of 3 independent determinations, after incubation of HUVECs, with or without respective PMP (100 ng/mL), for 24 h.

^a *P* < .01, compared with control.

^b *P* < .001, compared with control.

cells, live *C. pneumoniae* have been shown to induce the inflammatory mediators interleukin (IL)-6 and monocyte chemoattractant protein-1 (MCP-1) by this mechanism [9, 10]. Information on the particular chlamydial proteins involved in the initiation of these effects is sparse. Chlamydial heat shock protein 60 is one bacterial component that is responsible for such activation [12]. In a recent study, acellular components of *C. pneumoniae* were shown to stimulate the production of cytokines—such as tumor necrosis factor- α (TNF- α), IL-1, IL-6, IL-8, and MCP—in human blood mononuclear cells [13]; however, the bacterial components initiating these effects were not further characterized [13].

It is assumed that, because of their predicted location in the outer membrane of the bacterium, polymorphic membrane proteins (PMPs) of *C. pneumoniae* play a crucial role in the interaction of the pathogen and the host cell [14–16]. We investigated whether such PMPs of *C. pneumoniae* could affect the production of certain inflammatory mediators, namely IL-6 and MCP-1, in human endothelial cells in vitro.

MATERIALS AND METHODS

Cell culture. Human umbilical vein endothelial cells (HUVECs) were isolated from fresh umbilical cords by mild col-

lagenase treatment and were cultured and characterized, as described elsewhere [17]. Human skin microvascular endothelial cells (HSMECs) were isolated from skin-biopsy samples by trypsin digestion and by use of Dynabeads (Dyna) coated with *Ulex europaeus* agglutinin, as described elsewhere [17]. All cells used in this study were between passages 2 and 4. All human material was obtained and processed according to the recommendations of the hospital's Ethics Committee and Security Board.

Recombinant production of PMPs. PMPs were expressed in *Escherichia coli* and were purified as described elsewhere [15]. To exclude possible lipopolysaccharide (LPS)-mediated effects, in control experiments, aliquots of the respective PMPs were boiled for 5 min before being added to HUVECs.

Incubation of endothelial cells with PMPs. Endothelial cells were incubated with different concentrations of the respective PMP, for the indicated times, in medium 199 (M199; Sigma) containing 1.25% supplemented calf serum (Hyclone). After incubation, cell debris was removed by centrifugation, and culture supernatants were collected and were stored at -70°C until used. The total cell number of the respective cultures, after trypsinization, was counted by a hemocytometer.

Infection of HUVECs by live *C. pneumoniae*. HUVECs cultured in medium 199 containing 1.25% supplemented calf serum were infected by *C. pneumoniae* strain 2023, by the method of Krull et al. [12]. Strain 2023 (American Type Culture Collection) was originally isolated from a patient with pneumonia, and it forms inclusions that react with both specific anti-*C. pneumoniae* antibodies and antibodies directed against chlamydial LPS [18]. After incubation, the culture supernatants were collected and treated, as described above.

Transfection of HUVECs with I κ B α -adenovirus. The adenovirus construct for overexpression of I κ B α (rAd.I κ B α) contained the porcine I κ B α coding sequence fused to a nuclear localization signal under the cytomegalovirus promoter and was transfected into HUVECs, as described elsewhere [19]. In brief, confluent monolayers of HUVECs were washed once with PBS and were incubated with rAd.I κ B α , at an MOI of 1000, in PBS. After 30 min at 37°C, the adenovirus was removed by washing, and fresh medium was added. Cells were further cultivated, for 2 days, before stimulation by PMPs. As a control, an adenovirus construct containing the coding sequence for green fluorescence protein (rAd.GFP), whose ability to transfect HUVECs has been described elsewhere [20], was used. By use of this technique, a transfection rate of >90% was achieved (data not shown). To control for equal cell numbers, crystal-violet staining of the HUVEC monolayer was performed.

Assays for IL-6, IL-8, and MCP-1. IL-6, IL-8, and MCP-1 were quantified by specific ELISAs (R&D Systems).

Statistical analysis. Data were compared statistically by

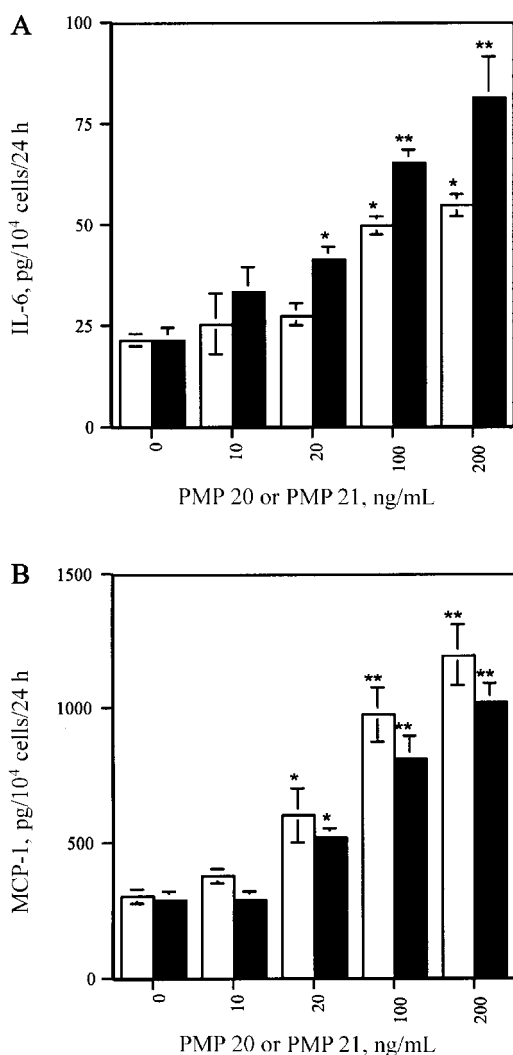


Figure 1. Dose-dependent effects of polymorphic membrane protein (PMP) 20 and PMP 21, on the production of interleukin (IL)-6 (A) and monocyte chemoattractant protein-1 (MCP-1) (B), in human umbilical vein endothelial cells (HUVECs). HUVECs were incubated for 24 h with or without either PMP 20 (open bars) or PMP 21 (solid bars), at concentrations of 10, 20, 100, or 200 ng/mL. Experiments were performed 3 times; a representative experiment is shown. Conditioned media of incubated cells were collected, and IL-6 and MCP-1 were quantified. Data are mean \pm SD of 3 independent determinations. * $P < .01$, ** $P < .001$, compared with control.

analysis of variance with the Bonferroni post hoc test for multiple comparisons, unless otherwise stated. $P \leq .05$ was considered significant. The SPSS statistical program (version 10.0; SPSS) was used for data analysis.

RESULTS

PMP 20 and PMP 21 induce IL-6, IL-8, and MCP-1 production in HUVECs. Of 15 PMPs tested, PMP 20 and PMP 21

were the strongest inducers of IL-6 and MCP-1 production in HUVECs (table 1). No further stimulation of production, however, was seen when PMP 20 and PMP 21 were added simultaneously to HUVECs. As can be seen in figure 1, these effects were dose dependent. In these cells, PMP 20 and PMP 21 increased IL-6 production by HUVECs ≤ 2.5 -fold and ≤ 3 -fold, respectively, and increased MCP-1 production ≤ 4 -fold and ≤ 3 -fold, respectively. When PMP 20 and PMP 21 were boiled for 5 min before being added to HUVECs, no effect on IL-6 and MCP-1 production was observed (IL-6: control, 17.0 ± 1.6 pg/10⁴ cells/24 h; PMP 20, 42.9 ± 2.2 pg/10⁴ cells/24 h; PMP 20 boiled, 18.8 ± 2.4 pg/10⁴ cells/24 h; PMP 21, 35.2 ± 5.0 pg/10⁴ cells/24 h; and PMP 21 boiled, 18.1 ± 2.0 pg/10⁴ cells/24 h) (MCP-1: control, 420.2 ± 31.4 pg/10⁴ cells/24 h; PMP 20, 1204.2 ± 31.0 pg/10⁴ cells/24 h; PMP 20 boiled, 361.2 ± 32.6 pg/10⁴ cells/24 h; PMP 21, 1025.4 ± 42.2 pg/10⁴ cells/24 h; and PMP 21 boiled, 415.7 ± 29.7 pg/10⁴ cells/24 h). This ruled out possible LPS contamination of the PMPs as a cause for their effect on IL-6 and MCP-1, in endothelial cells. PMP 20 and PMP 21 also stimulated the production of IL-8 in endothelial cells (table 2). Again, as shown for IL-6 and MCP-1, no further stimulation was seen when HUVECs were incubated simultaneously with PMP 20 and PMP 21. As can be seen in table 3, IL-6, IL-8, and MCP-1 production increased in cultured HUVECs infected with live *C. pneumoniae*, compared with that seen in noninfected control cells.

PMP 20 and PMP 21 induce IL-6, IL-8, and MCP-1 production in HSMECs. As can be seen in table 4, PMP 20 and PMP 21 stimulated production of IL-6, IL-8, and MCP-1, by HSMECs, to an extent similar to that seen by HUVECs.

NF- κ B-mediated activation of HUVECs. When HUVECs transfected with rAd.I κ B α were incubated with either PMP 20 or PMP 21, the stimulatory effect on IL-6 and MCP-1 production by these cells was abolished, whereas, when HUVECs transfected with the control virus rAd.GFP were incubated with either PMP 20 or PMP 21, an increased production of cyto-

Table 2. Effect of polymorphic membrane protein (PMP) 20 and PMP 21, on interleukin (IL)-8, in human umbilical vein endothelial cells (HUVECs).

Treatment	IL-8
Control	109.4 \pm 10.5
PMP 20	920.0 \pm 84.1 ^a
PMP 21	706.1 \pm 21.1 ^a
PMP 20 and PMP 21	912.7 \pm 84.4 ^a

NOTE. Data are mean \pm SD pg/10⁴ cells/24 h of 3 independent determinations, after incubation of HUVECs, with or without respective PMP (100 ng/mL), for 24 h.

^a $P < .001$, compared with control.

Table 3. Effect of live *Chlamydia pneumoniae* on interleukin (IL)-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1), in human umbilical vein endothelial cells (HUVECs).

Treatment	IL-6	IL-8	MCP-1
Control	23.0 ± 3.9	95.7 ± 13.8	397.7 ± 45.1
<i>C. pneumoniae</i>	562.3 ± 44.1 ^a	2215.0 ± 197.1 ^a	2177.9 ± 87.3 ^b

NOTE. Data are mean ± SD pg/10⁴ cells/24 h of 3 independent determinations, after incubation of HUVECs, without or with live *C. pneumoniae*, for 24 h.

^a *P* < .0001, compared with control.

^b *P* < .001, compared with control.

kines, similar to that seen in nontransfected HUVECs, was present (figure 2).

DISCUSSION

There is an increasing amount of intriguing evidence for a role of *C. pneumoniae* in the development and progression of atherosclerosis. On the one hand, this evidence is based on numerous clinical studies linking the presence of either antibodies against *C. pneumoniae* or the bacterium per se to the disease process [1–7]. On the other hand, several in vitro investigations have shown that *Chlamydia* species are capable of infecting vascular endothelial cells, smooth muscle cells, and macrophages, thereby leading to the initiation of inflammatory activation of these cells, a process that results in increased expression of adhesion molecules, tissue factor, PAI-1, and inflammatory cytokines [7, 9–12, 21–24]. In recent studies, chlamydial heat shock protein 60 has been identified as a bacterial component that is responsible for such activation, via the NF-κB pathway, of these cells [11, 25]. In addition, as-yet-uncharacterized acellular components of *C. pneumoniae* have been shown to stimulate cytokine production in human blood mononuclear cells [13]. Furthermore, LPS isolated from *C. trachomatis* and *C. pneumoniae* has been shown to induce the release of TNF-α from whole blood and macrophage foam cell formation, respectively [26, 27].

In the present study, we have investigated whether a particular class of chlamydial membrane proteins, the PMPs, may also play a role in the activation of endothelial cells. PMPs are predicted to be localized to the bacterial outer membrane and, as such, are likely to be primary mediators in pathogen infection and bacterium–host cell interaction [14–16, 28, 29]. In a recent study, particular chlamydial antigens, among them PMPs, have been shown to prime a CD8⁺ T cell–mediated immune response in mice [30].

In the present study, we have shown that, of 15 recombinant PMPs, 2 (PMP 20 and PMP 21) stimulated the production of IL-6 and MCP-1, in human endothelial cells in vitro. IL-6, as an important mediator of inflammation that is associated with cardiovascular disease and is found in atherosclerotic plaques,

seems to be involved in plaque instability and the pathogenesis of acute myocardial infarction [31–33]; MCP-1, by the induction of chemotaxis of monocytes, is thought to contribute to the accumulation of monocytes from circulation into the arterial wall, in early atherogenesis [34]. This notion is supported by data showing that mice deficient in MCP-1 are less susceptible to experimental atherosclerosis [35, 36]. The effects that PMP 20 and PMP 21 have on the production of these 2 inflammatory cytokines were dose-dependent, with maximum stimulation seen between 100 and 200 ng/mL. Both PMPs also increased the production of IL-8 in endothelial cells. It should be noted that no additive or synergistic effect on the production of these cytokines in endothelial cells was seen when PMP 20 and PMP 21 were added simultaneously. Thus, one might speculate that both PMPs act through the same receptor and/or activate the same intracellular pathway in endothelial cells. It should, however, be emphasized that no data on specific receptors for PMPs of *C. pneumoniae* are yet available.

As have the authors of other studies [9, 10], we have been able to demonstrate that live *C. pneumoniae* induce IL-6, IL-8, and MCP-1, in endothelial cells. Thus, our data suggest that PMP 20 and PMP 21 are responsible for a specific activation of endothelial cells, as reflected by the expression of a certain set of cytokines. Furthermore, we have been able to demonstrate that these effects that PMP 20 and PMP 21 have on the production of IL-6, IL-8, and MCP-1 were operative not only in macrovascular endothelial cells but also in microvascular endothelial cells. It should be noted that HUVECs seem to be more responsive to PMP 20 and PMP 21 than do HSMECs. However, whether this is due to differences between large- and small-vessel endothelia, in general, or whether this reflects differences in the responsiveness of the tissue from which the cells were isolated cannot be answered conclusively on the basis of our data. That LPS contamination of the recombinant PMPs is a possible reason for the observed effects could be ruled out, because of the fact that, when boiled for 5 min before being added to endothelial cells, PMP 20 and PMP 21 lost their ability

Table 4. Effect of polymorphic membrane protein (PMP) 20 and PMP 21, on interleukin (IL)-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1), in human skin microvascular endothelial cells (HSMECs).

Treatment	IL-6	IL-8	MCP-1
Control	53.4 ± 4.3	203.7 ± 23.0	406.7 ± 43.9
PMP 20	132.8 ± 6.3 ^a	439.3 ± 11.2 ^b	920.3 ± 45.0 ^b
PMP 21	99.0 ± 8.1 ^b	394.4 ± 41.3 ^b	888.5 ± 39.7 ^b

NOTE. Data are mean ± SD pg/10⁴ cells/24 h of 3 independent determinations, after incubation of HSMECs, with or without respective PMP (100 ng/mL), for 24 h.

^a *P* < .001, compared with control.

^b *P* < .01, compared with control.

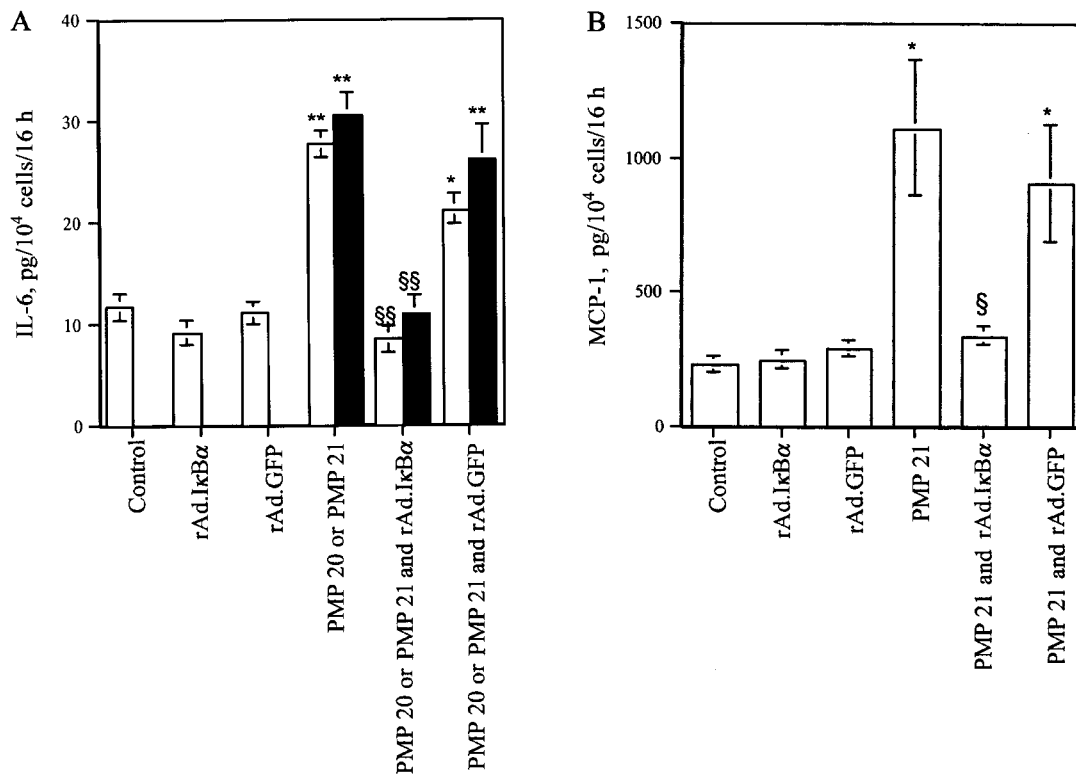


Figure 2. Inhibitory effect of adenovirus construct for overexpression of IκBα (rAd.IκBα) on polymorphic membrane protein (PMP)-mediated activation of interleukin (IL)-6 (A) and monocyte chemoattractant protein-1 (MCP-1) (B). Either untransfected human umbilical vein endothelial cells (HUVECs) and HUVECs transfected with adenovirus construct for overexpression of IκBα (rAd.IκBα) or a control virus (rAd.GFP) were incubated in presence or absence of 200 ng/mL of either PMP 20 (open bars) or PMP 21 (solid bars), for 16 h. Experiments were performed 3 times; a representative experiment is shown. Conditioned media of incubated cells were collected, and IL-6 and MCP-1 were quantified. Data are mean ± SD of 3 independent determinations. **P* < .01, ***P* < .001, compared with control; [§]*P* < .01, compared with PMP 21-treated untransfected or rAd.GFP-transfected HUVECs; ^{§§}*P* < .001, compared with PMP 20- or PMP 21-treated untransfected or rAd.GFP-transfected HUVECs.

to increase production of the cytokines. Last, we were able to demonstrate that PMP 20 and PMP 21 induce their effects on endothelial cell cytokine production by activation of the NF-κB pathway, a major pathway of inflammatory activation. In activated cells, translocation of NF-κB to the nucleus leads to the increased transcription of several markers of inflammation, including IL-6 and MCP-1 [9, 10, 19, 37]. Here we present evidence that, in endothelial cells transfected with an IκBα-adenovirus and thus overexpressing IκBα (an inhibitor of NF-κB translocation to the nucleus [19]), production of IL-6 and MCP-1 was not affected by either PMP 20 or PMP 21, whereas endothelial cells transfected with a control adenovirus responded to treatment with these PMPs with an increase in cytokine production similar to that seen in untransfected endothelial cells. As mentioned above, it is not known whether PMP 20 and PMP 21 interact with the same or different receptors on endothelial cells. Thus, differences in the pathway of endothelial cell activation by PMP 20 and PMP 21 might exist upstream from NF-κB, although our data showing that the effects of the 2 PMPs on endothelial cells are neither additive

nor synergistic lead us to speculate that they act through the same intracellular pathway.

In summary, our study has identified that specific chlamydial proteins, namely particular members of the PMP family of *C. pneumoniae*, are mediators of inflammatory activation of human endothelial cells in vitro via the NF-κB pathway. Thus, one could speculate that this effect of PMP 20 and PMP 21, in addition to the effect of chlamydial heat shock protein, could contribute to NF-κB activation in endothelial cells induced by live *C. pneumoniae* [7, 9–12, 21–24]. If such a proinflammatory mechanism is also operative in vivo, *C. pneumoniae* could, by interactions of its PMPs with the endothelium, contribute to the process of vascular injury during the development and progression of atherosclerotic lesions.

References

1. Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? *Lancet* 1997; 350:430–6.
2. Saikku P. Epidemiology of *Chlamydia pneumoniae* in atherosclerosis. *Am Heart J* 1999; 138:S500–3.

3. Davidson M, Kuo CC, Middaugh JP, et al. Confirmed previous infection with *Chlamydia pneumoniae* (TWAR) and its presence in early coronary atherosclerosis. *Circulation* **1998**; 98:628–33.
4. Sander D, Winbeck K, Klingelhofer J, Etgen T, Conrad B. Enhanced progression of early carotid atherosclerosis is related to *Chlamydia pneumoniae* (Taiwan acute respiratory) seropositivity. *Circulation* **2001**; 103:1390–5.
5. Elkind MS, Lin IE, Grayston JT, Sacco RL. *Chlamydia pneumoniae* and the risk of first ischemic stroke: The Northern Manhattan Stroke Study. *Stroke* **2000**; 31:1521–5.
6. Kuo CC, Grayston JT, Campbell LA, Goo YA, Wissler RW, Benditt EP. *Chlamydia pneumoniae* (TWAR) in coronary arteries of young adults (15–34 years old). *Proc Natl Acad Sci USA* **1995**; 92:6911–4.
7. Ngeh J, Anand V, Gupta S. *Chlamydia pneumoniae* and atherosclerosis—what we know and what we don't. *Clin Microbiol Infect* **2002**; 8:2–13.
8. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* **1993**; 362:801–9.
9. Dechend R, Maass M, Gieffers J, et al. *Chlamydia pneumoniae* infection of vascular smooth muscle and endothelial cells activates NF-kappaB and induces tissue factor and PAI-1 expression: a potential link to accelerated arteriosclerosis. *Circulation* **1999**; 100:1369–73.
10. Molestina RE, Miller RD, Lentsch AB, Ramirez JA, Summersgill JT. Requirement for NF-kappaB in transcriptional activation of monocyte chemotactic protein 1 by *Chlamydia pneumoniae* in human endothelial cells. *Infect Immun* **2000**; 68:4282–8.
11. Kol A, Bourcier T, Lichtman AH, Libby P. Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. *J Clin Invest* **1999**; 103:571–7.
12. Krull M, Klucken AC, Wuppermann FN, et al. Signal transduction pathways activated in endothelial cells following infection with *Chlamydia pneumoniae*. *J Immunol* **1999**; 162:4834–41.
13. Netea MG, Selzman CH, Kullberg BJ, et al. Acellular components of *Chlamydia pneumoniae* stimulate cytokine production in human blood mononuclear cells. *Eur J Immunol* **2000**; 30:541–9.
14. Pedersen AS, Christiansen G, Birkelund S. Differential expression of Pmp10 in cell culture infected with *Chlamydia pneumoniae* CWL029. *FEMS Microbiol Lett* **2001**; 203:153–9.
15. Christiansen G, Pedersen AS, Hjerno K, Vandahl B, Birkelund S. Potential relevance of *Chlamydia pneumoniae* surface proteins to an effective vaccine. *J Infect Dis* **2000**; 181(Suppl 3):S528–37.
16. Shirai M, Hirakawa H, Ouchi K, et al. Comparison of outer membrane protein genes omp and pmp in the whole genome sequences of *Chlamydia pneumoniae* isolates from Japan and the United States. *J Infect Dis* **2000**; 181(Suppl 3):S524–7.
17. Wojta J, Zoellner H, Gallicchio M, Hamilton JA, McGrath K. Gamma-interferon counteracts interleukin-1 alpha stimulated expression of urokinase-type plasminogen activator in human endothelial cells in vitro. *Biochem Biophys Res Commun* **1992**; 188:463–9.
18. Chirgwin K, Roblin PM, Hammerschlag MR. In vitro susceptibilities of *Chlamydia pneumoniae* (*Chlamydia* sp. strain TWAR). *Antimicrob Agents Chemother* **1989**; 33:1634–5.
19. Wrighton CJ, Hofer-Warbinek R, Moll T, Eytner R, Bach FH, de Martin R. Inhibition of endothelial cell activation by adenovirus-mediated expression of I kappa B alpha, an inhibitor of the transcription factor NF-kappa B. *J Exp Med* **1996**; 183:1013–22.
20. de Martin R, Raidl M, Hofer E, Binder BR. Adenovirus-mediated expression of green fluorescent protein. *Gene Ther* **1997**; 4:493–5.
21. Bianchi A, Dosquet C, Henry S, Couderc MC, Ferchal F, Scieux C. *Chlamydia trachomatis* growth stimulates interleukin 8 production by human monocytic U-937 cells. *Infect Immun* **1997**; 65:2434–6.
22. Fryer RH, Schwobe EP, Woods ML and Rodgers GM. *Chlamydia* species infect human vascular endothelial cells and induce procoagulant activity. *J Investig Med* **1997**; 45:168–74.
23. Godzik KL, O'Brien ER, Wang SK, Kuo CC. In vitro susceptibility of human vascular wall cells to infection with *Chlamydia pneumoniae*. *J Clin Microbiol* **1995**; 33:2411–4.
24. Summersgill JT, Molestina RE, Miller RD, Ramirez JA. Interactions of *Chlamydia pneumoniae* with human endothelial cells. *J Infect Dis* **2000**; 181(Suppl 3):S479–82.
25. Bulut Y, Faure E, Thomas L, et al. Chlamydial heat shock protein 60 activates macrophages and endothelial cells through Toll-like receptor 4 and MD2 in a MyD88-dependent pathway. *J Immunol* **2002**; 168:1435–40.
26. Ingalls RR, Rice PA, Qureshi N, Takayama K, Lin JS, Golenbock DT. The inflammatory cytokine response to *Chlamydia trachomatis* infection is endotoxin mediated. *Infect Immun* **1995**; 63:3125–30.
27. Kalayoglu MV, Byrne GL. A *Chlamydia pneumoniae* component that induces macrophage foam cell formation is chlamydial lipopolysaccharide. *Infect Immun* **1998**; 66:5067–72.
28. Christiansen G, Boesen T, Hjerno K, et al. Molecular biology of *Chlamydia pneumoniae* surface proteins and their role in immunopathogenicity. *Am Heart J* **1999**; 138:S491–5.
29. Grimwood J, Olinger L, Stephens RS. Expression of *Chlamydia pneumoniae* polymorphic membrane protein family genes. *Infect Immun* **2001**; 69:2383–9.
30. Wizel B, Starcher BC, Samten B, et al. Multiple *Chlamydia pneumoniae* antigens prime CD8(+) Tc1 responses that inhibit intracellular growth of this vacuolar pathogen. *J Immunol* **2002**; 169:2524–35.
31. Kukielka GL, Youker KA, Hawkins HK, et al. Regulation of ICAM-1 and IL-6 in myocardial ischemia: effect of reperfusion. *Ann NY Acad Sci* **1994**; 723:258–70.
32. Seino Y, Ikeda U, Ikeda M, et al. Interleukin 6 gene transcripts are expressed in human atherosclerotic lesions. *Cytokine* **1994**; 6:87–91.
33. Yazdani S, Simon AD, Vidhun R, Gulotta C, Schwartz A, Rabbani LE. Inflammatory profile in unstable angina versus stable angina in patients undergoing percutaneous interventions. *Am Heart J* **1998**; 136:357–61.
34. Seino Y, Ikeda U, Takahashi M, et al. Expression of monocyte chemoattractant protein-1 in vascular tissue. *Cytokine* **1995**; 7:575–9.
35. Gu L, Okada Y, Clinton SK, et al. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor—deficient mice. *Mol Cell* **1998**; 2:275–81.
36. Gosling J, Slaymaker S, Gu L, et al. MCP-1 deficiency reduces susceptibility to atherosclerosis in mice that overexpress human apolipoprotein B. *J Clin Invest* **1999**; 103:773–8.
37. Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. *Cell* **2002**; 109(Suppl):S81–96.