Atherosclerosis involves genetic and environmental influences. In addition to gradual atherosclerotic progression, plaque erosion and rupture may occur with mural thrombosis, sudden luminal narrowing, or abrupt vessel closure. The clinical consequence is the acute coronary syndrome (ACS). Recent data from epidemiological, histopathological, and intervention studies suggest a role of *C. pneumoniae* infection in the pathogenesis of ACS. However, the molecular mechanisms are poorly understood. Macrophages may be recruited to the plaque causing weakening of the fibrous cap and subsequent plaque rupture.5 Thrombogenicity was accompanied by sustained activation of NF-κB and interleukin-6 expression. This cellular response is accompanied by activation of NF-κB. Our results demonstrate how *C. pneumoniae* infection may initiate acute coronary syndromes. (Circulation. 1999;100:1369-1373.)

**Key Words:** plasminogen activator inhibitor 1 ■ tissue factor ■ interleukins ■ atherosclerosis ■ *C. pneumoniae*

*Chlamydia pneumoniae* Infection of Vascular Smooth Muscle and Endothelial Cells Activates NF-κB and Induces Tissue Factor and PAI-1 Expression

A Potential Link to Accelerated Atherosclerosis

Ralf Dechend, MD; M. Maass, MD; J. Gieffers, MD; R. Dietz, MD; C. Scheidereit, PhD; A. Leutz, PhD; D.C. Gulba MD, MSc

**Background**—Recent reports link *C. pneumoniae* infection of arteriosclerotic lesions to the precipitation of acute coronary syndromes, which also feature tissue factor and plasminogen activator inhibitor 1 (PAI-1) overexpression. We investigated whether or not *C. pneumoniae* can induce thrombogenicity by upregulation of procoagulant proteins. **Methods and Results**—Human vascular endothelial and smooth muscle cells were infected with a strain of *C. pneumoniae* isolated from an arteriosclerotic coronary artery. Tissue factor, PAI-1, and interleukin-6 expression was increased in infected cells. Concomitantly, NF-κB was activated and IκBα degraded. p50/p65 heterodimers were identified as the components responsible for the NF-κB activity. **Conclusions**—These data provide evidence that *C. pneumoniae* infection can induce procoagulant protein and proinflammatory cytokine expression. This cellular response is accompanied by activation of NF-κB. Our results demonstrate how *C. pneumoniae* infection may initiate acute coronary syndromes.

**Materials and Methods**

*C. pneumoniae* infection was established in primary cultures of human VSMCs (Clonetics, San Diego, Calif) and in the immortalized human venous endothelial cell line ECV-304 (ATCC CRL 1998). Stock suspensions were prepared from the vascular *C. pneumoniae* strain CV-4, isolated from an arteriosclerotic coronary artery as described earlier. *C. pneumoniae* growth was monitored 72 hours after infection using a fluorescein isothiocyanate-conjugated *C. pneumoniae*-specific monoclonal antibody (DAKO, Copenhagen, Denmark). Cells were also continuously checked for detachment from plates and cell lysis. Cells were harvested at different time points for electrophoretic mobility shift assay (EMSA) and lysed in whole cell lysate buffer. Labeling and binding reaction were performed as described.8 The DNA probe contained the κB site from the MHC-enhancer (H2K) or the H2B octamer binding site. EMSAs were repeated 3 times and representative gels are shown. Polyclonal antiserum to NF-κB proteins p50, p65, c-Rel, and RelB were purchased from Santa Cruz Biotechnology Inc, Calif. For immunoblotting, whole cell lysates were prepared, quantified by standard Bradford assay, and immunoblotting was performed as described.9 TF and PAI-1 were obtained from Loxo (GmbH, Dossenheim, Germany); IL-6 and tubulin from Genzyme (Framingham, Mass). Protein concentrations of the cellular supernatants were quantified for ELISA using the Bradford method. IL-6 (Quantikine Immunoassay, R&D Systems, Minneapolis Minn) and PAI-1 (American
IL-6 expression. Moreover, we demonstrated that *C. pneumoniae* infection resulted in NF-κB activation. NF-κB remained upregulated for at least 72 hours and was accompanied by IkB degradation. Although *C. pneumoniae* belong to the eubacteria, they have no close relatives among the known bacterial genera and a direct activation of NF-κB by *C. pneumoniae* infection has not been shown thus far.

Plaque rupture and increased thrombogenicity are prime mechanisms of ACS.2,6 Because chronic *C. pneumoniae* infection is linked to the precipitation of ACS,3,4 we examined whether infections with a *C. pneumoniae* strain isolated from such a coronary plaque would increase the expression of prothrombotic proteins in vascular cells. We found induced and sustained expression of functionally active TF and PAI-1. Our results agree with reports from *Rickettsia rickettsii*-infected ECs overexpressing TF, PAI-1, IL-1, IL-6, and IL-8.16 Furthermore, infection of ECs with different *Chlamydia* strains also induce TF overexpression.17 Thus, the alteration of the epi/pericellular hemostatic protein expression observed here is not necessarily specific for the vascular *C. pneumoniae* strain that we used.

In addition to an increased procoagulation protein expression, functional cytokines are expressed in human atherosclerotic lesions.1 IL-6 is an important mediator of inflammation in cardiovascular tissue.18 IL-6 is highly expressed in atherosclerotic lesions,19 implicated in plaque instability,20 and in the pathogenesis of acute myocardial infarction.18 *C. pneumoniae* infection induced sustained cellular overexpression and secretion of IL-6. Recruitment of mesenchymal and immunocompetent cells, proliferation, and migration of VSMCs are the consequences of cytokine overexpression in the atherosclerotic plaque.21 This state of affairs further perturbs the anticoagulant activities. The functional cooperation between products of the coagulation cascade and cytokine-mediated inflammatory response has been shown to transform a stable plaque into an unstable plaque.21 In this context, colocalization of human heat shock protein 60 (hsp) and chlamydial hsp 60 in macrophages was demonstrated. Both hsp forms induced proinflammatory cytokines such as TNF-α and metalloproteinases.5

TF and IL-6 are transcriptionally regulated by NF-κB.11,12 Furthermore, data have been presented that PAI-1 protein expression is also under the control of NF-κB.13 However, these data are less compelling. In addition, inhibition of TNF-α-induced PAI-1, TF, and IL-6 activation was achieved when NF-κB activity was inhibited with the antioxidant pyrrolidine dithiocarbamate.14 Therefore, current knowledge suggests a common link between *C. pneumoniae* infection, TF, PAI-1, IL-6 expression, and NF-κB. We therefore investigated whether NF-κB activity is induced in response to *C. pneumoniae* infection. We showed that after infection, p50/p65 heterodimer activation and expression of procoagulant and proinflammatory proteins.

**Discussion**

The important findings in this study were that *C. pneumoniae* infection of human EC and VSMC resulted in TF, PAI-1, and IL-6 overexpression. Moreover, we demonstrated that *C. pneumoniae* infection resulted in NF-κB activation. NF-κB remained upregulated for at least 72 hours and was accompanied by IkB degradation. Although *C. pneumoniae* belong to the eubacteria, they have no close relatives among the known bacterial genera and a direct activation of NF-κB by *C. pneumoniae* infection has not been shown thus far.

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Figure 1. A. C. pneumoniae infection of EC and HASMC. Immunofluorescence staining reveals large intracellular inclusion bodies containing actively replicating C. pneumoniae, 72 hours after infection. B. Effects of C. pneumoniae infection on cellular expression of TF, PAI-1, and IL-6. Immunoblot analysis of cellular extracts prepared from ECs (left) and HASMCs (right) at different time points after C pneumoniae or mock infection. Tubulin expression was determined as a control. Densitometry quantification values (NIH Image Pro-gram Version 1.61) are given in arbitrary units (AU) below each time point. C, Open and solid bars represent TF activity in EC extracts of mock-infected and infected cells, respectively (left). ■ and ○ represent supernatant PAI-1 and IL-6 levels in infected and mock-infected endothelial cells, respectively, determined by ELISA (center and right). Data and standard deviation represent mean of triplicates.
Figure 2. A, Top, kinetics of NF-κB activation in cellular extracts of ECs (left) and HASMCs (right) analyzed by EMSA. Arrows 1, 2, and 3 represent p50/p65, p50/p50, and unspecific complexes, respectively. Bottom, OCT-1 DNA binding activity on the H2B site, used as a control; oct-1 indicates octamer-binding transcription factor 1. B, Subunit composition of C. pneumoniae-induced NF-κB DNA binding activity. The antibody-supershifted complexes (AS) are indicated. Competition with unlabeled NF-κB and AP-1 oligonucleotide is demonstrated. C, IκBα protein level after infection with C. pneumoniae or mock infection analyzed by immunoblotting.
data showing that R. rickettsii-induced TF overexpression was controlled by NF-κB. R. rickettsii are not phylogenetically related; however, they share characteristics with C. pneumoniae, including obligate intracellular lifestyle, Gram-negative cell wall composition, and the ability to infect C. pneumoniae, including obligate intracellular lifestyle, calmodinically related; however, they share characteristics with C. pneumoniae infection. Interestingly, R. rickettsii has been shown to mediate NF-κB induced transcriptional activation, resulting in the inhibition of EC apoptosis. A similar mechanism in C. pneumoniae infection might provide an explanation for chlamydial persistence in the endothelium and a continuous inflammatory stimulus within the vascular wall. We are the first to show that NF-κB is activated in response to C. pneumoniae infection.

Our data suggest that chronic C. pneumoniae infection in the cellular components of plaques can result in increased cellular and epi/pericellular procoagulant protein expression and increased chemoattractant activity through activated NF-κB. Both effects could enhance the vulnerability of arteriosclerotic plaques. Our results add to the understanding of the role of C. pneumoniae bacteria in the mechanism of accelerated arteriosclerotic disease.

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References
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