munodeficiency virus incidence [letter]. J Infect Dis **2000**;181:1865–6 (in this issue).
3. Cold CJ, Young H. To promote circumcision as a preventive measure against human immunodeficiency virus transmission is irresponsible [letter]. J Infect Dis **2000**;181:1864–5 (in this issue).
4. Moench TR. Neglected opportunities [letter]. J Infect Dis **2000**;181:804–5.
5. Moses S, Bailey RC, Ronald AR. Male circumcision: assessment of health benefits and risks. Sex Transm Infect **1998**;74:368–73.
6. Halperin DT, Bailey RC. Male circumcision and HIV infection: 10 years and counting. Lancet **1999**;354:1813–5.
7. Rakwar JP, Lavreys L, Thompson ML, et al. Cofactors for acquisition of HIV-1 among heterosexual men: prospective cohort study of trucking company workers in Kenya. AIDS **1999**;13:607–14.

Reprints or correspondence: Dr. Joan Kreiss, Harborview Medical Center, Center for AIDS and STDs, 325 Ninth Ave., Box 359931, Seattle, WA 98104.

The Journal of Infectious Diseases 2000;181:1866–8

0022-1899/2000/18105-0062$02.00

Oxidation of Low-Density Lipoproteins by Acellular Components of *Chlamydia pneumoniae*

To the Editor—We read with great interest about the work by Kalayoglu et al. [1] on the capacity of *Chlamydia pneumoniae* to promote oxidation of low-density lipoproteins (LDLs) [1]. Regarding the association between infection with *C. pneumoniae* and atherosclerosis [2], this study provides important new insight on pathogenetic mechanisms through which *C. pneumoniae* may induce atherogenesis. Although the synergism between hyperlipoproteinemia and *C. pneumoniae* infection in the induction of atherosclerosis in experimental models has indirectly suggested a connection between these two atherogenic risk factors [3], the study by Kalayoglu et al. [1] is the first to show a link between the oxidative LDL modification and the infectious hypothesis of atherogenesis.

In a recent study, we found that an acellular component(s) of sonicated *C. pneumoniae,* in as little as 10^3–10^4 infection-forming units (ifu)/mL, induces the proinflammatory cytokines tumor necrosis factor (TNF)–α and interleukin (IL)–1β and the chemokines IL-8, monocyte chemoattractant protein–1, and macrophage inflammatory protein–1 [4]. This component did not appear to be a lipopolysaccharide (LPS). Nevertheless, because proinflammatory cytokines may affect oxidative processes, we subsequently investigated whether stimulation of human peripheral blood mononuclear cells (PBMC) by low numbers of sonicated chlamydia can induce oxidation of freshly isolated human LDL. The hypothesis of Kalayoglu et al. [1] that oxidation of LDL by *C. pneumoniae* may be mediated through intermediary production of proinflammatory cytokines, such as TNF-α and IL-1β, is indirectly supported by the relatively long interval (18–40 h) necessary for chlamydia to induce significant LDL oxidation, suggesting mediation through endogenous factors. When 10^4 ifu/mL of sonicated *C. pneumoniae* were added to PBMC (5×10^5/mL) and incubated for 24 h in the presence of freshly isolated endotoxin-free LDL (2 mmol/L), there was a significant increase in the oxidation of LDL as determined by measuring concentrations of thiobarbituric acid–reactive substance (figure 1). Although the level of oxidation was lower than that observed by Kalayoglu et al. [1], likely owing to the fewer chlamydia used, our observation is important because it suggests that low amounts of *C. pneumoniae* can induce LDL oxidation. Minimally oxidized LDL has been suggested to have an important pathogenetic role in atherogenesis [5]. Although a quantitative analysis of the number of chlamydial particles in atherosclerotic plaques has not been done, it is unlikely that 10^5–10^6 ifu of organisms (as used by Kalayoglu et al. [1]) are present in 1 plaque.

An interesting observation by Kalayoglu et al. [1] was that oxidation of LDL by *C. pneumoniae* is independent of LPS. This is consistent with our own data. We too found that induction of cytokine synthesis by *C. pneumoniae* is largely independent of chlamydial LPS [4]. Moreover, sonicated and live chlamydia were equally potent in our study, suggesting that an acellular component(s), and not live bacteria, possesses the activity [4]. This may be particularly important because recent data suggest that, at late stages only, chlamydial antigens, and not live bacteria, persist and maintain the chronic inflammation in atherosclerosis [6]. In fact, induction of interferon-γ production suggested that the non-LPS component of *C. pneumoniae* that is responsible for the induction of cytokines has the properties of a superantigen [4]. It may be that the chlamydial hsp60 reported by Kalayoglu et al. [1] as responsible

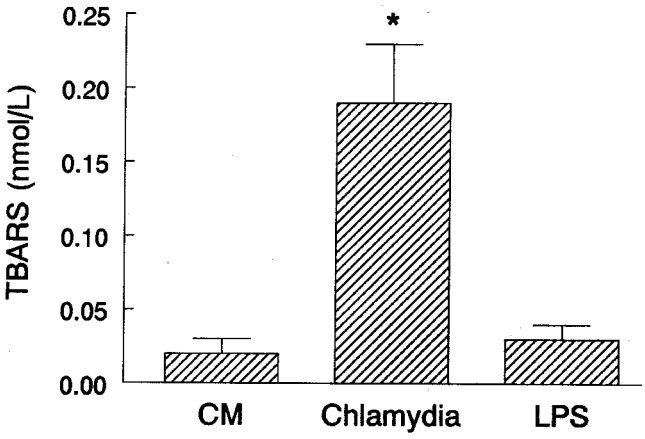

Figure 1. Human peripheral blood mononuclear cells, from 4 volunteers, were incubated for 24 h at 37°C with endotoxin-free low-density lipoprotein (LDL) with control medium (CM), sonicated *C. pneumoniae* (10^4 ifu/mL), and lipopolysaccharide (LPS; 10 ng/mL). LDL oxidation was assessed by measuring concentrations of thiobarbituric acid–reactive substance (TBARS). *$P < .01$, Mann-Whitney *U* test (mean + SD).

for LDL oxidation also may be involved in cytokine and chemokine induction in human PBMC, as suggested by studies of mouse macrophages [7].

Kalayoglu et al. [1] were surprised about the lack of involvement of superoxide in the mediation of LDL oxidation by *C. pneumoniae*. However, this may be less surprising if one considers that monocytes are low producers of oxygen radicals but major sources of nitrogen intermediates, which also are induced by proinflammatory cytokines [8]. It is tempting to speculate that this alternative mechanism may contribute to LDL oxidation by chlamydial antigens.

The observation that chlamydial antigens are able to induce LDL oxidation links the pathogenetic mechanisms through which hyperlipoproteinemia and *C. pneumoniae* infection contribute to the process of atherosclerosis and represents an important step forward in understanding the pathogenesis of this important disease.

Mihai G. Netea,[1] **Charles A. Dinarello,**[2] **Bart Jan Kullberg,**[1] **Trees Jansen,**[1] **Liesbeth Jacobs,**[1] **Anton F. H. Stalenhoef,**[1] **and Jos W. M.Van der Meer**[1]
[1]*Department of Medicine, University Hospital Nijmegen, The Netherlands;* [2]*Department of Medicine, University of Colorado Health Sciences Center, Denver*

References

1. Kalayoglu MV, Hoerneman B, LaVerda D, Morrison SG, Morrison RP, Byrne GI. Cellular oxidation of low-density lipoprotein by *Chlamydia pneumoniae.* J Infect Dis **1999**;180:780–90.
2. Danesh J, Collins R, Peto R. Chronic infections and coronary artery disease: is there a link? Lancet **1997**;350:430–6.
3. Hu H, Pierce GN, Zhong G. The atherogenic effects of chlamydia are dependent on serum cholesterol and specific to *Chlamydia pneumoniae.* J Clin Invest **1999**;103:747–53.
4. 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.
5. Partharasathy SD, Steinberg D, Witztum JL. The role of oxidized low-density lipoproteins in the pathogenesis of atherosclerosis. Annu Rev Med **1992**; 43:219–25.
6. Meijer A, van der Vliet JA, Roholl PJ, Gielis-Proper SK, de Vries A, Ossewaarde JM. *Chlamydia pneumoniae* in abdominal aortic aneurysms: abundance of membrane components in the absence of heat shock protein 60 and DNA. Arterioscler Thromb Vasc Biol **1999**;19:2680–6.
7. Kol A, Sukhova GK, Lichtman AH, Libby P. Chlamydial heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis factor–alpha and matrix metalloproteinase expression. Circulation **1998**; 98:300–7.
8. Nathan CF, Hibbs JB Jr. Role of nitric oxide synthesis in macrophage antimicrobial activity. Curr Opin Immunol **1991**;3:65–70.

Reprints or correspondence: Dr. Mihai G. Netea, Dept. of Medicine (541), University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands (M.Netea@aig.azn.nl).

The Journal of Infectious Diseases 2000;181:1868–9

0022-1899/2000/18105-0063$02.00

Reply

To the Editor—We thank Netea et al. [1] for interest in our work and for insight into how *Chlamydia pneumoniae* induce mononuclear phagocyte oxidation of low-density lipoprotein (LDL). We reported that *C. pneumoniae* induced macrophage oxidation of LDL and identified chsp60 as one chlamydial protein involved in activation of lipoprotein oxidation [2]. Data provided by Netea et al. [1] support this notion and suggest that lipoprotein oxidation involves indirect mechanisms, perhaps following induction of cytokines by macrophages [3]. One hypothesis is that *C. pneumoniae* hsp60 induces macrophage LDL oxidation by triggering cytokine release. Indeed, Kol and colleagues [4, 5] have demonstrated the capacity of chsp60 to induce inflammatory cytokine release by macrophages and by other cell types present in the atheroma. They also postulate that if a cytokine cascade is involved, then these inflammatory mediators may induce the release of macrophage nitrogen intermediates that oxidize lipoproteins. These are intriguing hypotheses that can be investigated directly. For example, *C. pneumoniae*–induced LDL oxidation can be measured in the presence or absence of neutralizing antibodies specific to the cytokines implicated by Netea et al. [1], such as tumor necrosis factor–α, interleukin (IL)–1β, and the chemokines IL-8, monocyte chemoattractant protein–1, and macrophage inflammatory protein–1.

We agree with Netea et al. [1] that the long interval (18–40 h) required to detect LDL oxidation suggests an indirect mechanism of lipoprotein modification, such as cytokine release. Indeed, *C. pneumoniae*–induced LDL oxidation by macrophages is a relatively late event [2], when compared with *C. pneumoniae*–induced LDL uptake and macrophage foam cell formation [6–8]. However, it also is possible that LDL oxidation begins immediately after macrophage exposure to *C. pneumoniae* but that oxidation byproducts accumulate to detectable levels only after a long interval. Direct and highly sensitive assays for lipoprotein oxidation are available [9] and may be used to determine the interval between macrophage exposure to *C. pneumoniae* and LDL oxidation.

Murat V. Kalayoglu, Michael Myint, and Gerald I. Byrne
Department of Medical Microbiology and Immunology, University of Wisconsin Medical School, Madison

References

1. Netea MG, Dinarello CA, Kullberg BJ, et al. Oxidation of low-density lipoproteins by acellular components of *Chlamydia pneumoniae* [letter]. J Infect Dis **2000**;181:1868–9 (in this issue).
2. Kalayoglu MV, Hoerneman B, LaVerda D, Morrison SG, Morrison RP, Byrne GI. Cellular oxidation of low-density lipoprotein by *Chlamydia pneumoniae.* J Infect Dis **1999**;180:780–90.
3. Netea MG, Selzman CH, Kullberg BJ, et al. Acellular components of *Chla-*