Interactions of *Chlamydia pneumoniae* with Human Endothelial Cells

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In order to fulfill the “biological plausibility” criterion of a role for infection with *Chlamydia pneumoniae* in the pathogenesis of human atherosclerosis, detailed studies on the interaction of this organism with the cell types involved are necessary. This article summarizes the current knowledge on the interaction of *C. pneumoniae* with human endothelial cells. In vitro, *C. pneumoniae* can infect human endothelial cells and induce the expression of many molecules that are important mediators of atherogenesis including cytokines, adhesion molecules, chemokines, and molecules with procoagulant activity.

Infection with *Chlamydia pneumoniae*, a common respiratory pathogen, has been implicated as an additional risk factor for development of atherosclerosis and coronary artery disease [1]. Three principle cell types are involved in the atherogenic process within the developing atheroma. These include the endothelial cell, the vascular smooth muscle cell, and the monocyte/macrophage. In order to fulfill the “biological plausibility” criterion of a direct role for *C. pneumoniae* within the atherogenic process, detailed studies on the interaction of this organism with the cell types involved are necessary. Here we summarize the current knowledge on the interaction of *C. pneumoniae* with human endothelial cells. Modifications of monocyte/macrophage functions by this organism are covered elsewhere in this supplement.

The endothelial layer has a number of characteristics necessary for the maintenance of normal vessel function. It provides a nonthrombogenic surface, acts as a permeability barrier, functions to maintain normal vascular tone, and provides a nonadherent surface for circulating leukocytes. Any alteration of these homeostatic functions can induce endothelial dysfunction, a precipitating event in atherosclerosis [2]. Since the process of atherogenesis is believed to be an “inflammatory event in response to injury,” activation of the endothelium can lead to generation of proinflammatory molecules necessary to initiate lesion formation [2].

Demonstration that *C. pneumoniae* is capable of replication within human endothelial cells is the first piece of data needed, and this has been demonstrated by several investigators. Kaukoranta-Tolvanen et al. [3] examined the ability of *C. pneumoniae* isolates to replicate in human umbilical vein endothelial cells (HUVEC) and an immortalized endothelial cell line (EA.hy 926). They reported that 3 *C. pneumoniae* isolates (Kajaani 6, Helsinki 12, and TW-183) exhibited similar replication patterns within both cell lines tested and that inclusion morphology appeared similar to those seen in control cells (HEp-2, HL, and McCoy). Furthermore, HL and EA.hy 926 cells could support a chronic infection with *C. pneumoniae* Kajaani 6 for up to 2 months. These observations were confirmed by 2 investigators who examined *C. pneumoniae* growth in endothelial cells and in smooth muscle cells and macrophages.

Godzik et al. [4] examined the replication of *C. pneumoniae* isolate AR-39 and reported the resulting inclusions in human arterial endothelial cells (HMEC-1) were typical in appearance when compared with those in HL cells. However, HMEC-1 cells were less permissive for *C. pneumoniae* growth than the control HL cells, reported as a “susceptibility index” of 0.32, with HL cells being assigned an index of 1.0. Gaydos et al. [5] examined the ability of endothelial cells of various origins to support the replication of 13 isolates of *C. pneumoniae*. Eight of 10 isolates were capable of growth in human aortic endothelial cells (HAEC), although there were fewer inclusions and inclusions were smaller than in control HEP-2 cells. Likewise, HUVEC supported the growth of 9 of 10 isolates tested, and all 11 isolates tested in human pulmonary artery endothelial cells showed appreciable replication.

Since one hallmark of atherogenesis in humans is an activated endothelium exhibiting an up-regulation of chemokines and adhesion molecules [2], several investigators have examined the ability of *C. pneumoniae* to stimulate this response in vitro. Kaukoranta-Tolvanen et al. [6] used flow cytometry to demonstrate the ability of *C. pneumoniae* Kajaani 6 to induce the expression of adhesion molecules on the surface of HUVEC. *C. pneumoniae* stimulated expression of endothelial leukocyte adhesion molecule 1, intercellular adhesion molecule (ICAM) 1, and vascular cell adhesion molecule (VCAM) 1 on the surface of HUVEC in a dose-dependent fashion. Expression of these molecules, however, was less than that seen with *Salmonella minnesota* Re lipopolysaccharide, which was used as a positive control. In addition, a preparation of viable *C. pneumoniae* elementary bodies (EBs) was a slightly better inducer of expression of these molecules than a heat-inactivated preparation.
Molestina et al. [7] examined the ability of *C. pneumoniae* to stimulate expression of soluble ICAM-1 from HUVEC and also measured the production of monocyte chemotactic protein (MCP)-1 and interleukin (IL)-8, proteins that serve as chemotactic cytokines with specificity for monocytes and neutrophils, respectively. With 3 *C. pneumoniae* respiratory isolates (BAL-16, T2634, and TW-183) and a single isolate from a human coronary atheroma (A-03) [8], they found a time- and dose-dependent induction of these molecules from HUVEC. Heat-inactivated EB preparations also lost a significant ability to induce these molecules, whereas EBs inactivated by exposure to UV irradiation retained partial stimulatory capability. This suggests that a potential heat-labile substance of *C. pneumoniae* was responsible for HUVEC activation. A significant amount of heterogeneity was observed between the isolates examined, with isolates A-03 and BAL-16 being significantly poorer inducers of this response than the other 2 respiratory isolates. However, in a subsequent publication [9], these differences were reported to be a function of the number of passages in cell culture prior to infection of the HUVEC.

The question of whether the stimulation of chemokines and adhesion molecules on the surface of an endothelial monolayer infected with *C. pneumoniae* would result in the chemotaxis and migration of leukocytes through the monolayer was addressed by Molestina et al. [9]. Infection of HUVEC monolayers with *C. pneumoniae* isolates from atherosclerotic tissue (A-03 and PS-32 [10]) and respiratory isolates (BR-393, BAL-16, T2634, and TW-183) stimulated significant transendothelial migration of neutrophils and monocytes. As mentioned above, the observed heterogeneity was addressed by Krull et al. [11]. Western blot analysis showed that infection of HUVEC and HAEC with *C. pneumoniae* strain GiD activated p42/p44 mitogen-activated protein kinase isoforms in these cells. *C. pneumoniae* also increased adhesion molecule expression (E-selectin, ICAM-1, VCAM-1) in HUVEC and HAEC as measured by an increase in specific mRNA and expression by cell surface ELISA. This resulted in a corresponding dose- and time-dependent increase in leukocyte rolling and adhesion and transendothelial migration when examined in HUVEC.

Much attention has been directed to the role that bacterial heat-shock proteins (hsp) may play in the pathogenesis of infectious diseases [12]. As a result, chlamydial hsp60 (Chsp60) was identified as a potential inflammatory mediator in atherogenesis. Kol et al. [13] reported that Chsp60, presumably produced in increased amounts after stress, as well as human hsp60 (Hhsp60), co-localize within macrophages during plaque formation. This group subsequently examined the effects of these proteins on human vascular endothelial cells in vitro [14] (table 1). Purified Chsp60 and Hhsp60 induced E-selectin, ICAM-1, and VCAM-1 expression on endothelial cells, but to a lesser degree and at earlier time points than seen with *Escherichia coli* lipopolysaccharide. Formalinized *C. pneumoniae* EB preparations did not induce the same response in endothelial cells, leading the authors to conclude that the heat-labile Chsp60 may be responsible for adhesion molecule expression. IL-6 was up-regulated in a similar fashion in endothelial cells treated with Chsp60 or Hhsp60. Since genes encoding these adhesion molecules and cytokines are transcriptionally activated by nuclear factor (NF) κB, the authors tested whether Chsp60 and/or Hhsp60 could trigger NF-κB activation of endothelial cells. Indeed, the data showed that both proteins could activate NF-κB in nuclear extracts from treated cells and that this ability was abolished by heat treatment. Thus, purified Chsp60 functions in a fashion similar to live *C. pneumoniae* in its ability to induce cytokine and adhesion molecule expression in endothelial cells.

The relationship of Chsp60 to atherosclerosis was further studied by Mayr et al. [15], who examined the cytotoxicity of antibodies to hsp for endothelial cells. Purified antibodies against Chsp60 and *E. coli* hsp (GroEL) from patients with atherosclerosis were cytotoxic in a dose-dependent fashion for HUVEC as determined by a complement-mediated 51Cr release assay. This effect was seen only in heat-stressed (42°C for 30 min) HUVEC and could be reversed by the addition of recombinant GroEL. Similar effects were seen when the experiments were done with HAEC. These authors concluded that an antibody response to bacterial hsp, particularly Chsp60, may be

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<th>Table 1. Activity of chlamydial heat-shock protein 60 (Chsp60) on endothelial cells.</th>
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C. pneumoniae and Endothelial Cells

Figure 1. Infection of human endothelial cells with C. pneumoniae results in stimulation of wide variety of cytokines, adhesion molecules, chemokines, and proteins with procoagulant activity. VCAM, vascular adhesion molecule; ICAM, intercellular adhesion molecule; ELAM, endothelial leukocyte adhesion molecule; MCP, monocyte chemotactic protein; IL, interleukin; PDGF, platelet-derived growth factor; TF, tissue factor; PAI, plasminogen activator inhibitor.

a source of endothelial injury and thus could play an important role in atherogenesis.

The process of atherosclerosis can culminate in a weakening of the fibrous cap of the plaque, subsequent rupture, and thrombosis, leading to an acute event. Several investigators have examined the ability of C. pneumoniae to interact with endothelial cells and participate in this event by inducing expression of molecules with procoagulant activity. Fryer et al. [16] found that C. pneumoniae–infected HUVEC produced tissue factor (TF) in a dose-dependent fashion following 18 h of infection. Similar results were seen when two serovars of C. trachomatis (L2/434/BU and H) were tested. This increase in TF production correlated with a significant (six-fold) increase in platelet adhesion to C. pneumoniae–infected HUVEC when compared with uninfected controls. More recently, Dechend et al. [17] examined C. pneumoniae–induced TF, plasminogen activator inhibitor 1, and IL-6 expression in an immortalized human endothelial cell line (ECV-304). With a coronary atheroma isolate (CV-4), C. pneumoniae was shown to induce these proteins in a time-dependent and significant manner when compared with mock-infected controls. Further study led to the finding that these molecules were induced via an NF-κB-mediated pathway. Taken together, these two studies [16, 17] suggest that C. pneumoniae infection of human endothelial cells results in the up-regulation of procoagulant activity in these cells and provide additional evidence for the role of infection with this bacterium in the atherogenic process.

One hallmark of atherogenesis in humans is the migration and proliferation of vascular smooth muscle cells (SMC) into the arterial intima [2]. This has been postulated to occur via soluble factors secreted from endothelial cells (i.e., platelet-derived growth factor, epidermal growth factor, and insulin-like growth factor 1) [2]. In a recent study by Coombes and Mahony [18], infection of HUVEC with C. pneumoniae (strain VR-1310) resulted in the production of soluble factor(s) that stimulates [3H]-thymidine incorporation into SMC and increased SMC replication. The kinetics of the secreted factor(s) was dose and time dependent with regard to the MOI, showing more mitogenic activity at 48 h than at 24 h. Experiments with heat-inactivated C. pneumoniae EB preparations showed that live or heat-killed EBs were equally capable of stimulating these factors. Studies are underway to identify the specific endothelial-derived factors.

In summary, recent data support the biological plausibility criterion of a significant role for infection with C. pneumoniae and atherogenesis in humans. The organism infects a critical cell type involved in the atherogenic process, the endothelial cell. Furthermore, after infection, C. pneumoniae can stimulate the production of several molecules deemed key factors in the initiation and/or progression of atherogenesis (figure 1). These
include cytokines, adhesion molecules, chemokines, and molecules with procoagulant activity. Future research should determine which biologic and/or genetic attributes of *C. pneumoniae* are responsible for this ability.

References