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## Is *Chlamydia pneumoniae* infection a risk factor for age related macular degeneration?

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The association between a pathogen and AMD is worth further investigation because a new approach might treat this incurable vision threatening disease

Age related macular degeneration (AMD) is a leading cause of decreased central vision in older people throughout the world. The pathogenesis of AMD is very complex and has still not been determined. In addition to some genetic and environmental factors, several kinds of risk factors have been proposed; sunlight exposure,<sup>1</sup> smoking,<sup>2</sup> and low levels of nutritional components such as antioxidants.<sup>3</sup> Hypertension,<sup>4</sup> hyperlipidaemia,<sup>4</sup> and atherosclerosis,<sup>5,6</sup> which may lead to cardiovascular diseases, are also considered to be risk factors.

Chronic inflammatory events have recently been identified as plausible causes of atherosclerosis. In particular, much interest has been focused on infections by *Chlamydia pneumoniae*, which was previously known as the TWAR strain. This strain is one of the chlamydial species that has been recognised as a causal mediator of respiratory infections such as bronchitis, pneumonia, and upper respiratory tract infections.<sup>7</sup>

*Chlamydia* can multiply in various host cells including macrophages and endothelial cells.<sup>8</sup> *C pneumoniae* is like a parasite and consumes energy that is needed by the host cells and, in the end, destroys them and then infects nearby cells. Thus, the pathogens tend to cause a chronic infection.

The first study showing a positive interaction between systemic vascular disease and infection with *C pneumoniae* was made by Saikku *et al* in 1988.<sup>9</sup> They reported increased immunoglobulin G (IgG) and IgA antibody titres against *C pneumoniae* in male patients with myocardial infarction or chronic coronary heart disease. Since then, attention has been focused on its association with atherosclerotic diseases.<sup>10</sup> A strong piece of evidence indicating a close interaction between *C pneumoniae* infection and vascular systemic diseases was the direct detection of *C pneumoniae*<sup>11,12</sup> or the detection of heat shock proteins of *C*

*pneumoniae* in the plaques of the coronary and carotid arteries.<sup>13</sup> The organisms have been shown to proliferate in vitro in vascular endothelial cells, vascular smooth muscles, and in macrophages.<sup>8</sup> Interestingly, it has been demonstrated that macrophages infected with *C pneumoniae* adhere to endothelial cells and transfer the pathogen to endothelial cells. Endothelial cells infected with *C pneumoniae* show an increase expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), accelerating the trapping of macrophages.<sup>14</sup> In addition, aortic sclerosis has been experimentally produced in rabbits in vivo following infection of *C pneumoniae* through the respiratory organs.<sup>15</sup> Thus, *C pneumoniae* can spread to various organs. A working hypothesis is that *C pneumoniae* interacts with the lipid metabolism through IgG antibody mediated immunoresponse in the vascular tissue which may then lead to atherosclerosis. Additionally, the potential contribution of infectious agents induced by *C pneumoniae* has recently been clarified, and chlamydial lipopolysaccharide (LPS) or infected macrophages may produce inflammatory cytokines such as interleukin 6 (IL-6), tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ), and matrix metalloproteinase (MMP), which impair the endothelial cells, trigger thrombus formation, and promote vascular obstruction.<sup>13,16</sup>

Future studies will be necessary to obtain direct evidence of *C pneumoniae* infection by demonstrating its presence in choroidal neovascular tissue harvested during vitreous surgery

These observations are relevant to AMD because it has been demonstrated that there is an association of the incidence of coronary heart diseases in patients with AMD.<sup>5,17</sup> Recent immunohistochemical investigations have demonstrated that

oxidised lipids and proteins are related to the pathogenesis of AMD.<sup>18,19</sup> Besides, an apolipoprotein E (ApoE) gene polymorphism has been shown in AMD,<sup>20</sup> and an infection by *C pneumoniae* leads to an acceleration in the progression of atherosclerosis in ApoE deficient mice, a hyperlipidaemic animal model.<sup>21</sup> In addition, our colleagues have reported that the serum level of oxidised low density lipoproteins (LDL) in patients with AMD was significantly higher than that in healthy controls, and that genetic polymorphism of paraoxonase, a gene involved in lipid metabolism to prevent LDL oxidation, is implicated in the pathogenesis of AMD.<sup>22</sup>

These findings strongly support the suggestion that atherosclerosis is a risk factor for AMD. Furthermore, the recent consideration of AMD as an inflammatory event was supported by the identification of several inflammation linked proteins in drusens.<sup>23</sup>

We have thus hypothesised that an infection with *C pneumoniae* may be an additional risk factor for AMD. To test this hypothesis, we analysed the specific antibody titres of *C pneumoniae* in the sera of patients with AMD. Informed consent was obtained from all patients after an explanation of the purpose of this study. To ensure uniformity in age distribution, the age of the patients was limited to 60–79 years. There were 27 patients with AMD (aged 71.1 (SD 6.4) years, 19 men and eight women) and 22 age matched controls (aged 69.5 (6.5) years, 12 men and 10 women). All AMD patients had the wet form of AMD which is more common in Japanese patients.

The level of IgA and IgG antibodies to *C pneumoniae* in the serum was determined by a specific enzyme linked immunosorbent assay (ELISA) kit (Hitazyme *C pneumoniae*, Hitachi Chemical, Tokyo, Japan) as previously reported.<sup>24</sup> Briefly, serum samples were reacted with an outer membrane antigen of chlamydia that was purified from the YK41 strain of *C pneumoniae* to form immune complexes with anti-human IgA or IgG antibodies. Then, p-nitrophenyl phosphate was added to the wells, and the absorbance was measured at 405 nm. The level of IgA and IgG to *C pneumoniae* in each sample was expressed as the IgA index and the IgG index. The mean (SD) index for 592 healthy adults has been reported to be 1.27 (0.87) for IgG and 1.20 (0.78) for IgA.<sup>24</sup>

The mean index (SD) of IgG antibody for anti-*C pneumoniae* was 2.08 (0.95) in the AMD group and 1.32 (0.85) in the control group, while that of the IgA antibody was 1.96 (0.80) in the AMD group and 1.39 (0.84) in the control group. Both antibody titres were significantly elevated in the AMD patients ( $p = 0.006$  for IgG;  $p = 0.005$  for IgA, Mann-Whitney test). No significant difference

was found between the men and women for both IgG and IgA.

We used the ELISA method to detect antibodies to the chlamydial outer membrane complex produced in infected monocytes/macrophages. Although the significance of the increased titres of specific IgG and IgA antibodies against *C pneumoniae* is not fully understood, higher IgA and IgG antibody titres may indicate an exposure to greater amounts of *C pneumoniae* and recurrent or chronic infections.

Increased specific titres against *C pneumoniae* in our AMD patients suggest a possible association between AMD and *C pneumoniae* infection. As mentioned, although *C pneumoniae* primarily infects the respiratory organs, *C pneumoniae* organisms are found in the atherosclerotic lesions. Thus, macrophages infected with *C pneumoniae* may enter the circulatory system and spread to various organs. The choroid, especially the region close to the macular area, is often the target of metastatic tumours and infections such as toxoplasmosis and histoplasmosis because the largest vascular supply is around the macular area. Therefore, we suggest that macrophages infected with *C pneumoniae* are trapped in the vascular net in the posterior choroid and inflammatory cytokines such as TNF- $\alpha$  and MMP can be produced. These agents impair vascular architecture and even trigger a rupture of choroidal vessels, which may help in the development of AMD.

Other than AMD, infection with *C pneumoniae* has been proposed as a risk factor for ischaemic optic neuropathy. Two research groups have investigated this association, but different results were reported; one showed significantly higher IgG titres in patients with ischaemic optic neuropathy,<sup>25</sup> and the other reported no significant differences from the controls.<sup>26</sup>

The limitations of our study are that it was a retrospective study of a small sample and combined systemic factors were not matched. However, because AMD might be associated with various systemic vascular disorders, a higher incidence of infection with *C pneumoniae* seen in AMD patients should be noted. Future studies will be necessary to obtain direct evidence of *C pneumoniae*

infection by demonstrating the presence of *C pneumoniae* in choroidal neovascular tissue harvested during vitreous surgery. Our findings do not necessarily justify *C pneumoniae* infection as the primary cause of AMD. However, inflammatory events caused by a focal infection around the macular area with *C pneumoniae* might affect the surrounding atherosclerotic environment as an additional factor. The association between a pathogen and AMD is worth further investigation because a new approach using antibiotics might treat this incurable vision threatening disease.

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