

## *Mycobacterium tuberculosis* Complex in Atherosclerosis

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In recent years, the results of some studies have revealed the possible potential role of several infectious agents in the inflammatory mechanism of atherosclerosis. The detection of specific antibodies against microorganisms such as *Chlamydia pneumoniae* and *cytomegalovirus* as well as antibodies directed to heat shock proteins in the sera of atherosclerotic patients and the presence of genomic material in atheromatous plaques all provide evidence supporting the presumptive role of infectious agents in atherosclerosis. There are some findings that can be accepted as clues for the possible involvement of *Mycobacterium tuberculosis* in atherosclerosis. These consist of the presence of high levels of mycobacterial heat shock protein 65 in atherosclerotic patients, and in animal studies, the detection of atherosclerotic changes in the vascular wall of animals vaccinated with recombinant heat shock protein 65, and *Mycobacterium tuberculosis* containing heat shock protein 65. The probable proatherogenic effect of the specific immune response to BCG-associated heat shock protein was also suggested. The mycobacterium cell wall contains a phospholipid, phosphatidylinositol, which was shown to have a procoagulant effect similar to that of a cytomegalovirus possessing phosphatidylserine, another phospholipid showing a procoagulant effect. These data suggest that *Mycobacterium tuberculosis* may also be involved in the pathogenesis of atherosclerosis.

**Key words:** *M. tuberculosis*, BCG, phospholipid, heat shock protein, atherosclerosis

Coronary artery disease (CAD) is among the major health problems of mankind resulting in death. The main underlying process in CAD is atherosclerosis [1]. Atherosclerosis is a chronic disease which may begin in childhood [2]. Some of the traditional risk factors for atherosclerosis are diabetes, hypertension, gender, age, smoking, obesity, hypercholesterolemia, hyperhomocysteinemia and heredity. Whatever the triggering factor for the development of atherosclerosis, the underlying process is inflammation, which occurs as a

response to injury. C-reactive protein (CRP), fibrinogen, serum amyloid, interleukins, tumour necrosis factor alpha (TNF- $\alpha$ ), and vascular and cellular fibrinogen adhesion molecules are the inflammatory markers that have been found in relation to the process of CAD [3]. In this process, there is an accumulation of lipids within the vessel wall, with mononuclear cell infiltration and smooth muscle cell proliferation leading to the formation of atheromatous plaques [1]. Activated T lymphocytes and macrophages are also characteristically involved in all phases of atherosclerosis [2].

A number of triggering factors suggested to be involved in CAD have been investigated by many researchers. Among these possible factors, the role of

infections in atherosclerosis has been proposed by several authors since the late 1800s [4]. In recent years, results of some serological [5-7] and experimental [8, 9] studies have revealed the presumptive role of several infectious agents in the inflammatory mechanism of atherosclerosis [10]. Many viruses, bacteria, and even parasites are claimed to affect atherosclerotic plaque formation [11]. It was also hypothesized that single or multiple infectious agents may have a direct or long-distance proinflammatory effect on the cell wall in the atherothrombosis process [4]. It was also stated that infections acquired during childhood could be responsible for atherosclerosis [12]. The most frequently accused infectious agents are *cytomegalovirus* (CMV), *Herpes simplex virus* (HSV), *Chlamydia pneumoniae* (*C. pneumoniae*), and *Helicobacter pylori* (*H. pylori*). While some serological data obtained from some studies has revealed the association of viral and bacterial agents such as CMV [13], HSV [6], *C. pneumoniae* [6, 14] and *H. pylori* [15] with atherosclerosis, the same relations were not shown in other studies [16, 17]. *C. pneumoniae* has been reported to possess the strongest association with atherosclerosis among the infectious agents [18]. The presence of a relationship between *C. pneumoniae* infection, specific IgG and IgM titers, and increased evidence of cerebrovascular, peripheral vascular diseases and myocardial infarction was reported [11]. In a meta-analysis including 9 trials with 11015 patients treated with macrolide antibiotics, contradictory to the findings mentioned above, it was reported that there was no significant reduction in recurrent cardiac events and mortality [19]. Slow or hardly growing microorganisms are the main subjects of investigation as atherosclerosis triggering agents. The failure to detect the direct or phenotypic parts of the organisms in some studies might be due to the slow progression of atherosclerosis. With the introduction of nucleic acid amplification techniques, many investigators have been able to demonstrate bacterial or viral genomic material in atherosclerotic tissue samples [20-23]. The contradictory results of studies on the presence of genomic materials of infectious agents in atheromatous plaques [24-27] and serological studies have led investigators to conduct additional research on this subject and propose new postulates about the pathogenesis of atherosclerosis.

## Heat shock proteins

Direct invasion of the infectious agents to the vessel wall and an autoimmune reaction are the infection-triggered thrombosis mechanisms among the several postulated atherosclerosis pathogenesis mechanisms [3, 16]. Heat shock proteins (HSP), which are expressed by prokaryotic and eukaryotic cells, protect cellular proteins from denaturation [28]. The expression of heat shock proteins on endothelial cells, macrophages and smooth muscle cells, which may have a role in atherosclerosis, are increased when they are exposed to stress such as inflammation, infection and mechanical stress [29]. Due to the molecular mimicry of the bacterial HSP60 with human heat shock protein [3], the induction of immune reactions against HSPs in bacterial infections may result in autoimmune reactions, which have also been considered an important aspect of atherosclerosis, as stated above [30]. The triggering of an autoimmune reaction by infection, association of the HSP60 antibody and the severity of CAD was first shown by Zhu *et al.* [31]. An increased antibody response against the bacterial stress protein HSP60 has been detected in atherosclerotic patients. It was reported that elevated serum antibody levels to mycobacterial HSP65 have a predictive value in the progression of atherosclerosis [29]. Studies have displayed significantly higher levels of mycobacterial HSP65 antibodies in individuals with carotid artery thickening and coronary atherosclerosis. It was suggested that mycobacterial HSP65 antibodies interacting with human HSPs which are overexpressed in stressed vascular wall cells result in atherogenic changes by immunopathological mechanisms [31]. In a prospective study by Mukherjee *et al.*, patients with angioplasty were determined to have the antibody to HSP65. While a decrease in the level of the antibody was detected in the group who did not develop restenosis after percutaneous transluminal coronary angioplasty, the same reduction was not observed in the group who developed restenosis. The authors stated that anti-HSP65 antibody appears to be a potential marker of coronary angioplasty [32]. In two experimental studies, in mice fed a high cholesterol fat diet, the acceleration of fatty streak formation by immunization with recombinant HSP65 and *M. tuberculosis* containing the HSP65 were shown by George J *et al.* [1, 9]. Neointimal thickening was also demonstrated in immunization with mycobacterial HSP65 in a rat model by the same authors [33]. The results obtained from the studies

related with mycobacterial HSP65 raise questions considering the possible role of *M. tuberculosis* in atherosclerosis. Based on these results, the clinical outcome of atherosclerosis in patients with tuberculosis should be evaluated, although to our knowledge, there are no studies concerning about the possible clinical relationship in patients with tuberculosis. In our opinion, it may be to investigate the clinical impact of tuberculosis on atherosclerosis.

BCG (Bacillus Calmette Guerin) is a live, whole organism vaccine employed for protection against tuberculosis [34]. Approximately 70% of the world's children receive this vaccine. The vaccination is administered more than once during childhood in some countries such as in Turkey. In a recently done experimental study, rabbits were immunized with BCG vaccine, and the anti HSP60 titres in the BCG immunized rabbits were found to be correlated with the atherosclerotic plaque formation. As a result of this study, the authors suggested that the specific immune response to BCG-associated HSP might be proatherogenic [35]. It was postulated that, while the morbidity and mortality of tuberculosis are reduced by the administration of BCG vaccine, the vaccine might be causing stress and stimulating proatherogenic mechanisms [34]. In a recent study by Perschinka *et al.*, evidence was provided for 8 atherosclerosis-associated epitopes shared between hHSP60 and HSP60 of *E. coli*, *C. trachomatis* and *M. tuberculosis*. In advanced atherosclerotic lesions, all 8 epitopes were recognized. In particular, the antibody against epitope 8 was found to be very reactive in healthy arterial specimens of children. The authors suggested that antibodies reactive to epitope 8 might be involved in the development of early inflammatory disorders of the arterial wall, and the cross-reactive epitopes serve as autoimmune targets in the early stage of atherosclerosis [36]. The results of this study led us to consider the risks and benefits of administering BCG vaccine in the early years of life, as well as to consider how *M. tuberculosis* infection may trigger and/or accelerate atherogenesis. Taking into account the information discussed above, administration of BCG vaccine several times throughout childhood, as is done in Turkey, might elicit a marked immune response against heat shock protein, and possibly contribute to atherosclerosis which begins in early ages of life. As proposed by Lamb *et al.* [34] as a precaution, the development of HSP-free BCG might reduce the possible effect on coronary artery disease.

## Lipid composition

Phospholipids are among the constituents of the herpes virus envelope and mycobacteriaceae cell wall. In a previous study, we tested the ability of phospholipids to support the assembly of prothrombinase complex and the generation of thrombin. The results of the study displayed that the highest amount of thrombin generation is supported by specific phospholipids as phosphatidylinositol, phosphatidylethanolamine and phosphatidylserine. Among these, phosphatidylinositol provided the greatest support for the assembly of the prothrombinase complex and thrombin generation [37]. In a study by Pyrzidal *et al.* [38] it was shown that purified *cytomegalovirus* express phosphatidylserine-like procoagulant activity. In addition, several groups reported data supporting the hypothesis that several *herpesviruses*, including *CMV*, act as prothrombotic agents by activation of the coagulation. It was postulated that this activity might be due to the surface molecules containing a procoagulant phospholipid in *CMV* that promotes the assembly of a functional complex between factor Xa and cofactor Va and the ability to form prothrombinase complex, leading to thrombin generation [26]. Although bacteremia does not occur that often in tuberculosis, it was reported that *M. tuberculosis*, which harbours phosphatidylinositol in the cell wall, has been detected in the blood samples of tuberculosis patients up to a frequency of 33% in non-HIV infected patients [39, 40]. In this respect, we can suggest that mycobacterial phosphatidylinositol might also stimulate or take part in thrombin generation resulting in procoagulant activity. This raises the question as to whether *M. tuberculosis* can also exert an atherosclerotic effect as proposed for *CMV* due to the constituent of its cell wall.

## Conclusion

In conclusion, although there is no clinical evidence of atherosclerosis in patients with tuberculosis, and no case reports of progressive coronary heart disease in patients with tuberculosis, there is a considerable amount of data supporting the hypothesis that HSP65 can be involved in the progression of atherosclerosis. There was no convincing proof of this in many of the reports themselves. But taken together, by the data obtained for the possible atherogenic effect of mycobacterial HSP65 and the phospholipid constituent of the *M. tuberculosis* cell wall, it can

be postulated that *M. tuberculosis* is also one of the several infectious agents contributing to the atherosclerotic process. As tuberculosis infection is a major health problem in developing countries, clarifying whether *M. tuberculosis* is involved in atherosclerosis pathogenesis will require further studies focusing on *M. tuberculosis*, and this area of research may be an interesting one for researchers.

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