The role of the infectious agents in the pathogenesis and evolution of atherosclerosis

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Atherosclerosis is a chronic inflammatory process due to the endothelial reaction to stress risk factors, only some of which are known. Clinical and experimental observations have suggested that several infectious agents are involved in this process. These agents, particularly the germ *Chlamydia pneumoniae*, and their relationship to the atheromata are described. Two hypotheses concerning how these infectious agents act are suggested. Both hypotheses are based on the capacity of these agents to induce the production, by endothelial cells, of the so-called heat shock protein (HSP), one of whose characteristics is to provoke an immune system reaction: 1) induction of a cross immune reaction, due to “molecular mimicry”, between the HSP of infectious origin and the one that is produced by the endothelium as a consequence of stress due to the risk factors; 2) infection of the endothelial cells, followed by the synthesis and exposure on their surface of the HSP and activation of innate immune surveillance. Numerous experimental studies have been performed and are still being performed with the aim of verifying the efficacy of antibiotic treatment in preventing or reducing the rate of acute cardiovascular events. The results are still inconclusive. Probably, to be effective, treatment should be started at an earlier age. Prevention through vaccination against the involved microorganisms and the consequent induction of immune tolerance toward the HSP is also being investigated. As the mechanisms of action of infectious agents are further clarified, effective therapeutic and preventive measures could be taken with important clinical spin-offs.

(Ann Ital Med Int 2004; 19: 249-261)

Key words: Atherosclerosis; *Chlamydia pneumoniae*; Cytomegalovirus; Heat shock protein; Infectious agents.

Introduction

Atherosclerosis is a chronic pathology of the internal wall of the artery where inflammatory processes are present. It is a complex process with diverse etiologies (risk factors) acting simultaneously on the endothelium through two main mechanisms: bio-mechanical stress and immune reaction. The natural chemical factors (such as smoking, dyslipidemia, hyperglycemia, etc.) as well as mechanical factors (e.g., high blood pressure) are well known. In about one third of the cases, conventional risk factors are absent. This leads us to suppose that other yet unknown factors may play an important role. Among them, in recent years, a number of infectious agents – both viral and bacterial – have been considered. They could act by means of a not yet completely understood complex mechanism linked to damage to and reaction of the endothelium. According to the prevalent interpretation of the origin of atherosclerosis, in fact, the various risk factors cause an increase, within the endothelial cells, of ionized oxygen molecules (“reactive oxygen species” or “superoxides”). Such ions have a strong tendency to react with proteins causing serious damage: the so-called “oxidative stress”. Endothelial dysfunction, constituting a reaction to the damage, is the result. The transfer of information contained in specific genes that control this reaction on messenger RNA molecules is activated by means of a nuclear transcription factor (NF-kB). This leads to the synthesis of molecules (cytokines, factors of attraction and adhesion to the endothelial surface of lymphocytes, monocytes, etc.) that initiate the inflammatory process and reduce the antithrombotic properties of the surface. Also activated is the production of a protein called “heat shock protein” (HSP) that has a very important part in the role played by infectious agents. Its exposure to the surface of the endothelial cells is one of the antigenic stimuli activating the immune component of atherosclerosis (the other are low-density lipoproteins [LDL] that have penetrated into the subendothelial space due to the increased permeability and have been oxidized).

Aside from describing the microorganisms involved, we will also illustrate the mechanisms by which they are supposed to act and the possible therapeutic and preventive interventions.
Origins of the infective hypothesis for atherosclerosis

The idea that atherosclerosis could be related to an infection is over a hundred years old. This hypothesis had already been made at the end of 1800 in Europe, and was again put forth at the turn of the century by Osler and other American physicians. In the 1970s, the discovery that cells present in the plaque were of monoclonal origin led to the supposition of the action of cell transformation factors such as viruses. Moreover, researches were able to induce atherosclerotic lesions in chickens infected with herpes virus. In the 1980s, a group of Finnish researchers noted a significant increase in antibodies against a bacterium, Chlamydia pneumoniae, in subjects at greater risk of acute coronary events. Numerous studies following these first observations confirmed this relationship between infectious agents and atherosclerosis.

It is interesting to note that the association is with microorganisms that share two characteristics: they are obligate intracellular prokaryotic pathogens and are able to provoke chronic infections with periodic reactivations that are accompanied by systemic immune reactions. Diabetes mellitus, of which atherosclerosis is the chief complication, could constitute a favorable condition, because it also alters the defense capacity of the organism to infections and increases the number of infectious processes which it undergoes (“increased infectious burden”).

The infectious agents involved

Before making a detailed examination of the agents implicated, the concept of “infectious burden” should be clarified. It has been observed that the greater the number of microorganisms to which an individual has been exposed, the greater the impact of the infectious factor on atherogenesis. This suggests either a possible synergism between the agents on the initial and subsequent development of lesions, or an accumulative effect of the infections that cause endothelial dysfunction and inflammation to precipitate.

Viruses

Genus: herpes. The role of these viruses in man has not yet been well-defined, even though there is conclusive epidemiological evidence of their involvement. Other evidence includes: the presence, in the atherosclerotic lesions, of their antigens or of amino acid sequences of their DNA (although the same viral tracks have been found in non-involved aortic tissue); the demonstration of their capacity to provoke atherosclerosis in animal models, and, finally, in vitro experiments that have induced inflammatory and prothrombotic responses in infected endothelial cells. Their infection amplifies the effect of hypercholesterolemia in experimental models.

Cytomegalovirus. This virus is implicated in both the pathogenesis of systemic atherosclerosis and in atherosclerosis subsequent to some types of heart surgery. Many studies have demonstrated a significant association between the presence of antibodies against the virus, the incidence of atherosclerosis and the severity of coronaropathy. In experimental models with elevated hypercholesterolemia, infection with this virus induces aortic lesions similar to those observed during the first phases of the atherosclerotic process. Such an effect could be brought about either by its capacity to infect the endothelial cells and the smooth muscle cells (where it could persist for a long time in a latent form) or could be mediated by a mechanism of immune origin. The second possibility seems more probable. In fact, human antibodies against viral peptides provoke a cross-reaction with HSP-60 molecules present on the surface of stressed endothelial cells and contribute to the process of endothelial dysfunction.

With regard to pathology following surgery, the virus could be responsible for the proliferation of the smooth muscle cells leading to restenosis after angioplasty. In fact, it has been observed that 43% of the patients who are seropositive for the virus have this complication as opposed to 8% of those who are seronegative. Moreover, a correlation between antibody levels and the appearance of a rapidly-progressive particular form of atherosclerosis in the coronary arteries of transplanted hearts has been observed; it is characterized by diffuse and concentric intimal hyperplasia associated with proliferation of the smooth muscle cells and inflammation of the arterial wall. This is the leading cause of heart transplant failure. Moreover, transplanted patients previously infected by the virus have a significantly greater lumen stenosis than those who have not had the infection.

Two hypotheses have been proposed: either a direct action of the virus on the vessel wall or an action mediated by the formation of antigens on the endothelial surface that could further stimulate the rejection reaction. In any case, prophylactic action with a drug that inhibits viral replication for 28 days following transplant had lowered the incidence (compared to treatment with a placebo) of this form of atherosclerosis.

Herpes simplex type 1 and type 2. A strong association has been noted between the presence of antibodies against this virus and coronaropathy (particularly among smokers).
**Epstein-Barr virus.** The presence of antibodies against this virus is significantly associated with cardiovascular events\(^4\). It is an obligate intracellular pathogen that persists in B lymphocytes for the patient’s entire lifetime, although it is not present in the plaques.

**Hepatitis C and A virus.** Seropositivity for hepatitis C and hepatitis A virus is associated with the carotid plaque and with myointimal carotid thickening independently of other risk factors\(^3\).

**Enterovirus.** The presence of antibodies against these viruses is associated with an increased risk of myocardial infarction\(^4\). The mechanism of action could consist in an immune response provoked by the HSP\(^3\) the production of which is induced by the virus.

**Bacteria**

*Helicobacter pylori.* This bacterium, object of one of the most important pathogenetic discoveries in recent years (and emblematic of the difficulties that new ideas are faced with in being accepted by the medical environment\(^3\)) also seems to be involved in atherosclerosis. A direct relationship between an increased concentration of antibodies against it and cardiovascular diseases has been noticed\(^3\). Its DNA has been found in carotid plaques\(^3\). In this case too, it is suspected that its link to atherosclerosis passes through the immune cross-reaction against the HSP produced by the endothelium\(^1\). In fact, this bacterium also produces its own HSP, and the presence of anti-HSP antibodies in patients with this infection has been described\(^4\). For reasons that are not yet clear to us, the contribution to atherosclerosis seems to be provided above all by the most cytotoxic *Helicobacter pylori* species, the one characterized by the presence of gene A, associated with cytotoxin\(^4\). In particular, an association between this bacterial species and cerebral ictus caused by emboli originating in particularly advanced and unstable carotid plaques has been observed\(^3\). The mechanism of this association is not clear. Here too, cross immune processes are invoked\(^3\).

*Anaerobic gram-negative bacteria of the oral cavity.* The pathology they provoke, “periodontal disease”, consists of a chronic, destructive infection of the gums, the connective tissue and the alveolar bone. It causes bone reabsorption, the formation of gingival pouches, progressive damage to the fixing apparatus of the teeth and finally their loss\(^4\). The greater risk of coronaropathy in the presence of untreated periodontitis, with respect to healthy subjects, is 25% (increasing to 70% in men < 50 years)\(^4\). The DARIC study (Dental Atherosclerotic Risk in Communities) pointed out the direct relationship between periodontal disease and the middle-intimal thickness of the arteries\(^2\). These germs produce HSP, and in patients with gingivitis, the level of anti-HSP IgA antibodies in the saliva is increased\(^5\). It is thus hypothesized that the same type of immune reaction described for other microorganisms occurs\(^2,2\).

**Chlamydia pneumoniae.** This is by far the infectious agent most involved in the pathogenesis of atherosclerosis\(^4\), Consequently, it will be discussed in more detail. It is an ubiquitous gram-negative bacterium that depends on the host cell for its survival and development. It is forced to multiply within the host cell because it is unable to generate the adenosine triphosphate molecule autonomously and consequently depends on the cell it occupies for its energy requirements\(^4\). It is transmitted among humans by means of aerosolized droplets and may infect the upper and lower respiratory tracts (atypical pneumonia, sinusitis, pharyngitis, bronchitis); it causes middle ear inflammation\(^5\) and is at the origin of many cases of adult asthma. The infection often causes very mild symptoms (low grade fever, cough and hoarseness) or no symptoms at all, and hence, generally goes untreated\(^4\). Once an acute infectious episode is resolved, it may persist in a latent form\(^4\). This depends on the particular vital cycle of the bacterium that consists of an extracellular phase characterized by the so-called “elementary bodies” which are surrounded by a protective coating; these are metabolically-inactive and may penetrate the host cell and remain alive there for long periods (with inclusion aspects similar to spores) without replicating. The bacterium may also initiate an intracellular phase of transformation into metabolically active but non-infectious “reticulate bodies” that modify the host cell physiology to their advantage: they reproduce by binary fission and again change into “elementary bodies”. After about 72 hours, following cell lysis, they may return to the external environment and initiate a new cycle\(^4\). Still, in the presence of inhibitory factors (such as, for example, the immune-mediated cytokines interferon-gamma and tumor necrosis factor [TNF-\(\alpha\)]), the bacterium may remain for a long time in both a metabolically and infectiously inactive form (“persistent bodies”). This form, important because of its pathogenic mechanism that will be described later, has particular characteristics: a diverse morphology and, from the immunological point of view, the absence of proteins that play the dominant antigenic role, except for the increased expression of the HSP, on its external coating\(^4\). This immune tactic, which enables it to escape control and
not be eliminated by the host, explains how the principal characteristic of *Chlamydia pneumoniae* infection is to set off a chronic infectious state and how it is able to gain a role in the pathogenesis of atherosclerosis, particularly the precocious type. Its natural history, consisting of a progressive exposition over time, is very similar to that of the *Helicobacter pylori*.

**Relationship between the presence of anti-*Chlamydia pneumoniae* antibodies and atherosclerosis**

The previously-cited observations by Saikku et al., which, during the course of the Helsinki Heart Study, highlighted a significant association between serological positivity for this germ and acute cardiovascular events (50% with acute myocardial infarction and 68% with angina pectoris with respect to 17% of serologically negative patients), were the first clues to the possible involvement of this microorganism in atherosclerosis. As it is a widespread microorganism, many people are repeatedly infected by it during their lifetime. The prevalence of seropositivity at 20 years of age is 50% and increases with age, reaching, at 65 years of age, 80% in men and 70% in women. The higher seropositivity in men, as yet unexplainable, corresponds singularly with the higher incidence of atherosclerosis among males. This association has been confirmed in 30 other studies carried out in 8 different countries. The most useful antibodies as reliable indicators of infection (primary, chronic and recurrent) are the IgA that appear after 6-8 weeks, like the IgG but, unlike the latter, disappear rapidly due to their brief half-life (5-6 days). Moreover, in contrast to the IgG, they permit us to distinguish between a past and a current infection.

**Relationship between hypercholesterolemia and *Chlamydia pneumoniae* infection**

While some experiments showed that it is also possible to provoke atheromatous lesions by infecting animals with normal cholesterol levels and by the pro-atherogenic action of *Chlamydia pneumoniae* cannot occur in the absence of an increase in blood lipoproteins. In animals fed a diet rich in cholesterol, repeated infections via the nose (to simulate the port of entry of human infection) have induced the rapid development of aortic atherosclerosis. Treatment with the antibiotic azithromycin, to which the bacterium is particularly sensitive, initiated within a week after infection, has impeded this effect. In man too, a correlation between increased cholesterolemia and the role of the microorganism in the atheromatous process has been observed. The incidence of atherosclerosis in the tropics, where the diet is poor in fats, is low despite the widespread diffusion of *Chlamydia pneumoniae* infections. It is here interesting to note, that while the early phase of atherosclerosis, that is the formation of lipidic stria, is widespread throughout the world, its evolution to the sclero-calcific plaque is typical in societies characterized by diets rich in saturated fats and high levels of cholesterolemia.

Some interpretations have been made concerning the possible mechanisms that relate hypercholesterolemia to infection. It is believed that LDL, if present in increased quantities in the circulation, cross the endothelial barrier where they are oxidized due to the action of the oxygen ions (“hypothesis of modified LDL”). One of the virulence factors of the bacterium, is able to cause oxidation of the LDL by monocytes, even in the absence of superoxides. Monocytes, attracted into the subendothelial space by the molecules produced by the stressed cells, transform into macrophages and incorporate the oxidized LDL. In macrophages there is normally an accurate equilibrium between the LDL that enter and those that exit. This depends on the genetic control of the activity of the receptors for these lipoproteins (the apo-B/E receptor for the LDL). On the other hand, the oxidized LDL are captured by the so-called “scavenger” receptors that cannot be saturated, that is that cannot be counter-regulated by an excess of phagocytized oxidized LDL. The macrophages accumulate fat and transform into the so-called “foam cells.” Even the macrophages infected in vitro by *Chlamydia pneumoniae* lose this capacity to control, and increase the captation and accumulation of LDL. This effect is induced by one of the constituents of the external cell membrane of the bacterium, the lipopolysaccharide (LPS), and could be finalized to its survival strategies.

The oxidized LDL themselves represent, moreover, an important antigenic stimulus that takes part in the immune reaction at the endothelial level together with that induced by bacterial HSP (to be discussed further on). According to a convincing theory, at the beginning of the process, the immune-mediated inflammatory reaction provoked by the microbial antigen and the oxidized LDL dominates the hyperlipidic effect but, due to the auto-alimentation of the phenomenon, the coexisting situation of hypercholesterolemia impedes its resolution which would otherwise take place within 2-3 weeks.

**The presence of *Chlamydia pneumoniae* in atherosclerotic lesions**

*Chlamydia pneumoniae* has often been seen with the electron microscope inside the atheromatous plaques. Many other investigations, carried out with various tech-
niques (the search for immunocomplexes with immuno-cytochemistry, the search for antibodies by means of immuno-cytocentrifugation, the finding and amplification of DNA of a bacterium by means of the polymerase chain reaction and of in situ hybridization) have confirmed its presence. According to a recent literature review, the bacterium is identified in 46% of the samples of arteries with atheromatous plaque compared to less than 1% of the samples of healthy arteries. It has been identified in the plaque of all types of arteries, particularly the carotids of heavy smokers. It has also been isolated from atheromatous tissue and cultivated in vitro, although with some difficulty.

Experiments on animal models that do not spontaneously develop atherosclerosis have demonstrated that this particular strain of Chlamydia, once introduced through the nose, may cause initial atheromatous lesions that continue to grow if the animals are fed an atherogenic diet. Thus, the germ can aim at the internal arterial wall, infect it and constitute the primum movens of the diseased arteries. It could favor the formation of thrombi by means of its strong adherent capacity and activation of their aggregation mechanisms.

The role of Chlamydia pneumoniae in modulating the inflammatory reaction of the artery

Local action

Chlamydia pneumoniae is able to infect most of the cells present in the atheromatous lesion and to influence their activity. It acts, above all, by means of two “virulence factors” (factors responsible for its capacity to settle in and reproduce): the above-cited surface LPS and the HSP.

Actions induced by lipopolysaccharide

Aside from the already-mentioned stimulus to the formation of foam cells, the surface LPS (by means of the κB genic transcription factor) is one of the responsible factors for the cascade of the molecules at the origin of inflammation in the endothelial cells. It is able to act independently of the presence of live bacteria. Moreover, the smooth muscle cells are stimulated by it to proliferate and, once transformed into fibroblasts, produce the fibrous capsule of the plaque that may be considered a defense mechanism against further passage of lipids and inflammatory cells. On the other hand, the LPS also stimulates the leukocytes to produce “metalloproteinase” enzymes that degrade the collagen fibers of the capsule, rendering the plaque unstable and predisposed to rupture.

Actions of heat shock proteins

The often-cited HSP is a protein produced by the cells of the organism in response to stress, that is, by any stimulus, internal or external, that leads to a central effect: the denaturation of the proteins within the cells and the consequent activation of the gene for HSP. These stimuli may include, aside from increased heat, infection, presence of free oxygen radicals, lack of nutrition, etc. It has a double function: one inside and the other outside the cell. The former consists of the role of “chaperon” of the young proteins, that is to stabilize and protect them from denaturation, from erroneous folding and from aggregation (both induced by stress). It facilitates the folding of rising proteins, renders the already-formed protein aggregates soluble, transports the proteins across the mitochondrial membrane and assists in the new, correct folding of the denatured proteins. All of this has the purpose of impeding the cascade formation of altered protein aggregates that are lethal to the cell. The second function is carried out on the cell surface to which it may move in response to stress, activating the innate immune monitoring system, as a danger signal to the system (the “danger” theory). Once the cell is identified, the latter determines activation of NF-κB and consequent expression of the pro-inflammatory cytokines.

HSPs are present in nature in about 24 molecules, grouped into five families according to their molecular weight. They have an amino acid sequence that is very similar even in distant species such as bacteria and man.
(particularly the HSP-60 family)\(^{61,62}\). All organisms respond to potentially dangerous situations by inducing the transcription of genes responsible for HSP synthesis\(^{49,66}\). The molecule produced by the endothelial cells of man has an amino acid sequence that is very similar (> 50%) to that produced by *Chlamydia pneumoniae*\(^{62}\). Thus, the risk of immune cross reactions between the two proteins is high\(^{62}\).

**Heat shock protein and the immune response**

*Chlamydia pneumoniae* (in the form of “persistent bodies” of its vital cycle) may elaborate great quantities of HSP-60 (from its molecular weight)\(^{79,80,86}\), which is highly antigenic to man\(^{84,89}\). On the other hand, in order to maintain homeostasis in the arterial wall, the endothelium produces a great deal of HSP-60 in response to the stress-causing factors represented by the various risk factors for atherosclerosis\(^{49,84}\), including oxidized LDL\(^{93}\) and the immune reaction against the oxidized LDL itself\(^{86}\). It has been hypothesized that the immune system, already sensitized by bacterial HSP-60, may set off an allergic reaction\(^{40}\) in the presence of the HSP-60 produced by the endothelium and translocated onto its surface. This mechanism is part of the immune surveillance against cells altered by stress. It has the purpose of eliminating them and protecting, in this way, the rest of the cellular community\(^{83}\). This so-called “cross” reaction, due to the resemblance between the two molecules, has cytotoxic effects on the endothelial cells through complement activation or antibody-linked toxicity\(^{40,49,93}\). This could contribute to endothelial dysfunction and to cell apoptosis as well, which are a part of the atherosclerotic process\(^{26,91}\).

Clinical and experimental data confirm that indeed this auto-immune reaction against HSP-60 is produced in the early stages of atherosclerosis\(^{40,43,49,74,86,94,95}\). In man, a strong increase in IgG against HSP has been observed in concomitance with acute coronary events\(^{96}\). Moreover, percutaneous angioplasty may induce an increase in the antibodies against *Chlamydia pneumoniae*\(^{96,97}\), and a relationship between elevated antibody levels and coronary restenosis after angioplasty with or without stent implantation\(^{55}\) has been ascertained. The anti-HSP antibodies, too, provoked by the cross immune reaction between bacterial HSP and human HSP could play a role in the restenosis that follows angioplasty interventions, inasmuch as the antibodies against HSP could provoke an acceleration of the neointimal abnormal growth that narrows the arterial lumen\(^{98}\).

The fact that there is an immune component in the inflammation at the origin of atherosclerosis is further suggested by the presence, in the plaque, of activated T-lymphocytes\(^{98}\) and also by memory T “asleep” lymphocytes that may be reactivated in the presence of antigens similar to those to which they had previously been sensitized\(^{93}\). The T-cells found in atherosclerotic lesions respond *in vitro* to *Chlamydia pneumoniae*, human and bacterial HSP and oxidized LDL\(^{92}\).

Finally, a bacterium of the same species, *Chlamydia trachomatis*, causes trachoma blindness and the obstruction of the fallopian tubes with a fibrosis that follows the infiltration of lymphocytes and macrophages, through a delayed sensitization reaction in which the key antigen is HSP-60\(^{99}\), a process that has interesting analogies with the development of atherosclerotic plaque\(^{99}\).

**Other atherogenic reactions of heat shock proteins**

HSP would also act in an atherogenic sense by activating pro-inflammatory genes both in the endothelial cells and in the macrophages and smooth muscle cells\(^{84}\), thus inducing a classic atherogenic effect: 1) the introduction of inflammatory cytokines; 2) the production of adhesion and recruitment molecules of the inflammatory cells; 3) the production of metalloproteases; 4) the oxidation of the LDL by monocytes\(^{49}\).

**Pathogenetic hypotheses**

The first, also called “molecular mimicry” may be synthesized as follows\(^{40,43,54,86}\) (Fig. 1): a) the immune reaction to bacterial HSP is set off in the course of the first infection; b) stress risk factors provoke the dysfunction of the endothelium followed by the activation of the inflammatory process; moreover, they provoke cell protein denaturation which is followed by the production of endothelial HSP; c) HSP, translocated to the cell surface and identified by immune surveillance, triggers the allergic reaction set off by the antibodies followed by their cytotoxic effect; also intervening are the T-lymphocytes that, aimed against the HSP, reach the target, proliferate and complete the attack; d) the monocytes with the bacteria settled within the initial infection site, become their vehicles of spread. Attracted to the atheromatous lesion, they cross the endothelium and transport the bacteria there; the HSP and the LPS of *Chlamydia pneumoniae* contribute to the inflammation as well as to the oxidation and subsequent phagocytosis of the LDL, and thus to the evolution of the atheroma, the rupture of the fibrous lining through the action of the metalloproteases and TNF-\(\alpha\) (to the production of which the presence of bacteria contributes) and the formation of the thrombus may follow.

This process makes one think of a sophisticated strategy of survival and reproduction of *Chlamydia pneu-

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pose and that might be co-evolved with the species subject to atherosclerotic disease.

The second hypothesis, not necessarily alternative to the first, could be applied particularly for the initial phases of atherosclerosis, those that occur at a young age\cite{8, 28, 60, 70, 83} (Fig. 2). It maintains that the infective agent reaches the endothelial wall of an artery and stabilizes a persistent low-grade chronic infection there\cite{50}. The infection would provoke the formation of HSP and its expression on the surface, the activation of innate immune surveillance, of NF-κB, the production of pro-inflammatory molecules\cite{70, 85}, and the setting off of the atheromatous process.

The subintimal infiltration of the immuno-competent cells, in young people, occurs before lipids are deposited\cite{8}. This leads us to believe that the process is subsequently sustained by the oxidized LDL. These, in turn, play the role of antigen in an autoimmune reaction with a cytotoxic effect that induces the production of HSP\cite{86}. In fact, the autoimmune process and the inflammation deriving from it are amplified by high serum levels of cholesterol\cite{100}. The immune-mediated inflammatory response, in the absence of hypercholesterolemia, is unable to make atherogenesis last, and it tends to regress. On the other hand, the lowering of cholesterol levels is much less effective in reducing the media-intimal thickness in individuals positive for anti-*Chlamydia pneumoniae* antibodies with respect to those who are negative\cite{2}. Thus, especially in the early, juvenile phases, atherosclerosis could be the effect of a com-

**FIGURE 1.** Hypothesis of autoimmune pathogenesis of atherosclerosis provoked by infectious agents, called “molecular mimicry” (see text).

HSPb = bacterial heat shock protein; HSPe = endothelial heat shock protein; LDL = low-density lipoprotein; LDL-OX = oxidized LDL.

**FIGURE 2.** Hypothesis of autoimmune pathogenesis of atherosclerosis provoked by direct action of infectious agents on the endothelial cells (see text).

Abbreviations as in figure 1.
plex interaction between lipid imbalances and an immune reaction induced by infectious agents. These hypotheses could also be valid for the other infectious agents involved in the pathogenesis of atherosclerosis. As illustrated, the HSP expressed by them (with regard to bacteria) or incorporated into their membrane when they abandon the infected cell (with regard to viruses) is suspected to cause the immune cross reaction with the human HSP at the level of the endothelium. Moreover, the various bacteria and viruses both act by inducing the production of HSP by the infected cells. In this way, they also stimulate the response of the innate immune system. Finally, we cannot exclude the possibility that the risk factors themselves determine the activation of the immune reaction. The mechanism would be: endothelial stress, consequent denaturation of the cellular proteins and activation of the genes that lead to the synthesis of HSP and its expression on the cellular surface.

It is interesting to note that *Chlamydia pneumoniae* is associated with an increasing number of chronic diseases including Alzheimer’s disease. Some of them such as multiple sclerosis, Reiter’s syndrome, reactive arthritis, Guillain-Barré syndrome, some types of thyroiditis, sarcoidosis, erythema nodosum and rheumatoid arthritis are considered autoimmune diseases. The relation between these diseases, *Chlamydia pneumoniae* infection and atherosclerosis could derive from the fact that a particular genetic characterization (the presence of the ε4 allele in the lipoprotein apo-E locus) is linked to an increased risk of these diseases and also of *Chlamydia pneumoniae* infection and of atherosclerosis. Thus, the pathogenic mechanism would have a common autoimmune basis.

**Treatment and prevention**

This aspect will be examined mainly for *Chlamydia pneumoniae* on which the researchers’ studies were concentrated.

**Antibiotic treatment**

Theoretically, antibiotic treatment effective for *Chlamydia pneumoniae* could prevent or attenuate the cardiovascular events brought about by atherosclerosis. Still, this bacterium is difficult to fight due to the characteristics of its life cycle. Both during the inactive metabolic form of its “elementary bodies” and during the latent form of its “reticulate bodies” it is not sensitive to antibiotics (which are able to act only during the transformation from “reticulate bodies” to “elementary bodies”) Moreover, when within the monocytes, its sensitivity is particularly reduced. Treatment with some antibiotics favors the formation of the “persistent” and chronic form which is difficult to eradicate. The macrolides, particularly azithromycin, are the most effective. The chinolones, tetracyclines and some anti-tubercular drugs are also effective.

**Studies carried out**

Various pilot studies have been carried out to ascertain the usefulness of antibiotic treatment in patients with atherosclerosis, in particular, those with coronaropathy. The results have been encouraging for some of them. After an acute myocardial infarction, patients with high levels of antibodies against the bacterium have been treated with azithromycin or with placebo: those treated with the antibiotic had a significantly reduced incidence of subsequent cardiovascular events. Still, other studies of brief duration (5 days), such as the AZACS study (Azithromycin in Acute Coronary Syndrome) that have involved about 1500 subjects, did not confirm a significant preventive effect either on the recurrences of infarct or on the appearance of acute events in subjects with high levels of antibodies. Studies with more prolonged treatment, involving several thousand patients, were subsequently carried out. In one of these, the WIZARD study (Weekly Intervention with Zithromax for Atherosclerosis and its Related Disorders), completed in 2002 and including over 7000 subjects with a personal history of heart attack and positivity for anti- *Chlamydia pneumoniae* antibodies, azithromycin was administered for 3 months. This resulted in a significantly decreased mortality due to a recurrent acute myocardial infarction and occurring among patients treated with the antibiotic during the first 6 months of the experiment with respect to those treated with placebo. In the ACADEMIC study (Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia), that utilized azithromycin versus placebo in about 300 patients for a period of 3 months, a tendency towards the reduction of cardiovascular events was observed after 1 year and after 1 year and a half; this tendency, however, did not reach statistical significance. A retrospective study, carried out on a large number of patients, to show the effects of the primary prevention of antibiotic treatment on the onset of myocardial infarction has also yielded encouraging results. Other large experiments such as the ACES trial (Azithromycin and Coronary Events Study) that will involve thousands of coronar-
pathic patients, sponsored by the United States National Institutes of Health are presently underway, indicating the importance that is attributed to the subject. Some studies have shown, in antibody-positive patients, a significant reduction in the levels of inflammatory markers such as C-reactive protein, interleukin-6 and TNF-α, that constitute the so-called “acute-phase response” of the liver to inflammation. Still, the reduction of these markers could be due to the aspecific anti-inflammatory effects of the antibiotics. Among other things, antibiotics stabilize plaque: this could explain the improvement observed in patients with coronary syndromes, independently of any antibacterial effect.

It is hoped that new studies on primary prevention will be undertaken as the pathogenic mechanisms are further clarified. They should be conducted during the first stages of disease progression, particularly on young subjects. In fact, the natural history of atherosclerosis develops over the course of decades, and more than 50% of young subjects between 10 and 12 years old and living in western countries already have coronary atherosclerotic lesions in progress. Already before 20 years of age the typical succession of intimal thickening begins, followed by partial healing and new relapses linked to recurrent episodes of inflammatory stimuli; this goes hand in hand with episodes of infection and reinfection (or reactivation of a chronic infection) by Chlamydia pneumoniae. Traces of the presence of the bacterium have been found in the lipidic striae already in young adults. Moreover, it is probable that antibiotic therapy is not particularly effective in preventing cardiovascular events in grown-ups when the atherosclerotic process has already reached its advanced stages.

Methods to evaluate the efficacy of antibiotic treatment

One could hypothesize that prevention may be achieved by the periodic administration of an antibiotic on the basis of the course of reference values, something like what was done to prevent the cardiac alterations of acute rheumatic fever on basis of the course of the antistreptolysin titer and erythrocyte sedimentation rate. Still, what is missing here is a standardization of the various methods to prove the presence and evolution of Chlamydia pneumoniae, and this reduces the possibility of evaluating with certainty the possible efficacy of treatment. It could be useful to utilize the course of the IgA antibodies the titer of which normalize rapidly after effective treatment or parameters of the immune response to members of the HSP family of bacterial origin. The patients to treat could be selected on the basis of the titer of anti-HSP antibodies.

Prevention via vaccine

An anti-Chlamydia pneumoniae vaccine that would prevent infection from the very beginning is being researched in various laboratories. To date, no molecules towards which vaccines with a guaranteed protective action could be developed have been identified. The hypothesis of the contribution of infectious agents to the origin of atherosclerosis, after many years since its first formulation, has not yet been confirmed because proof of a direct causal relationship is still lacking and is
extremely difficult to obtain\textsuperscript{44,70}. The most frequently-raised objection to this hypothesis is that the treatment of subjects at risk for cardiovascular events with antibiotics (in particular macrodilids for \textit{Chlamydia pneumoniae}) has not yielded very encouraging results in terms of reducing such events. Still, as already stated, it is difficult to think of reaching this objective when the plaques are already in an advanced state of evolution. This is because the action of the infective agents probably occurs, in most cases, very prematurely, at the beginning of the natural history of the lesion when the patient is still young\textsuperscript{44,70}. The hypothesis, then, could be proposed in terms of probability rather than of certainty\textsuperscript{73}. Still, as it has been attempted to demonstrate, it is based on a notable amount of associative and correlative evidences\textsuperscript{70}.

To date, two mechanisms through which the infectious agents could contribute to the development of the atheromatous process have been proposed: 1) by inducing an immune cross reaction due to “molecular mimicry” against the HSP produced by the endothelium as a response to the traditional risk factors; 2) by directly infecting the endothelial cells, inducing the expression of the HSP on their surface and activating the immune system. The two mechanisms could coexist, and the second one could be responsible for the most precious and juvenile phases of atherosclerosis. Both of them would integrate with the fundamental role of hyperlipidemia and of the other risk factors.

From a therapeutic and prophylactic point of view, antibiotic treatment should probably be initiated at a young age. Moreover, vaccination before 20 years of age against various microorganisms (above all against \textit{Chlamydia pneumoniae}) and the modulation of the immune reactivity against HSP are promising.

All of this is particularly important for diabetics, who undergo a notable “infectious burden” and are characterized by an altered immune response\textsuperscript{18}. It has been demonstrated, for example, that \textit{Chlamydia pneumoniae} increases the risk of coronaropathy induced by the metabolic syndrome\textsuperscript{26}.

Many steps must still be taken\textsuperscript{65}. When the pathogenetic mechanisms of the infectious component of atherosclerosis are further clarified, a properly aimed therapeutic or prophylactic strategy could lead, in clinical terms, to extremely significant outcomes.

**Riassunto**

L’aterosclerosi costituisce un processo infiammatorio cronico legato alla reazione dell’endotelio allo stress provocato da fattori di rischio, alcuni ancora sconosciuti. Osservazioni cliniche e sperimentali hanno condotto a ritenere che anche alcuni agenti infettivi siano coinvolti in questo processo. Vengono descritti i vari microrganismi e i loro rapporti con le lesioni ateromasiche, in particolare la \textit{Chlamydia pneumoniae}. Si possono proporre due ipotesi su come agirebbero. Ambedue si basano sulla loro proprietà di indurre la produzione da parte delle cellule endoteliali di una proteina chiamata “proteina da shock di calore” (HSP) che ha la caratteristica di provocare l’attivazione del sistema immunitario: 1) induzione di una reazione immunitaria crociata, da “mimetismo molecolare”, tra la HSP di origine infettiva e quella prodotta dall’endotelio in risposta ai fattori di rischio; 2) infezione delle cellule endoteliali, conseguente sintesi ed esposizione sulla loro superficie della HSP con attivazione della sorveglianza immunitaria innata. Studi sperimentali sono stati eseguiti e sono tuttora in corso allo scopo di accertare l’efficacia del trattamento antibiotico nel ridurre l’incidenza di eventi cardiovascolari acuti come l’ictus e l’infarto del miocardio. I risultati sono ancora inconcludenti. Probabilmente, per essere efficace, il trattamento deve essere iniziato in età giovane. È stata anche intrapresa la via della prevenzione tramite la vaccinazione contro i microrganismi coinvolti o l’induzione di tolleranza immunitaria verso la HSP. Quando verranno ulteriormente chiariti i meccanismi attraverso i quali gli agenti infettivi contribuiscono o sono all’origine dell’aterosclerosi potrebbero essere messe in atto delle misure terapeutiche o preventive efficaci con importanti ricadute sul piano clinico\textsuperscript{9}.

**Parole chiave:** Agenti infettivi; Aterosclerosi; \textit{Chlamydia pneumoniae}; Cytomegalovirus; Proteina da shock da calore.

**Acknowledgments**

The author wishes to thank Dr. Alessio Tommassetti for his skill and kind help in creating the figures.

**References**


*La versione italiana dell’intero articolo è disponibile su richiesta all’autore.
49. Kalayoglu MV. Chlamydial heat shock protein 60 and lipo-
poly saccharide: potential virulence determinants in atheroge-
50. Stratton CW, Sriman S. Association of Chlamydia pneumoni-
52. Campbell LA, Kuo CC. Chlamydia pneumoniae and athero-
53. Lindholt JS, Stovring J, Andersen PL, Hennegaer EW, Oester-
gard LA. A review of macrolide treatment of atheroscle-
rosis and abdominal aortic aneurysms. Curr Drug Targets
54. Siscovick DS, Schwartz S, Caps M, Wang SP, Grayston JT. Chlamydia pneumoniae and atherosclerotic risk in popu-
64. Ludewig B, Zinkernagel RM, Hengartner H. Arterial inflam-
68. Ciervo A, Oetzel SP, Gieffers J, Byrne GI. Chlamydia pneu-
70. Kälvegren H, Majeed M, Bengtsson T. Chlamydia pneumoniae
binds to platelets and triggers P-selectin expression and aggregation. A causal role in cardiovascular disease? Arteri-
E28.
72. Lopes-Virella MF, Virella G. The role of immune and inflam-
matory processes in the development of macrovascular disease in diabetes. Front Biosci 2003; 8: S750-S768.
74. Baer JT, du Laney TV, Wyrick PB, et al. Nuclear factor-xB acti-
77. Choi EY, Kim KS, Hong BK, et al. Upregulation of extra-
78. Kol A, Sukhova GK, Lichtman AH, Libby P. Chlamydia heat shock protein 60 localizes in human atheroma and regulates macrophage tumour necrosis factor-α and matrix metallopro-
80. Zügel U, Kaufman SH. Role of heat shock proteins in protec-
84. Lamb DJ, El-Sankary W, Ferns GA. Molecular mimicry in ath-
85. Kol A, Lichtman AH, Finnberg RW, Libby P, Kurt-Jones EA. Cutting edge: heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP 60 acti-