

Atherosclerosis, autoimmunity, and vascular-associated lymphoid tissue

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CLASSICAL CONCEPTS FOR THE DEVELOPMENT OF ATHEROSCLEROSIS

Atherosclerosis is a multifactorial disease induced by the effects of various risk factors on appropriate genetic backgrounds. It is characterized by vascular areas containing mononuclear and proliferating smooth muscle cells, as well as extracellular matrix (ECM)² components resulting in hardening and thickening (arteriosclerosis) of the arterial wall. In a strict sense, atherosclerotic lesions are localized in the intima; they also contain foam cells and deposits of cholesterol crystals manifested as fatty streaks and, finally, as atherosclerotic plaques.

Fatty streaks are whitish, cushion-like lesions of the arterial intima harboring abundant lipid-laden macrophages, so-called foam cells, which are considered precursors of the fully developed plaques that may finally exulcerate and even calcify. The main theories of atherogenesis are the "response to injury" (1) and the "response to altered lipoprotein" (2) hypotheses. The response to injury hypothesis postulates an alteration of the intima by various risk factors (mechanical injury, chemically altered low density lipoproteins [LDL], viruses, toxins) that initiates a primary endothelial dysfunction and subsequent changes in permeability, expression of adhesion molecules, and release of chemotactic and growth factors. Consequently, platelets and monocytes become activated and attach to these endothelial cells. Blood-derived monocytes transmigrate into the subendothelial space and transform into macrophages; smooth muscle cells (SMC) are attracted from the media to the same site. Both monocytes/macrophages and SMC possess the so-called scavenger receptor, which binds chemically altered (oxidized LDL [oxLDL]), but not native, LDL in a nonsaturable fashion. By uptake of oxLDL, macrophages and SMC develop into foam cells, and the deposition of collagenous and noncollagenous ECM components, especially in the peripheral "shoulder" region and the superficial "cap" area, complete the pathohistological appearance of fatty streaks and atherosclerotic plaques, respectively.

The response to altered lipoprotein hypothesis is based on the concept that lipoproteins can be chemically modified and are then able to induce foam cell formation by monocytes/macrophages and SMC. In recent years it has become evident that modification of lipoproteins does not occur primarily in the circulation or during transgression through the endothelium, but they rather accumulate in native form in the subendothelial space, where the lipoproteins are retained and oxidized. Accumulation of oxLDL, therefore, is not only the result of increased influx from the serum into the arterial intima but, conversely, is also due to a diminished efflux with subsequent foam cell formation. This "response to LDL retention" hypothesis (3) is thus a special variant of the response to altered LDL concept. Thus far, these latter theories have not explained why atherosclerotic lesions develop at certain arterial predilection sites or why the disease does not affect the venous vascular bed.

THE "IMMUNOLOGICAL HYPOTHESIS" FOR ATHEROGENESIS

During the past few years we have developed a new immunological hypothesis for atherogenesis (4–6) based on observations in humans and in animal experiments that encompass major components of both the response to injury and the response to altered lipoprotein hypotheses. Our studies were originally triggered by three factors: our work on the immunomodulatory role of lipids (7, 8), our long-

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² Abbreviation: hsp, heat shock proteins; ECM, extracellular matrix; LDL, low density lipoprotein; SMC, smooth muscle cells; oxLDL, oxidized LDL; IL, interleukin; MHC, major histocompatibility complex; IFN, interferon; TNF, tumor necrosis factor; MALT, mucosa-associated lymphoid tissue; GALT, gut-associated lymphoid tissue; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule-1; ELAM-1, E-selectin; HLA, human MHC; VALT, vascular-associated lymphoid tissue.

standing interest in autoimmunity (9, 10), and the increasing number of reports in the literature pointing to the possible primary or secondary contribution of humoral and cellular immune reactions to atherosclerosis (11–15). Thus, immunoglobulin and complement deposits can be demonstrated in advanced lesions, and evidence for complement activation via the alternative pathway has also been provided (16). A considerable number of lymphoid cells can be demonstrated within atherosclerotic plaques, most of which are CD4⁺ and interleukin-2 (IL-2) receptor⁺, i.e., activated, although CD8⁺ cells are also present (17). Major histocompatibility complex (MHC) class II (HLA-DR) antigens are expressed by the majority of T cells as well as by neighboring macrophages and SMC, indicating the production of interferon γ (IFN- γ) by the former (17). This fact is also supported by increased levels of neopterin, an IFN- γ -induced monocyte/macrophage product, in the serum of atherosclerotic patients (18). B cells are scarce in all stages of atherosclerotic lesions, rendering local antibody production rather improbable. Also, the number of intralésional granulocytes is surprisingly low. The cells constituting atherosclerotic lesions produce many different cytokines that stimulate growth, differentiation, chemotaxis, and cytotoxicity, including IL-1 (endothelial cells, SMC, macrophages), IL-8 (endothelial cells, macrophages), tumor necrosis factor α (TNF- α) (SMC, macrophages, T cells), monocyte chemoattractant peptide 1 (endothelial cells, SMC, macrophages), platelet-derived growth factor (PDGF) (endothelial cells, SMC, macrophages), and IFN- γ (T cells) (19).

In contrast to general belief, quantitative immunohistochemical analyses of the mononuclear cell infiltrate in the earliest stage of atherosclerotic lesions—in the transition zone between normal intima and fatty streaks—revealed a clear preponderance of T cells over macrophages, thus supporting the primary role of immune reactions in disease development (17). Furthermore, HLA-DR expression by endothelial cells was observed only in areas overlying T cell infiltrates in the intima, excluding the possibility of endothelial cells acting as primary antigen-presenting cells. We postulated sensitization of T cells at more distant sites, such as draining lymph nodes. However, Bobryshev et al. (20–22) have clearly shown that the presence of dendritic cells in the normal arterial intima suggests T cell sensitization at that site as well.

The majority of T cells in early lesions carry the T cell receptor α/β (TCR- α/β), but a surprisingly high percentage (10–15%) express TCR- γ/δ at levels far exceeding that in the peripheral blood of the same donors (23). Whereas the proportion of V $\delta^9/\delta 2$ TCR⁺ cells in peripheral blood remained low and constant throughout all lesional stages, the TCRV $\delta 1^+$ subpopulation made up most of the TCR- γ/δ cells in

very early lesions. These cells are characteristic for mucosa-associated lymphoid tissue (MALT), such as the gut-associated lymphoid tissue (GALT), and preferentially react to heat shock proteins (hsp) (24).

Whereas the T cell repertoire in early atherosclerotic lesions has not yet been determined, no indications of restricted TCR V-gene usage have been found in analyses of T cell clones derived from atherosclerotic plaques (25).

Candidate antigens that may lead to atherosclerosis-associated humoral and/or cellular immune reactions include (12): 1) modified lipoproteins; 2) partly degraded or denatured macromolecules derived from the necrotic core of plaques; 3) intracellular cryptic antigens exposed through cytolysis or necrosis; 4) neoantigens: hsp or cytokine-induced cell surface antigens; and 5) antigens of infectious organisms such as herpes virus, cytomegalovirus, and *Chlamydia pneumoniae*.

To date, definitive experimental proof that one of these antigens may initiate the pathogenesis of atherosclerosis is available only for hsp (26). Thus, autoantibodies to oxLDL are now considered to have a protective effect (27), and induction of disease by immunization of experimental animals with oxLDL has not been achieved. There may, of course, be exceptions to the concept that immune reactions are instrumental in atherogenesis, especially where excessive overloading of the system with cholesterol occurs due to severe genetic conditions, such as the lack of certain lipoproteins or lipoprotein receptors with or without concomitant immune deficiency (28), homocystinuria, etc.

ATHEROSCLEROSIS: AN AUTOIMMUNE DISEASE

Our own clinical and experimental data provide evidence that atherosclerosis starts as an inflammatory immunological disease due to an autoimmune reaction against the stress protein hsp 60.

Stress proteins (or hsp) comprise a family of proteins and its cognates that are induced by various forms of stress such as temperature, mechanical irritation, infections, and toxins (29). Hsp are classified according to their molecular mass into four main groups: hsp 90, hsp 70, hsp 60 kDa, and low molecular mass families.

They fulfill various physiological functions such as the assembly, intracellular transport, and controlled breakdown of proteins; many hsp act as chaperones to protect other proteins from denaturation when cells are subjected to stress. Hsp are phylogenetically highly conserved. Thus, mycobacterial hsp 65 show greater than 50% sequence homology with human hsp 60 on the DNA and protein levels. Hsp constitute major antigenic determinants of microorganisms and

are thus important for induction of protective humoral and cellular immune responses, although we “pay” for this protection by risking consecutive autoimmune reactions that may result in the development of autoimmune disease (30, 31).

Evidence that the initial stages of atherosclerosis are mediated by an immune reaction against hsp 60 can be summarized as follows: Immunization of rabbits with hsp 65-containing material, such as complete Freund’s adjuvant alone or recombinant mycobacterial hsp 65, develop arteriosclerotic lesions at typical predilection sites that are subject to increased hemodynamic stress, including the aortic arch and major arterial bifurcations. Pathohistologically, these lesions consist of activated T cells, macrophages, SMC and ECM deposition, but no foam cells (17). Concomitant feeding of a cholesterol-rich diet induces the development of classical fatty streaks and atherosclerotic plaques, including foam cells, that are more severe than those induced by cholesterol feeding alone and completely parallel the human situation (26). Whereas the first inflammatory stage is reversible, the later cholesterol-augmented stage is not, at least under our experimental time schedule (32). The peripheral blood of hsp 65-immunized rabbits contains hsp 65-specific antibodies and T cells. T cells derived from arterial lesions of these rabbits show a significantly increased reactivity to hsp 65 compared to T cells from peripheral blood of the same animals. T cells isolated from atherosclerotic lesions of rabbits that were not immunized, but received a cholesterol-rich diet, also revealed a preferential reactivity with hsp 65 (33).

Since hsp 65/60 has been incriminated as a possible (auto)antigen in a variety of autoimmune diseases (rheumatoid arthritis, diabetes, systemic lupus erythematosus, scleroderma) (30, 34–37), it is of relevance that immunization of rats with hsp 65 led to adjuvant arthritis without arteriosclerosis (34; our own unpublished observations), whereas the reverse is true for rabbits (26). Inducing hsp 65-dependent arteriosclerosis in mice has also met with limited success. These differing responses may be due to both recognition of different hsp 65/60 epitopes by the immune system and differing target cell susceptibility (i.e., endothelial cells vs. synovial cells) to different stressors in different species.

Establishing T cell lines and clones from very early human lesions has so far been complicated by technical problems such as the relative paucity of infiltrating cells, difficulties excluding T cells adhering to arterial endothelium or contained in *vasa vasorum*, or insufficient supply of antigen-presenting cells. Nevertheless, we have demonstrated significantly higher titers of anti-hsp 65 antibodies in the sera of many clinically healthy volunteers with sonographically proven atherosclerotic carotid artery lesions vs. controls without such lesions (38), an observation re-

cently corroborated in coronary atherosclerosis patients. Immediately after myocardial infarction, these patients experience a drop in anti-hsp 65 titers, probably due to the sudden liberation of hsp 60 and subsequent immune complex formation (39). The anti-mycobacterial hsp 65 antibodies cross-react with recombinant human hsp 60 and also yield a 60 kDa band in Western blots with extracts of human atherosclerotic plaques. Furthermore, these antibodies react with GroEL, the *Escherichia coli* hsp 60 homologue and *Chlamydia* hsp 60 (Fig. 1). The presence of anti-hsp 65/60 antibodies has been defined as a new risk factor for atherosclerosis independent of classical risk factors such as high blood cholesterol values, smoking, overweight status, and gender. So far, three linear hsp 65 epitopes have been shown to be recognized by human anti-hsp 65 antibodies, two with high homology for human hsp 60 (40). The reactivity with—probably more important—conformational epitopes is still under investigation.

Certain hsp 60 epitopes could be demonstrated on the surface of stressed (heat, TNF- α , H₂O₂, bacterial endotoxin), but not unstressed, cells (41–44). The mechanism whereby hsp 60 or portions of this intracellular, mainly mitochondrial protein are transported to the cell surface is still under investigation in several laboratories (45). It has, however, been firmly established that anti-hsp 65/60 antibodies exert a cytotoxic effect on stressed, but not unstressed, endothelial cells and macrophages in a complement-mediated fashion or via antibody-dependent cellular cytotoxicity (46, 47). This effect can also be blocked by *E. coli* GroEL.

To allow for interaction of hsp 65/60-specific T cells with endothelial cells, the expression of adhesion molecules and hsp 60 is required. The latter

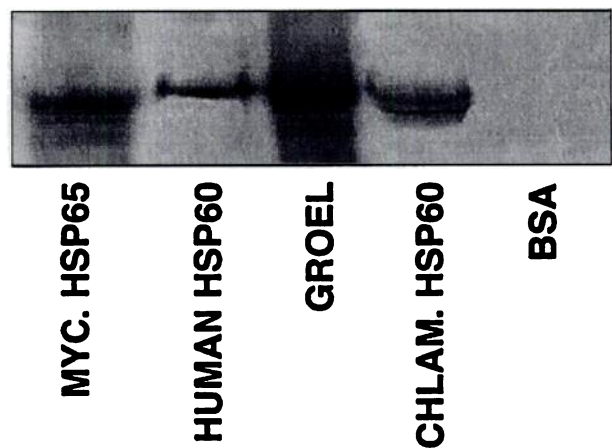


Figure 1. Western blot showing the cross-reactivity of human anti-hsp antibodies (affinity chromatography purified from a pool of 5 high titer sera of clinically healthy persons with sonographically demonstrable atherosclerotic lesions in the *A. carotis* with recombinant mycobacterial hsp 65, human hsp 60, *E. coli* GroEL, and hsp 60 from *Chlamydia pneumoniae*. Bovine serum albumin (BSA) was used as a negative control antigen.

must be presented in association with HLA class I or class II molecules. However, as mentioned earlier, a primary aberrant HLA class II expression has only been observed on human endothelial cells after initial T cell infiltration (17). On the other hand, HLA class II-positive macrophages and dendritic cells are present at predilection sites in the intima, and may stimulate hsp 65/60-specific T cells after their transmigration to this site (22). Since the affinity of the TCR for the MHC-peptide complex is far too low for interaction of hsp 65/60-specific T cells with stressed endothelial cells under arterial blood flow conditions, adhesion molecule expression is critical to the initiation of an immune response. Application of various types of stress to human arterial and venous endothelial cells leads to the simultaneous expression of hsp 60 and adhesion molecules (intercellular adhesion molecule 1, ICAM-1; vascular cell adhesion

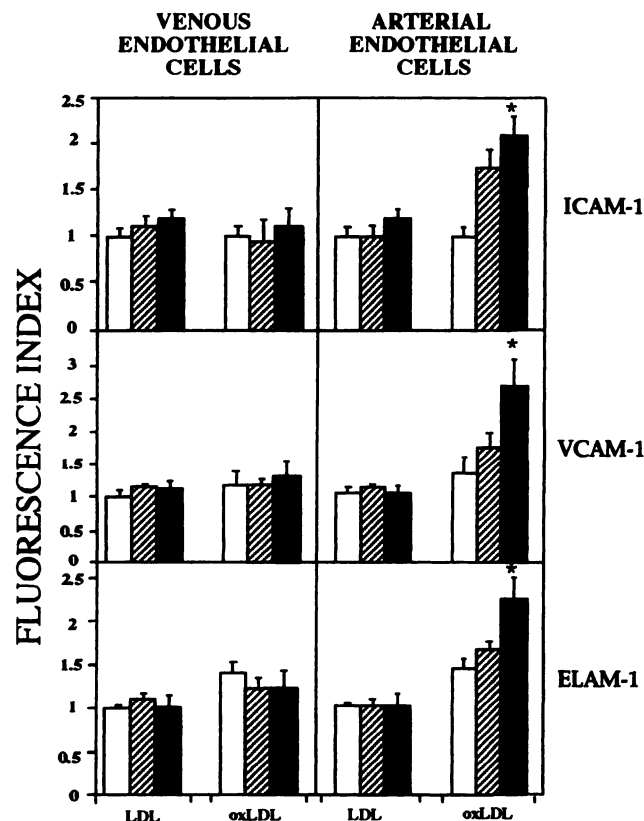


Figure 2. Fluorescence activated cell sorter (FACS) analysis of adhesion molecule expression by human venous and arterial endothelial cells after stress by incubation with oxLDL and native LDL as a control. Confluent arterial endothelial cell monolayers were treated with 80 μ g/ml native or oxLDL. The fluorescence index was calculated as the ratio of the mean fluorescence intensity of stressed vs. unstressed endothelial cells. Expression of ICAM-1, VCAM-1, and ELAM-1 in terms of mean values and standard deviations of four independent experiments are shown. Preparation and characterization of oxLDL as well as incubation conditions are detailed in ref 49. Incubation for 12 h (\square); 24 h (\blacksquare); 48 h (\blacksquare). *Significant difference from control cells $P < 0.05$.

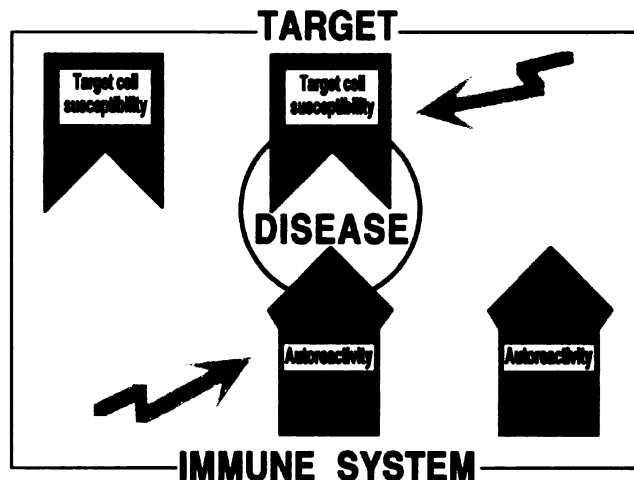


Figure 3. The development of autoimmune disease depends on the presence of essential genes coding for autoreactivity of the immune system and susceptibility of the target cell for the autoimmune attack, respectively. Nonessential factors modulating the final outcome of the disease are symbolized by arrows. Essential genes influencing autoreactivity may, for example, code for certain MHC haplotypes, T cell receptor specificity, and interleukin-2 (IL-2) hyperproduction. Essential genes affecting a target cell or target organ may code for susceptibility to virus infections. Modulatory factors include dietary components (such as iodine in the case of Hashimoto thyroiditis) and hormones (glucocorticoids, sex hormones, and others).

molecule-1, VCAM-1; E-selectin, ELAM-1) (48, 49). There are distinct differences in the susceptibility of arterial and endothelial cells to different stressors, the former generally giving a more sustained response, especially with respect to ELAM-1. In fact, oxLDL acts as a potent inducer of adhesion molecules and hsp 60 exclusively on arterial endothelial cells, and the efficiency of oxLDL as a stressor depends on its degree of oxidation (Fig. 2). However, the group of Fogelman (50) has clearly shown that prolonged action of minimally modified LDL is also atherogenic.

Based on our experience with animal models of spontaneous or experimentally induced organ-specific and systemic autoimmune diseases, we earlier developed a general concept for the development of autoimmune diseases postulating that *two* sets of essential genes must be present for autoimmune disease development in an individual or an inbred strain of animals (51). One set of genes codes for an abnormal autoimmune reactivity of the immune system, the other for a primary susceptibility of the target organ/structure to attack by humoral and/or cellular autoimmune effector mechanisms. The definitive outcome of a given disease is then fine-tuned by modulatory factors such as diet and hormones (Fig. 3).

This concept also applies to our immunological hypothesis for atherogenesis, which can be summarized as follows (Fig. 4). Most human beings possess hsp 65-reactive humoral antibodies and T cells that afford

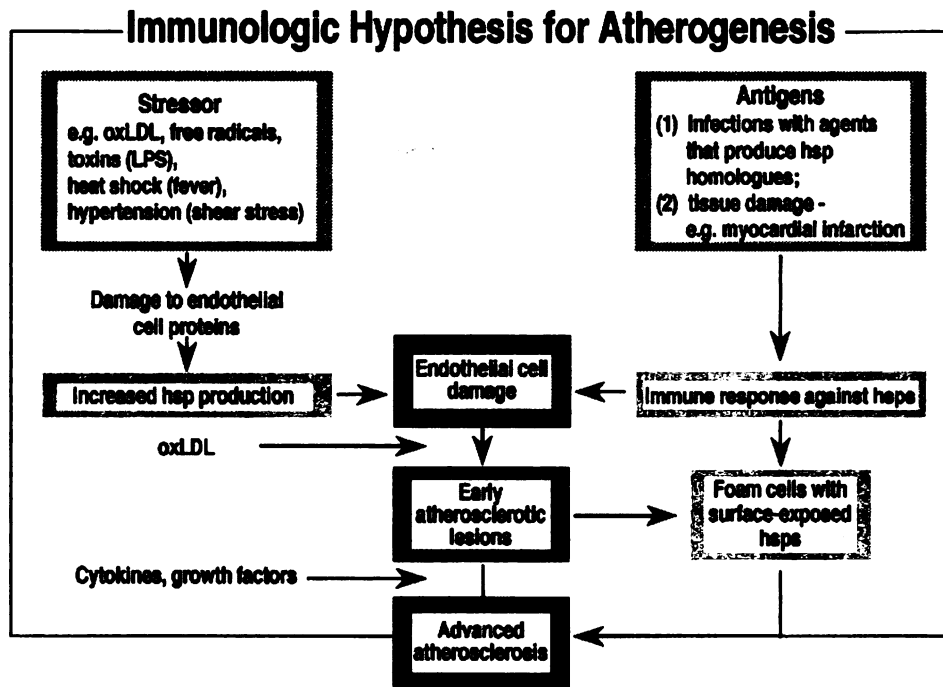


Figure 4. Schematic representation of the immunologic hypothesis for atherogenesis and the potential role of the immune response to hsp 65/60. Different stressors such as hypertension, oxLDL, free radicals, bacterial toxins, and heat shock lead to the expression of hsp 60 by endothelial cells, arterial cells showing a lower threshold to various risk factors due to their previous, life-long exposure to higher hemodynamic stress as compared to veins. Preexisting antibodies and T cells against hsp 60 from infectious agents or hsp 60 released from damaged autologous tissue can cross-react with hsp 60 expressed on the surface of endothelial cells leading to a local inflammatory autoimmune reaction. LDL may be chemically modified (for instance, oxidized) by stressed endothelial cells or LDL present in the intima can be oxidized and retained at this site. Blood-derived macrophages and smooth muscle cells present at those sites can take up chemically modified LDL via the scavenger receptor and transform it into foam cells. These foam cells are important constituents of early atherosclerotic lesions and are themselves subjected to stress due to the production of free radicals during metabolism of oxLDL. Therefore, foam cells also express high amounts of hsp 60 that lead to stimulation of locally present hsp 65/60-reactive T cells and thus perpetuation of the disease. These foam cells may then be killed by cytotoxic antibodies and/or T cells and become components of the necrotic core of advanced atherosclerotic lesions.

vital protection from various potentially dangerous microorganisms. These polyclonal effector mechanisms recognize various hsp 65 epitopes, several of which provide the basis for potential pathogenetic cross-reactions with human hsp 60. The emergence of a bona fide autoimmune reaction, therefore, depends exclusively on expression of specific disease-determining hsp 60 epitopes at certain target structures, such as vascular endothelial cells in atherosclerosis or synovial cells in rheumatoid arthritis. In an individual patient, the development of atherosclerosis or rheumatoid arthritis, or both, thus depends not only on the specificity and potency of the immune response to hsp 65/60 (determined, among other factors, by the HLA constellation), but also on the expression site of the appropriate antigenic determinant (or determinants). The development of the first inflammatory stage of atherosclerosis therefore depends on how we "treat" our endothelial cells, i.e., whether hsp 60 expression is induced via classical risk factors such as high blood pressure, overweight, smoking, and increased serum cholesterol levels. Other atherosclerosis-inducing factors also need to be considered, such as viral and bacterial in-

fections of the vessel wall itself (52–54). Humans develop atherosclerosis rather than venosclerosis because arterial endothelial cells are subjected to higher blood pressure than veins throughout life, thus lowering the threshold for the adhesion molecule and hsp 60-inducing effect of other risk factors. Hsp 60 and adhesion molecule expression can first be found at predilection sites for atherosclerotic lesions where special hemodynamic conditions prevail.

This concept is also supported by the observation that venous coronary bypasses also become "atherosclerotic" once subjected to arterial pressure and flow.

In conclusion, the immunological hypothesis for atherogenesis logically encompasses the classical response to injury and response to altered lipoprotein theories, but views them from a new and unconventional aspect.

VASCULAR-ASSOCIATED LYMPHOID TISSUE (VALT)

In the course of studies that led us to formulate the above-noted concept of a primary immunologic in-

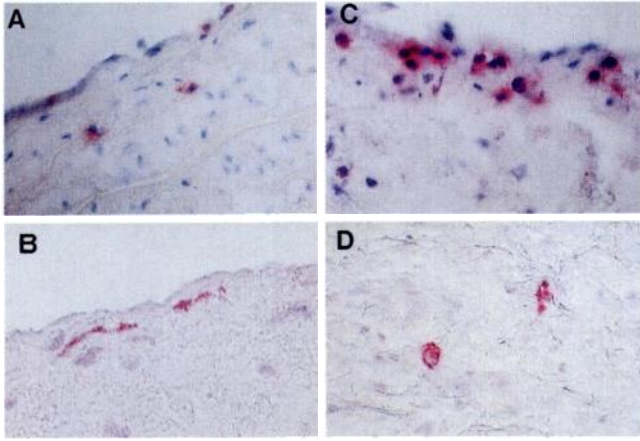


Figure 5. Immunohistochemical demonstration of cells contributing to the formation of the VALT using the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. Positive cells are stained red using the naphthol AS-TR phosphate/fast red RC reaction product of alkaline phosphatase. Different cell types in intima of the carotid bifurcation: A) CD68⁺ macrophages; 8-year-old child; B) CD1a⁺ dendritic cells; 10-year-old child; C) CD3⁺ T lymphocytes; 8-year-old child; D) tryptase⁺ mast cells; 10-year-old child. Original magnification $\times 630$.

flammatory pathogenesis of atherosclerosis, we also investigated normal, unaffected arteries for control purposes. These included carotid bifurcations (i.e., the *A. carotis communis* dividing into the *A. carotis interna* and *externa*) from essentially healthy children, aged 8 wk to 8 years, who were either victims of accidents or died from sudden infant death syndrome.

These investigations led to the unexpected and previously unknown finding that mononuclear cells preexist in the intima at bifurcation sites, which are known to be predisposed to development of atherosclerotic lesions later in life. Analogous to the classical system providing a local defense mechanism, the mucosa-associated lymphoid tissue (MALT), we called these accumulations of mononuclear cells in the arterial intima the vascular-associated lymphoid tissue (VALT) (5), and provisionally assigned a similar function to this newly discovered system: monitoring of internal vascular surfaces for potentially harmful blood-borne agents. However, mononuclear cells making up the VALT are, of course, far less abundant than those accumulations observed in the MALT, e.g., GALT. Our findings concerning the VALT can be summarized as follows: Mononuclear cells are always found in certain arterial regions that are subject to major hemodynamic stress, but rarely, if ever, in the intima of veins. Although the proportion of monocytes/macrophages vs. CD3⁺ T cells (Fig. 5A, C; Fig. 6) differs in different specimens, overall the latter clearly predominated.

In general, CD4⁺ predominate over CD8⁺ T cells. Most T cells express TCR- α/β , but TCR- γ/δ -carrying cells are also present, paralleling earlier observations

in very early atherosclerotic lesions and also supporting the notion that hsp 65/60 may be involved in initiating atherogenic immune responses later in life. Mononuclear cells were found not only in the intima, but also around the *vasa vasorum*. We have no functional data indicating whether the intimal cells had immigrated from the vascular lumen or from the *vasa vasorum* via the media.

In several recent publications, Bobshyrev et al. (20–22) compellingly described the presence of dendritic cells in the intima of atherosclerotic lesions. In preliminary experiments (G. Falkensammer and G. Wick, unpublished results), we also identified dendritic cells in the intima of healthy arteries from children (Fig. 5B).

Another population of cells receiving increasing attention in atherosclerosis research are mast cells. However, in contrast to earlier observations by others (55, 56) describing mast cells in the normal intima and adventitia, but not in the media, we have not yet been able to corroborate these findings. We detected chymase/tryptase-positive mast cells in the normal adventitia and in the media only in close association with the *vasa vasorum* (Fig. 5D). Mast cells may contribute to atherogenesis in several ways, such as by the chymase-induced, granula-mediated uptake of LDL by macrophages and SMC (57) and chymase-dependent inhibition of the reverse cholesterol transport, and thus promote foam cell formation (58). Moreover, histamine leads to increased endothelial permeability, another important factor contributing to formation of atherosclerotic lesions. Other important properties of histamine include a mitogenic effect on fibroblasts and SMC-contracting activity (59). Mast cells also produce lymphocyte chemotactic factor, which contributes to the recruitment of T cells into inflammation sites (60). In addition, mast cells store a variety of cytokines (TNF- α and IL-1) known to induce a expression of adhesion molecules and hsp 60. Finally, mast cells have been shown to express

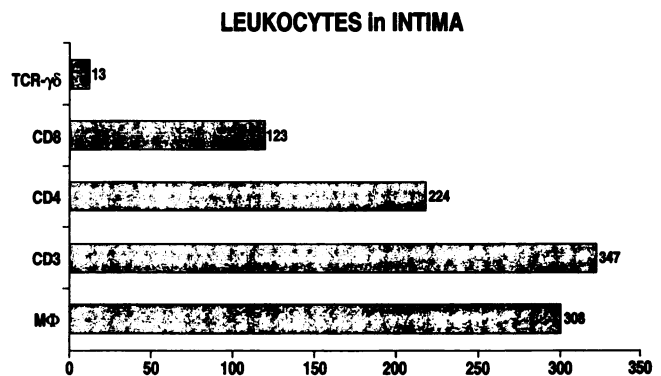


Figure 6. The vascular-associated lymphoid tissue (VALT). Distribution of immunohistochemically visualized mononuclear cells in frozen sections of carotid arteries from six children aged 8 wk–8 years. Total sum of cells counted per 5 mm² section area. TCR- γ/δ = T cells γ/δ ; MØ = macrophages.

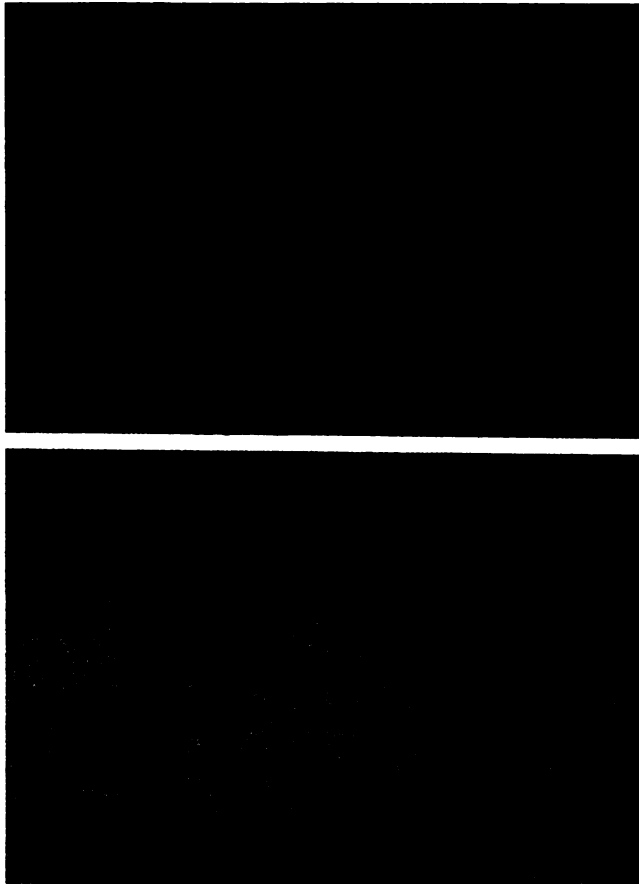


Figure 7. Demonstration (using the APAAP technique) of P-selectin in endothelial cells at the bifurcation area of the carotid artery of an 8-wk-old child. A) Staining of the carotid endothelium as well as the endothelium of *vasa vasorum*. Original magnification $\times 100$. B) Staining of the endothelium of *vasa vasorum*. Original magnification $\times 400$.

MHC class II molecules and may function as antigen-presenting cells (61). Thus, this type of cells will be included in ongoing and future studies of the VALT and early atherosclerotic lesions.

K/NK cells, polymorphonuclear granulocytes, and B cells were virtually absent from the carotid arteries of all children. Hsp 60 expression was observed in endothelial cells only at sites subjected to increased mechanical stress, such as the side walls of the bifurcations, and also the endothelium of the *vasa vasorum*. Furthermore, hsp 60-positive macrophages that simultaneously express MHC class II molecules were found in the intima.

Immunohistochemical analyses of adhesion molecules revealed the following: There seems to be a constitutive basic expression of ICAM-1 and P-selectin in endothelial cells of normal carotid arteries, confirming data published by others (62–64), as well as the *vasa vasorum* (Fig. 7), but not of any other cell type within the intima, media, or adventitia. This expression is independent from underlying elements of the VALT.

ELAM-1 was absent throughout all specimens investigated. Since adhesion of neutrophils is dependent mainly on the presence of this molecule, this partly explains the lack of this cell type within the VALT.

VCAM-1 can be demonstrated on both endothelial cells and macrophages within the VALT, and may be instrumental in recruiting further mononuclear cells and the later possible development of atherosclerotic lesions.

The simultaneous up-regulation of adhesion molecules ICAM-1, ELAM-1, and VCAM-1, together with hsp 60 in endothelial cells subjected to classical risk factors for atherogenesis (mechanical stress, oxLDL, cytokines) shown in previous experiments in our laboratory, is considered the primary prerequisite for initiation of the inflammatory-immunological process and subsequent lesion development.

CONCLUSIONS

Our new immunological hypothesis for the development of atherosclerosis postulates an autoimmune reaction against hsp 60 as a main initiating factor. Since essentially all individuals possess cellular and/or humoral immune reactivity against bacterial hsp 65 due to previous infections or vaccinations, this protective immunity harbors the risk of cross-reactivity with the highly homologous autologous hsp 60. The emergence of disease based on this cross-reactivity depends on the specificity of hsp 65/60-reactive T cells and antibodies (i.e., a given individual's immune response genes and the type of infection, respectively); on the other hand, it also depends on the organ-specific or systemic expression of the cross-reactive hsp 60 epitopes. For unknown reasons, in rheumatoid arthritis the relevant epitopes seem to be expressed by synovial cells, whereas in atherosclerosis, the target is the endothelial cell. The latter cell type has also been shown to express hsp 60 epitopes on the cell surface, rendering it susceptible to cytotoxic antibodies. We do not dispute the importance of classical risk factors for atherogenesis such as high blood pressure, oxLDL, smoking, and even infections, but assign a different role to them: the simultaneous induction of adhesion molecule and hsp 60 expression by endothelial cells. The fact that arterial endothelial cells are subjected to higher hemodynamic stress during their lifetime lowers their threshold for the induction of adhesion molecules and hsp 60 by other risk factors. Therefore, it depends essentially on how we "treat" our vascular endothelial cells if the preexisting immune reactions entail intimal T cell infiltrations and antibody-mediated cytotoxic damage. Even though this first inflammatory stage of atherosclerosis has been shown to be reversible, continuous presence of risk factors, especially high blood

cholesterol levels, lead to irreversible, severe lesions. Predilection sites for atherosclerosis are known to be those vascular segments subjected to increased hemodynamic forces, such as the arterial bifurcations. We have observed previously unknown accumulations of mononuclear cells in the intima at these sites even in arteries of children before the emergence of atherosclerosis, and have tentatively termed this phenomenon vascular-associated lymphoid tissue, analogous to the mucosa-associated lymphoid tissue. Similar to MALT, VALT may monitor the internal vascular surface for blood-borne, potentially dangerous, or cross-reactive agents. VALT may thus also constitute a crystallization point for recruitment of hsp 65/60-reactive T cells during early atherogenesis.

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