

ATHEROGENESIS AND LYMPHSTASIS ...CONNECTING THE DOTS

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ABSTRACT

“The Role of Lymphstasis in Atherogenesis” appeared in the Annals of Thoracic Surgery in the March 1981 issue which examined the significance of lymphatic involvement in arteriosclerosis. Presently, the underlying premise of the original paper is reviewed with its relationship to recent information regarding reverse cholesterol transport.

Since then, an increasing number of articles have been published identifying the proteins and peptides that play a critical role in lymphatic clearance of arterial wall cholesterol. This process, its relationship to the 1981 article, and a concept of arteriosclerosis that explains the scientific merit of the beneficial effects of lifestyle is presented.

Keywords: arteriosclerosis, inflammatory Cells, inflammatory mediators, plasma lipids

The critical role of lymphatic clearance in atherogenesis was proposed in an article in the Annals of Thoracic Surgery in March 1981(1). The article was received with interest, generating requests for reprints in about 12 countries. However, few articles were published on this topic over the next two decades (2-5). I believe there were several reasons for this, namely: the novelty of the postulation, the difficulty in researching lymphatic function (6), limitations of biochemical, physiological and immunologic

analysis and understanding at that time, and perhaps the belief of cardiac surgeons that this area was outside their scope of interest.

The paper hypothesized “that lymphstasis slows the transit of the local proteins in the arterial wall, and that this prolonged presence increases tissue and lipid phagocytosis by the intramural foam cells” and “lymphstasis, either congenital (hereditary) or acquired (stress, sympathetic stimulation, poor diet, and anoxia, hypertension), could play a pivotal role in the development of atherosclerosis.” Of course, at that time, the role of inflammation, cell adhesion molecules, cytokines and neuropeptides had not been delineated. These significant contributions were acknowledged in the 2000 publication (7) of “the unifying concept of arteriosclerosis.” In the 1981 article the possibility of their existence was prefigured in the statement “local changes of type, amount, or physical properties of the mucopolysaccharides may represent the initial lesion (viz. atherosclerotic), and observation paralleling the connective tissue proliferation and increased cellularity seen in lymphstasis.” At that time, reverse cholesterol transport was an evolving concept with little consideration for the role of the lymphatics in the process. Nevertheless, the paper proposed that “since HDL can transport cholesterol from tissue back to the liver by way of the lymphatics for excretion, this enhanced lymphatic clearance may be the explanation for its beneficial effect.”

Much has changed in our approach to arteriosclerosis in the last 15 years. Great

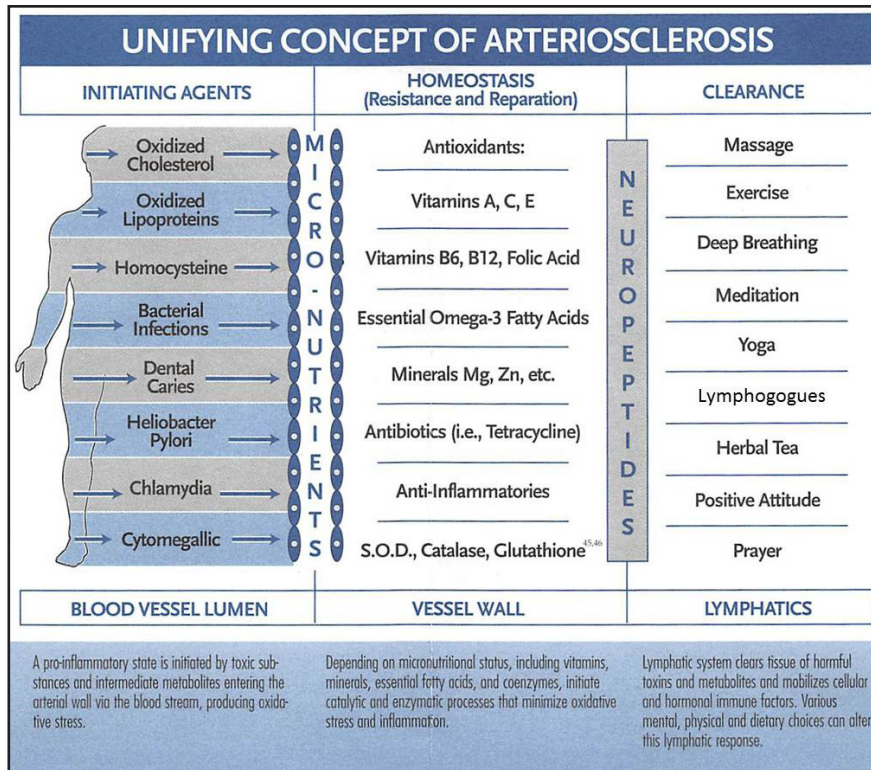


Fig. Unifying concept of arteriosclerosis (see text for further details).

strides have been made in identifying, labeling and mapping of the markers, proteins and pathways of inflammation, as well as normal and abnormal arterial metabolism. Investigators have taken a greater interest and participation in basic research (8). Most importantly, the recent investigation of the role of the lymphatics in reverse cholesterol transport has produced an increasing number of papers supporting this concept (9-15). This article is written to review the underlying premise of the original paper, to update it based on new scientific data and to present a way of explaining the overall disease process suggesting “the unifying concept of arteriosclerosis” (Fig. 1).

Cholesterol is normally delivered to the tissue through the capillaries, to be utilized to create cell membrane, bile salts, hormones, pro-vitamins and other necessary metabolites (16). Coincidentally, because of turbulence

(17), hypertension (18), aging, inflammation and metabolic disorders, the arterial endothelium becomes dysfunctional (19) or denuded and loses its integrity (20), permitting LDL-cholesterol (especially the small dense particles (21)) and HDL to pass into the intima. The LDL rapidly becomes oxidized (22) initiating an endothelial inflammatory process that involves vascular cellular adhesion molecules, selectins and other pro-inflammatory proteins (23). Monocytes are attracted; they subsequently migrate into the intima, becoming macrophages and enhancing the inflammation by secreting other pro-inflammatory cytokines and chemokines (22). It is well to remember that the inflammatory process can be initiated by other than oxidized LDL – for example, Apo-B particles (24), homocysteine (25), infection (26), radical oxygen species, etc. (27). Macrophages then engulf the

cholesterol, subsequently transform into foam cells and, unless they can transfer the cholesterol or migrate through the arterial wall, create further inflammation and deposition of the cholesterol.

Development of arteriosclerosis depends on how efficiently the cholesterol can be cleared from the sub-intimal space (28). In the smallest arteries this can occur with little difficulty but in the large and mid-sized arteries, the high-density lipoprotein and foam cells must pass from the intima through the internal elastic lamina and the inner half of the media (9) before coming into contact with lymphatic vessels. In the past, passage of cholesterol through the arterial wall was not considered essential in reverse cholesterol transport because of the thickness of the arterial wall, the integrity of the internal elastic lamina, and the distance to the lymphatic vessels.

The inflamed, hypertrophied arterial wall and foam cells manufacture enzymes, cytokines, and chemokines, creating oxidative stress, which immobilizes the macrophages and increases the fibroblasts and collagen matrix, further slowing down the cholesterol's progression to the lymphatics (23). Myeloperoxidase is expressed by the cells and alters the discoid HDL (29) rendering it unable to transfer the cholesterol from the foam cells. Egress is further delayed by the expression of certain neuropeptides like netrin-1 (30), neuropeptide Y (31) and neuropeptide substance P and Sema3A. (32). All of these neuropeptides play an inflammatory role, preventing reverse cholesterol transport. Netrin 1, a neural guidance cue, is secreted by the foam cells in the atheroma and is a powerful chemoattractant and smooth muscle cell recruiter and retardant to macrophage exit from the arterial wall to the lymphatics (30). It appears then that foam cells play a critical but reversible role in atherogenesis (33). These lipid laden macrophages remove significant amounts of cholesterol when unimpeded. However, in the presence of oxidized LDL, their migration

can be inhibited (22) due to NADPH oxidase mediated reactive oxygen species. The mechanism by which this occurs is the loss of plasticity of the cellular actin cytoskeleton. Normal plasticity can be restored by addition of the antioxidants resveratrol, N-acetyl cysteine, and NADPH oxidase inhibitors which normalize dynamic activation (22). This does not diminish the significance of cholesterol clearance by ABCA1 transporter transfer to nascent HDL. The 1981 article (1) recognized the significance of the HDL particle as an integral component of this hypothesis, proposing that the cholesterol laden HDL particle is returned by way of the lymphatic system to the liver. The reasoning behind this is that the mature cholesterol laden particle is an inanimate molecule that must respond to pressure convection and gradient in the arterial wall (34-36), unlike its potential egress from the capillary where there is a pressure gradient reversal and capillary spaces where the extravascular HDL can access the venous system. Furthermore, the *in vivo* work of Nordestgaard (3) clearly showed that the overwhelming percentage of HDL cholesterol that entered the arterial intima penetrated the arterial wall beyond the elastic lamina. The results suggested that the most important efflux route of HDL was through the lymphatics and vasa vasorum of the outer media and adventitia. Lastly, a number of studies (37-40) demonstrated a significantly higher percentage of cholesterol laden HDL in the lymph than in the plasma, implying the lymphatic route of reverse cholesterol transport.

The final factor in the delay of cholesterol clearance in the arterial wall is the function of the lymphatic system (10,12,14). The lymphatic system is not just a passive conduit (41) for interstitial fluid balance but is essential for lipid and protein transport (1). The lymph endothelial cell not only responds to inflammation-producing peptides and proteins, but also expresses them (42,43). Lymph channels contain contractile smooth muscle fibers and autonomic nerve endings

(1). The lymphatic system is the immune domain, warehousing lymphocytes and providing a conduit for them. It is intimately connected with the nervous and endocrine systems.

There are many causes for slowing of lymphatic clearance, such as inflammation (1), absence or diminished lymph channels, viscosity of lymph liquid, tissue pressure gradient, gravity, sclerosis of the lymphatic vessels, radiation, poor muscle pumping action, sluggish lymph peristalsis, shallow breathing, and/or a diet poor in flavonoids and polyphenols (1). Increasing lymphatic flow can diminish the oxidative stress, increasing cholesterol clearance and potentially decreasing atherosclerotic deposition (14). Recognizing the involvement of the lymphatic system in atherogenesis helps shed new light on the process. Evidence of dendritic cells, T cells and mast cells in the arterial wall opens the possibility of arteriosclerosis being an immune or even autoimmune disease (44-47).

Other than the pursuit of scientific understanding, there are two especially compelling reasons to pursue this area of special interest. The first is that lymphatic involvement in atherogenesis permits us to formulate an overarching, unifying concept in the development of arteriosclerosis (7) and helps explain the inverse relationship between cardiovascular disease, exercise and deep breathing, high vegetable diet, stress modification (1) and the direct relationship to sedentary lifestyle, stress, a pro-inflammatory diet and reactive oxygen species (7). Secondly, establishing the involvement of lymphatics with atherogenesis underscores, explains and encourages a lifestyle of exercise, healthy diet, and stress modification, to prevent or abate cardiovascular disease.

A diet high in vegetables and fruit has much scientific evidence that it is cardiovascular protective (48-51). All plants contain flavonoids and polyphenols which are lymph stimulating, (52-57). Lymphogogic products are essentially compounded flavonoids.

Daflon, consisting of the flavonoids hesperidin and diosmin, stimulates lymphatic drainage by increasing the total number of functional lymphatic capillaries, as well as intensity and frequency of lymphatic contractions (54-57). It reduces endothelial expression of the adhesion molecules ICAM-1 (intercellular adhesion molecule 1), VCAM-1 (vascular cell adhesion molecule 1), and L-selectin. The result is reduction of adhesion, migration, and activation of leukocytes, leading to lowering of prostaglandin PGE2, PGF2a and radical oxygen species (57). Vitamin D has been shown to slow the ingress of monocytes and dendritic cells into the intima and, in addition, downregulates the inflammation of the macrophages and monocytes (51,52).

Exercise is considered part of an optimum lifestyle for cardiovascular health. Besides positively affecting anti-inflammatory markers and reducing pro-inflammatory ones (60), exercise considerably increases lymphatic circulation (61). Since it is necessary for the lymph to egress through the chest cavity to the subclavian vein, where it returns into the bloodstream and ultimately reaches the liver, its circulation is affected by rapid, deep breathing (1). Such respiration creates larger diaphragmatic excursions and increases negative intrathoracic pressure, thus will stimulate lymphatic flow maximizing lipid clearance (1).

The complete cycle of lymphatic circulation is about two days (1). The life of an HDL particle is four days (62) in contradistinction to a macrophage lifespan of several weeks to months (63). This means that, with exercise, by tripling the lymphatic flow (1) we can significantly increase cholesterol clearance. This implies more rapid circulation and egress of the macrophages, dendritic cells and T cells that recent studies have shown to be increasingly significant in atherogenesis (10,15,23).

Both physical and mental stress play an important role in the development of inflammation and atherosclerosis (64,65). Stress has been documented to increase

cortisol and epinephrine (59,60), thus creating an immune suppression and a subsequent decrease in lymphatic function. Increases in long-term cortisol levels create lymphatic atrophy (66,67) which can then decrease reverse cholesterol transport. Smoking induces oxidative injury, promotes altered eicosanoid production and contributes to the dysfunction of lymphatics under normal conditions, as well as a variety of clinical disorders (68).

In summary, recent and developing information regarding the lymphatic system's significant role in reverse cholesterol transport and atherogenesis begs a series of questions regarding our current laboratory tests for detecting arteriosclerosis. Besides examining the standard lipid profile, C-reactive protein (CRP), LDL particle size and Apo A/Apo B ratio, should we also consider indicators of oxidative stress such as IL-6 and F2-isoprostane, along with the CRP? Should we measure the indicators of immune status such as the CD4/CD8 ratio, CD3 mature T cells and NK cells and antioxidant status? I believe that these are exciting times for research in the genesis of arteriosclerotic vascular disease, stimulating the development of new drugs and lifestyle modalities that can prevent or ameliorate this serious problem for a growing world population. Furthermore, it is not only opportune but appropriate that cardiovascular investigators should be encouraged to become more deeply involved in the understanding of the emerging aspects of the lymphatic system in atherosclerosis.

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