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Forum Review

Microvascular Responses to Hypercholesterolemia: The Interactions Between Innate and Adaptive Immune Responses

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ABSTRACT

Hypercholesterolemia is recognized as one of the major risk factors in cardiovascular disease. It promotes the development of a proinflammatory phenotype in large vessels, in particular arteries, with disease. Cells of the innate and adaptive immune system are localized within atherosclerotic plaques and participate in the initiation and progression of plaque formation. It is now recognized that each segment of the microvasculature also experiences inflammation due to hypercholesterolemia, and that this occurs long before events in the large vessels. More recently, it is has been established that the innate and adaptive immune systems participate in the responses of postcapillary venules, and possibly arterioles, to elevated cholesterol levels, and that T lymphocytes may be one of the early cell types activated by hypercholesterolemia. These cells initiate a series of steps that lead to leukocyte accumulation in postcapillary venules and endothelial dysfunction in the arterioles. This review discusses the microvascular alterations induced by hypercholesterolemia, with particular attention paid to the roles of the innate and adaptive immune responses, and how these two systems may communicate to induce the microvascular inflammation. *Antioxid. Redox Signal.* 8, 1141–1151.

INTRODUCTION

One of the dominant risk factors identified in cardiovascular disease and its associated complications is hypercholesterolemia. A key pathway through which elevated cholesterol levels contribute to the development of atherosclerotic lesions in large vessels is through the promotion of inflammation. More recently, it has been revealed that an inflammatory and thrombogenic phenotype is acquired by the microvasculature within days to weeks of cholesterol-enriched diet. This is characterized by endothelial activation, which is manifested as impaired vasorelaxation in arterioles (57), and recruitment of neutrophils and platelets in postcapillary venules (76, 81). Evidence has emerged that the adaptive immune system is engaged during the development of atherosclerosis, with T cells and their cytokines localized within atherosclerotic lesions (31, 68, 72, 89, 90). These immune cells also are central players in the generation of the inflammatory response to acute hypercholesterolemia through the release of cytokines such as interferon-γ (73, 74). This review focuses on the alterations of the microcirculation induced by elevated cholesterol levels and discusses the potential interactions between the innate and adaptive immune systems through which these responses may be invoked.

MICROVASCULAR RESPONSES TO HYPERCHOLESTEROLEMIA

One of the first alterations that can be observed on exposure of vessels to hypercholesterolemia is an oxidative stress, characterized by elevated reactive oxygen species (ROS) (in particular superoxide) generation, and impaired nitric oxide (NO) bioavailability (56, 60). These changes have been identified primarily in the macrovasculature and may be the underlying cause of many subsequent inflammatory and thrombogenic events. However, it is now appreciated that the microvasculature undergoes many similar events leading to oxidative stress, although these are not so

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well characterized (77). Both the innate and the adaptive immune systems are activated and participate in the progression of this response. The following segments discuss the impact of elevated cholesterol levels on the oxidative and inflammatory status of the microvasculature and role of the innate immune system therein. This is followed by a review of the contribution of the adaptive immune system in these alterations, with specific focus on the fact that oxidative stress is a potential link between the innate and adaptive immune responses to hypercholesterolemia.

OXIDATIVE STRESS INDUCED BY HYPERCHOLESTEROLEMIA

Hypercholesterolemia invokes both inflammatory and thrombogenic responses in the microvasculature within the first weeks of diet (39, 76, 81). These alterations occur when levels of cholesterol are in the range of what is considered borderline high/high in humans (*i.e.*, 180–240 mg/dl). Although hypercholesterolemia-induced inflammation is manifested differently in the arterioles, capillaries, and postcapillary venules, many underlying mechanisms are similar between the different vascular segments and are common to those responsible for atherosclerotic changes in large arteries. One of the first responses to elevated levels of cholesterol is endothelial dysfunction, which is characterized by an imbalance between NO bioavailability and ROS generation (56, 60, 77). This leads to impaired endothelium-dependent vasodilation in the arterioles (57) and the upregulation of adhesion molecule expression on venular endothelium (26), which supports the recruitment of leukocytes and platelets from the circulation. It is likely that these blood cells contribute to the maintenance of this oxidative stress (76).

The increased production of ROS that is invoked by an increase in cholesterol levels is due to the generation of a number of species. These are discussed in more detail elsewhere (9), but for the purpose of this review, superoxide is the primary focus. Excess superoxide generation primarily acts indirectly as a signaling molecule, which could lead to many of the inflammatory and thrombogenic events invoked by hypercholesterolemia. Several potential sources of superoxide exist. For example, elevated superoxide generation from xanthine oxidase in large arteries has been demonstrated through the use of xanthine oxidase inhibitors in hypercholesterolemic animals and humans (54, 60), although a comparable role for this enzyme has not been specifically identified in arterioles. NAD(P)H oxidase is a superoxide-producing enzyme present in many cell types, including endothelial cells, neutrophils, monocytes, smooth muscle cells, and platelets (4, 27). Last year, a functional form of this enzyme also was found in T cells (40). *In vivo* evidence of oxidative stress in the microvasculature comes from a study in which the oxidant-sensitive fluorescent probe dihydrorhodamine-123 was superfused over the cremaster muscle of hypercholesterolemic animals, and higher levels of oxidation (as indicated by fluorescence) were detected in postcapillary venules (74, 75). Release of ROS in the presence of low-density lipoprotein (LDL) also could result in the generation of oxidized low-density lipoprotein (oxLDL), which has been implicated as one of the mediators of hypercholesterolemia-induced vessel dysfunction and inflammation (47, 48). A key role for superoxide in the pathogenesis of endothelial dysfunction is its ability to interact almost instantaneously with NO [at a rate much faster than with endogenous superoxide scavengers such as superoxide dismutase (SOD)]. This not only generates the highly toxic peroxynitrite radical, but perhaps more important, reduces the bioavailability of NO. Although the exact mechanisms remain unclear, several other events also lead to impaired NO bioavailability during hypercholesterolemia. For example, endothelial NO synthase (eNOS), which is responsible for maintaining basal vascular tone in arterioles and an anti-inflammatory antithrombogenic phenotype in the venules, may become uncoupled because of insufficient levels of cofactors such as tetrahydrobiopterin (this can be caused by elevated ROS generation). This leads to superoxide generation from this enzyme, thereby propagating the imbalance between the two radicals (42). Furthermore, levels of the NOS inhibitor asymmetric dimethylarginine (ADMA) are increased during hypercholesterolemia, aided in part by a concomitant decline in the levels of the enzyme dimethylarginine dimethylaminohydrolase, which degrades ADMA (46). Taken together, these events culminate in the elevation of oxidative stress and the reduction of NO bioavailability.

ARTERIOLAR DYSFUNCTION

In both large arteries and arterioles, hypercholesterolemia invokes an impaired endothelium-dependent vasodilatory response that can be reversed by lipid-lowering strategies (49). This dysfunction is at least in part due to the altered balance between superoxide and NO. Thus, the level of vasodilation [invoked by substances such as acetylcholine (26) and bradykinin (34), which normally bind receptors on endothelial cells and stimulate NO release, thereby promoting smooth muscle relaxation] is reduced. If the role of the endothelial cell is "bypassed," and an artery or arteriole is directly stimulated with an NO donor, normal levels of vasodilation are achieved (26). This suggests that smooth muscle cells remain responsive to NO, supporting the concept that it is the bioavailability of this NO that is diminished. Nellore *et al*. (57) revealed a role for NO in the impaired perfusion of the microvasculature in a model of acute hypercholesterolemia by demonstrating that this could be reversed by administration of the NOS substrate, L-arginine. Although direct evidence is lacking for the microcirculation, supplementation with SOD can restore normal function in arteries of hypercholesterolemic rabbits (54), underlining the important of the superoxide/NO balance in the development of the endothelial dysfunction caused by increased cholesterol levels. Interestingly, events in the venules influenced the arteriolar responses, and evidence has been provided that neutrophils contribute to the arteriolar dysfunction (57), although these leukocytes do not accumulate in the arterioles. The proposal that leukocyte recruitment in venules may affect upstream arterioles or arteries is supported by findings in a model of ischemia–reperfusion injury in which adhesion molecule–deficient mice, unlike their wild-type counterparts, exhibit nor-

mal endothelium-dependent dilation responses to acetylcholine in the feeding artery (5). The exact mechanism through which the neutrophils act is unknown, but several possibilities exist. First, adherent neutrophils may release mediators that activate the venular endothelium, which, through intercellular communication, sends signals upstream to the endothelium of the arterioles, thus leading to limitation of NO bioavailability. Second, emigrated neutrophils that are in close proximity to nearby paired arterioles could release mediators that directly act on the arteriolar endothelium to promote the dysfunctional vasorelaxation responses. A third plausible explanation is that the interaction between adherent leukocytes and the vascular endothelium causes the release of soluble substances (*e.g.*, cytokines, with half lives longer than that of superoxide or NO) into the blood, and that these mediators enter the arterioles via the systemic circulation. There they bind receptors on the arteriolar endothelium and initiate a sequence of events that lead to impaired NO availability. In humans, neutrophil activation has been correlated with impaired arterial function (79), and it was postulated that these leukocytes may attack the endothelia, thereby contributing to dysfunction.

LEUKOCYTE RECRUITMENT DURING HYPERCHOLESTEROLEMIA

Although much of the emphasis has been placed on leukocyte infiltration of the arterial wall (where clinical disease is manifested) in the macrovasculature exposed to cardiovascular risk factors, in the microcirculation more specifics are known about the postcapillary venules. These are the primary sites of blood cell recruitment in small vessels. Adhesion molecules are upregulated or induced on both blood cells and the vascular endothelium during inflammatory states such as hypercholesterolemia, and the interaction between these molecules supports leukocyte recruitment (29). Three primary classes of molecules exist: selectins, integrins, and immunoglobulins. These support the three distinct stages of leukocyte recruitment:

1. Initially leukocytes are captured from the blood flow and slow down, rolling along the vascular endothelium, using L-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) on the leukocyte, which interact with sugars and P-selectin, respectively, on the endothelial cells;
2. Some of the rolling leukocytes become firmly adherent on the vessel wall via ligation of immunoglobulins such as intercellular adhesion molecule-1 (ICAM-1) on the endothelium by integrins such as CD11b/CD18 on the leukocyte;
3. The final stage of leukocyte recruitment is emigration, which involves many of the same molecules as the adhesion process, but different types of interactions between these receptors, such that the leukocytes can move through the vessel wall into the interstitium.

Unlike in major vessels during prolonged hypercholesterolemia, leukocytes are recruited predominantly to the venular side of the microvasculature and emigrate into the interstitium within 2 weeks of placement on a cholesterol-enriched diet. This leukocyte accumulation is not confined to distinct focal areas as in the large vessels; rather all postcapillary venules of the organs examined to date (*i.e.*, brain, small intestine, mesentery, and skeletal muscle) exhibit three- to fivefold increases in leukocyte adhesion (26, 39, 76, 81). Early during hypercholesterolemia, P-selectin and ICAM-1 are upregulated on the venular endothelium, allowing recruitment of leukocytes into the tissues (26). These molecules also are found in atherosclerotic lesions and contribute to lesion development (14, 38, 59, 70). Blocking P-selectin can attenuate the venular leukocyte recruitment, most likely by reducing rolling interactions, which are a prerequisite of firm adhesion (39). Tailor *et al.* (2004) also demonstrated a role for CD18 in the generation of the inflammatory phenotype in the postcapillary venules of the small intestine that accompanied an elevation in circulating cholesterol levels (81). During this early period of hypercholesterolemia, the recruited leukocytes are primarily neutrophils (73). Although limited evidence implicates neutrophils in the chronic responses to hypercholesterolemia, it is plausible that the multiorgan recruitment of neutrophils and other innate immune responses in the microvasculature lead to a low-level inflammatory milieu that feeds the developing pathologic manifestations of cardiovascular disease (78).

As mentioned earlier, an imbalance between superoxide and NO is one of the events responsible for leukocyte accumulation in the hypercholesterolemic microvasculature. Evidence for superoxide in these responses was gained from mice overexpressing the superoxide scavenger, CuZnSOD, which were placed on a cholesterol-enriched diet and failed to exhibit leukocyte adhesion (76). Although ample evidence exists for xanthine oxidase being a source of the superoxide generation in the endothelium of the large vessels, recent studies have focused on the role of NAD(P)H oxidase in different aspects of inflammation occurring in both large and small vessels because of hypercholesterolemia (32). This enzyme is present in many cell types, including neutrophils and endothelial cells, albeit the subunits of this enzyme differ slightly between cell types (4, 30). In the postcapillary venules, mice deficient in a subunit of NAD(P)H oxidase found in both of these cells types ($p47^{phox}$) were protected against hypercholesterolemia-induced neutrophil recruitment. Because these leukocytes are major producers of superoxide via NAD(P)H oxidase, bone marrow chimeras were used to determine whether leukocyte-associated or vessel wall–associated NAD(P)H oxidase was responsible for the inflammatory phenotype found in postcapillary venules. Interestingly, both blood and vessel-wall sources of this enzyme were equally important, suggesting that they may be in a positive-feedback loop with each other (76). Thus, superoxide is intimately linked with the recruitment of neutrophils from the circulation, and perhaps neutrophils themselves are contributing to the superoxide generation. It is likely that the superoxide is involved in signaling pathways that lead to adhesion molecule upregulation and leukocyte recruitment. Taken together with findings in animals injected with oxLDL in which leukocyte adhesion also is increased (47), the generation of oxLDL by superoxide from NAD(P)H oxidase may contribute to the leukocyte accumu-

lation observed in postcapillary venules of hypercholesterolemic animals.

ROLE OF NEUTROPHILS IN PLATELET RECRUITMENT

Platelets and neutrophils are recruited to the postcapillary venules once cholesterol levels increase two- to threefold. However, until recently, it remained unclear whether an interdependency exists between these two blood cell populations, such that one cell type is required to recruit the other. Although it has been established in large-vessel disease that platelets interact with the artery wall and deposit chemokines that attract monocytes to the area where plaque formation occurs (37), a similar link has only recently been investigated in the microvasculature. By using intravital microscopy, Tailor *et al*. (80) injected exogenously labeled platelets into recipient mice, and with rhodamine-6G to view the leukocytes simultaneously, they discovered that a majority of the platelet recruitment was dependent on leukocytes. The leukocytes physically supported the platelet adhesion, whereby platelets attached to leukocytes that were attached/attaching to the vessel wall. Thus, depletion of neutrophils abrogated platelet accumulation in the venules, and deficiency of CD18, and therefore leukocyte adhesion, had a similar effect. Taken together with their previous findings that platelet P-selectin and, to a lesser extent, endothelial P-selectin were key molecules in the generation of the thrombogenic phenotype in hypercholesterolemia (81), the following scenario could be proposed: platelet P-selectin binds to PSGL-1 on adherent leukocytes, and these leukocytes (also via PSGL-1) in turn bind endothelial P-selectin, with a small portion of platelets adhering directly to the vessel wall via a P-selectin interaction with an unknown ligand. This is in contrast to the accepted view of the interaction between the platelets and inflammatory cells in the large vessels, where it was demonstrated that platelets promoted leukocyte adhesion through the deposition of chemokines on the artery wall (37). However, it was not tested whether platelets exerted a reciprocal pro-inflammatory effect on the leukocytes. This question was answered recently by using hypercholesterolemic mice rendered thrombocytopenic for 24 h and bone marrow chimeras lacking platelet-associated P-selectin but expressing normal levels of endothelial P-selectin. The relatively short-term depletion of platelets was not enough to abrogate the inflammatory response; however, prolonged deficiency of platelet P-selectin, which would block platelet–leukocyte aggregation, was effective in attenuating the leukocyte adhesion response. This suggests that platelets are indeed an important component of the microvascular response to hypercholesterolemia and that they were not required to recruit leukocytes physically at the point of contact with the venular endothelium, but use a P-selectin–dependent mechanism through which they recruit leukocytes (unpublished observations). Unlike in the large vessels, it is unlikely that the platelets are depositing chemokines on the vessel wall, as platelet adhesion does not increase significantly before leukocyte adhesion, and platelets require leukocytes to attach to the vessel wall. Reports describing an altered responsiveness of coronary microvessels to products of platelet activation also suggest that platelets may contribute to impaired arteriolar vasodilation (67). Although this has yet to be fully investigated, findings in our laboratory support such roles for platelets and neutrophils and may have important implications in the overlap between inflammatory and thrombogenic pathways in cardiovascular disease.

CONTRIBUTION OF T CELLS TO LEUKOCYTE RECRUITMENT DURING HYPERCHOLESTEROLEMIA

The previous sections describe the manifestations of hypercholesterolemia-induced inflammation and platelet recruitment and the role of the innate immune response in these events. However, over the past several years, it has become appreciated that T lymphocytes are not only important in the progression of inflammation but that they have the capacity to mediate the early neutrophil recruitment to a site of inflammation [*e.g.*, after ischemia–reperfusion injury (35, 92)]. Both $CD4^+$ and $CD8^+$ T cells have been implicated in this response, through the release of inflammatory cytokines such as interferon-γ (IFN-γ). Interestingly, these leukocytes possess many properties that would be amenable to roles in many of the responses to hypercholesterolemia described earlier, including the upregulation of adhesion molecules on endothelial cells and the stimulation of oxidant release (6, 45). Although early studies demonstrated the presence of T cells within atherosclerotic plaques (33), whether these cells participated in the development of the plaque remained controversial for some time. However, it is now known that T cells are important early during the development of the plaque, and their role diminishes as the lesion progresses (16, 20, 24, 68, 72, 89–91). Much earlier, in the microvasculature, these lymphocytes also contribute to the inflammatory phenotype that is generated in mice on a cholesterol-enriched diet, as demonstrated by using mice that are genetically deficient in both T and B lymphocytes. More specifically, when the individual roles of T cells were assessed by depletion of either $CD4^+$ or $CD8^+$ T cells in hypercholesterolemic mice, the diet-induced leukocyte adhesion in postcapillary venules was attenuated. Depletion of both of these T-lymphocyte subsets was synergistic in its abrogation of leukocyte recruitment, suggesting that these cells may act together to invoke this response (73). Thus, a link between the innate (neutrophil adhesion) and adaptive (T lymphocytes) immune responses to hypercholesterolemia in the postcapillary venules was made. However, the question remained, are these cells acting directly by adhering to the endothelium or neutrophils themselves, or do they exert an indirect effect on the microvascular inflammation through the release of a soluble factor? This was answered by experiments in which the administration of lymphocytes to immunodeficient mice restored the venular inflammation in the absence of these T cells entering the circulation, suggesting that these lymphocytes are acting via one or more soluble mediators that promote the response (74).

SOLUBLE T CELL–DERIVED FACTORS

This led to the exploration of which factor could be responsible for these findings. One such factor is IFN-γ, which is released by both $CD4^+$ and $CD8^+$ T cells and is expressed in and participates in the development of atherosclerotic plaques (36, 88). Interestingly, oxLDL stimulates IFN-γ production by T lymphocytes *in vitro* (23), and IFN-γ is capable of promoting LDL oxidation under certain conditions (58). It was discovered that IFN-γ is also a prime mediator of the neutrophil adhesion that occurs in the microvasculature of acutely hypercholesterolemic mice (74). It is plausible that this cytokine is causing the upregulation of adhesion molecules such as ICAM-1 on the vascular endothelium, thereby promoting the recruitment of leukocytes from the circulation. When IFN-γ–deficient mice were examined, elevated cholesterol levels failed to invoke an inflammatory response in the postcapillary venules. However, the administration of splenocytes from wild-type mice rescued the inflammatory phenotype, and the reconstituted mice exhibited leukocyte adhesion. Further evidence was gained from experiments in which the transfer of IFN-γ–deficient splenocytes into a lymphocyte-deficient mouse was not capable of restoring the injury induced by hypercholesterolemia. The fact that natural killer cells (an alternative source of IFN-γ) are present in lymphocyte-deficient mice suggested that the T cells were producing this cytokine. Thus, a role for T cell–derived IFN-γ in the generation of inflammation after consumption of a cholesterol-enriched diet was established (74).

Evidence for other cytokines in the generation or progression of the inflammatory and prothrombogenic phenotype observed during hypercholesterolemia is limited. However, one other cytokine with particular relevance to the T lymphocyte–derived immune response is interleukin-12 (IL-12). IL-12 is generated primarily by monocytes/macrophages; however, it has been demonstrated that neutrophils and endothelial cells also are producers of this cytokine (10, 50). IL-12 is intimately related to the release of IFN-γ from T cells, in that IL-12 polarizes T cells toward the IFN-γ–producing Th1 phenotype, and IFN-γ in turn feeds back to promote IL-12 formation. These two cytokines are found in close proximity in atherosclerotic plaques and in areas where macrophages and lymphocytes are accumulated (84). Furthermore, a chemotactic role for IL-12 has been identified for neutrophils, which could be important early during hypercholesterolemia (8). Interestingly, it has been found that, similar to IFN-γ, IL-12 is a key player in the development of the inflammatory phenotype found in the microvasculature of hypercholesterolemic rodents. Leukocyte adhesion is abrogated in animals deficient in either the p35 or p40 subunits of IL-12. Findings from lymphocyte-deficient mice administered splenocytes from IL-12 knockout mice were consistent with this cytokine participating in the T cell-dependent pathways of inflammation during hypercholesterolemia, rather than directly acting to attract neutrophils to the venular wall (75).

The exact link between the superoxide-dependent neutrophil adhesion and the T cell–IFN-γ–IL-12–dependent adhesion response remains unclear; however, several potential pathways that could link the innate and adaptive immune components are worth consideration. For example, it is plausible that T lymphocytes contribute to the induction or upregulation of adhesion molecules through the release of cytokines such as tumor necrosis factor-α (TNF-α) and IFN-γ (7, 44). These cytokines are especially effective when they act in concert with each other to activate NF-κB, thereby inducing the expression of adhesion molecules such as E-selectin and P-selectin. IL-12 also has the capacity to increase ICAM-1, for example, in the liver (55). However, although such a scenario has been elegantly demonstrated in other models of inflammation and would fit in well with current knowledge in the hypercholesterolemic microvasculature, this has not been directly addressed to date in the small vessels of animals maintained on a cholesterol-enriched diet.

Another potential link between the innate and adaptive immune responses that has been investigated in the venules during hypercholesterolemia is the association between IFN-γ and ROS generation. This cytokine promotes superoxide production in endothelial cells (53) and NAD(P)H oxidase–mediated ROS generation in monocytes and granulocytes (18, 21). However, the oxidative stress experienced by postcapillary venules of IFN-γ–knockout mice when circulating cholesterol levels are increased twofold to threefold is comparable to levels usually observed in normal-diet mice (74). This finding extends beyond IFN-γ to IL-12, which also participates in the generation of the oxidative stress in the hypercholesterolemic microvasculature (75). Therefore, it is reasonable to speculate that hypercholesterolemia may lead to IL-12–induced activation of T cells, which release IFN-γ, and that IFN-γ in turn promotes an oxidative stress in the cells of the microvasculature (Fig. 1). This oxidative stress may induce adhesion molecule expression in postcapillary venules, ultimately leading to neutrophil adhesion. Bearing in mind that platelets depend on neutrophil accumulation to adhere, it follows that the same pathway is involved in platelet recruitment in the postcapillary venules.

Studies demonstrating that cytokines, such as IFN-γ, TNF-α, and IL-1, impair acetylcholine-induced arterial dilation evoke the theory that these immune cell–derived products may also contribute to the arteriolar dysfunction in hypercholesterolemia (17, 43, 86). Preliminary unpublished observations in our laboratory support such a novel role for T cell–derived IFN-γ in arteriolar dysfunction, although the exact mechanism through which this occurs remains unclear. The capacity for IFN-γ to alter the oxidative status and neutrophil infiltration of postcapillary venules would be consistent with this cytokine being able to influence arteriolar vasodilation. Alternatively, it has been proposed that the ability of IFN-γ to impair arteriolar vasodilatation in response to bradykinin is due to prolonged NO release during incubation with this cytokine, and therefore desensitization of the endothelium to bradykinin-induced NO generation (17).

CELL SURFACE–ASSOCIATED CYTOKINES

Another cytokine more recently implicated in the inflammatory and thrombogenic consequences of cardiovascular disease in humans is CD40 ligand (CD40L) and its receptor

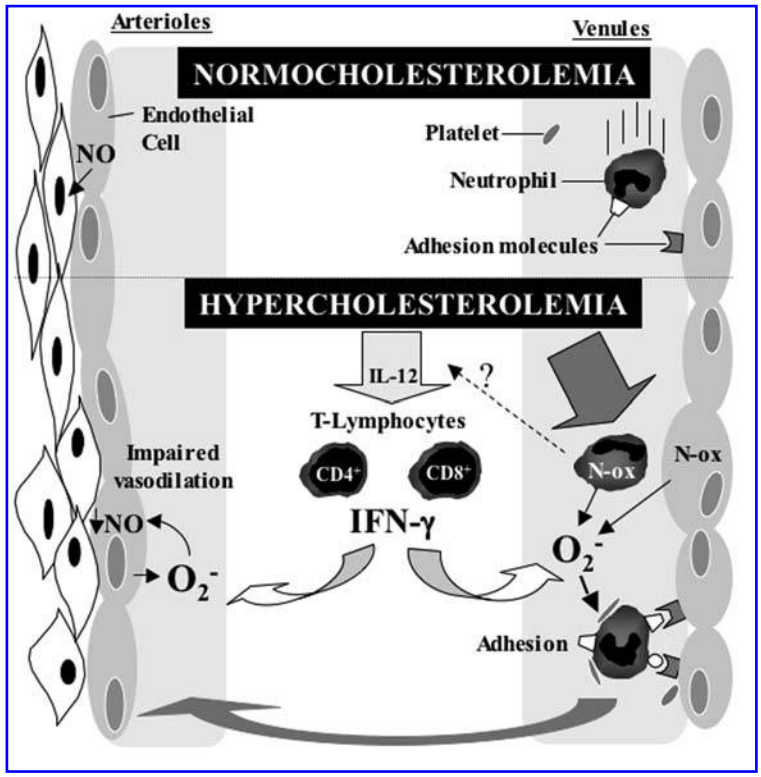

FIG. 1. A diagrammatic representation of the communication between innate and adaptive immune systems in the microvasculature responses to elevated cholesterol levels. In noninflamed normocholesterolemic individuals depicted on the top, arterioles (left) maintain tone by baseline release of nitric oxide (NO), and postcapillary venules (right) possess an antiinflammatory antithrombogenic phenotype in which few interactions occur between blood cells and the vessel wall. However, under hypercholesterolemic conditions, the innate system is activated, and superoxide (O_2^-) is released from the NAD(P)H oxidase (N-ox) in the blood cells and the vessel wall, leading to an oxidative stress and neutrophil adhesion. In addition, hypercholesterolemia promotes IL-12 release, which activates $CD4^+$ and $CD8^+$ T lymphocytes to produce IFN-γ. This IFN-γ can activate NAD(P)H oxidase to generate superoxide, thereby propagating the response. The arteriole experiences impaired endothelium-dependent vasodilation (most likely through reduced bioavailability of NO and increased superoxide), which is invoked by neutrophils and platelets, possibly in the venules, although the exact link remains to be elucidated. Nonetheless, the adaptive immune response also appears to participate in this arteriolar dysfunction.

CD40 (51, 64, 85). CD40L is a member of the TNF family of cytokines and is expressed on many cell types, in particular, in T lymphocytes and platelets. This molecule is cleaved off the surface of platelets during inflammation, and it has been suggested that its soluble form is not only a marker of platelet activation but also a proinflammatory soluble factor that can bind CD40 and initiate inflammatory responses such as adhesion molecule expression and cytokine release. CD40 also is ubiquitously expressed and is found on endothelial cells as well as cells that may participate in the adaptive immune response, including monocytes and B cells. CD40 and CD40L are upregulated on circulating monocytes and platelets, respectively, in hypercholesterolemic humans (25). Disruption of the CD40/CD40L system prevents the initiation and progression of plaque formation in large vessels (52, 71). This dyad also is important in the microvascular responses to hypercholesterolemia. The neutrophil adhesion observed in mice placed on a cholesterol-enriched diet for 2 weeks was found to be dependent on CD40 and T cell–associated CD40L. However, the exact nature of this association is not known. Recent *in vitro* data revealed that the ligation of CD40L on T cells promotes IFN-γ production, and binding of endothelial CD40 by T cell-associated CD40L could induce the expression of adhesion molecules such as ICAM-1 (61). However, the latter is unlikely to be the mechanism through which hypercholesterolemia induces leukocyte adhesion in postcapillary venules as the leukocyte population that interacts with the endothelium is neutrophils, and these cells are not known to express CD40 or CD40L. A more plausible explanation may be that the interaction of lymphocyte CD40L with B-cell or monocyte CD40 stimulates the generation of IL-12 and IFN-γ, which serve to propagate the release of each other and are responsible for leukocyte adhesion in the hypercholesterolemic microvasculature, although this requires further clarification.

CARDIOVASCULAR DISEASE THERAPIES AND THE MICROVASCULAR RESPONSES TO HYPERCHOLESTEROLEMIA

A growing body of evidence suggests currently used therapies in the field of cardiovascular medicine also may be effective in attenuating hypercholesterolemia-induced microvascular responses by modulating the innate and possibly the adaptive immune systems. For example, statins protect against hypercholesterolemia-induced platelet adhesion in postcapillary venules (82). This response was found to be independent of its cholesterol-lowering effects; rather, it was mediated by NO. However, statins also have the capacity to reduce IFN-γ–mediated adhesion molecule upregulation and the appearance of IFN-γ–positive T cells in the blood of patients (13, 83). Furthermore, these drugs attenuate NAD(P)H oxidase activation by interfering with some components of the multi-subunit complex (87). These are all actions that may bring the innate and immune components of the inflammation induced by hypercholesterolemia under control. Another therapy not specifically developed for hypercholesterolemia is the angiotensin II type 1a receptor (AT1a) antagonist, losartan. Treatment with this drug can prevent the leukocyte and platelet adhesion found in postcapillary venules of hypercholesterolemic mice (63). Interestingly, losartan also reduced the oxidative stress experienced by the

vessels. Taken together with the well-established capacity of AT1 activation to activate NAD(P)H oxidase in many cell types, including neutrophils (19) and endothelial cells (69), and to induce the expression of adhesion molecules in an oxidant-dependent manner (28, 62, 66), it is likely that AT1 activation is involved in the innate immune response observed during hypercholesterolemia. However, recent findings with angiotensin and angiotensin-converting enzyme inhibitors suggest that the angiotensin II that is produced in hypercholesterolemic individuals may bind AT1 on monocytes and lymphocytes to induce the generation of IL-12 and IFN-γ, respectively (12, 15, 22). IFN-γ can promote AT1 expression, which is an interesting observation, as mice placed on a high-cholesterol diet for 2 weeks exhibit elevated expression of this receptor in several vascular beds (63). This leads to the possibility that hypercholesterolemia-induced angiotensin II stimulates the innate and adaptive immune systems and that these responses may propagate themselves by acting on each other in that AT1 activation leads to NAD(P)H oxidase activation and IFN-γ production, and IFN-γ is a potent stimulator of NAD(P)H oxidase.

SUMMARY

Cardiovascular disease is responsible for more than 50% of deaths worldwide, and the associated cost of medical care is enormous. It results in a combined inflammatory and thrombogenic phenotype that develops over much of the adult life span. However, it is now recognized that before the clinical and pathologic evidence of cardiovascular disease is observed in large arteries, the microvasculature is altered by risk factors, such as hypercholesterolemia, and exhibits inflammatory and thrombogenic events that possess underlying mechanisms similar to those responsible for large-vessel disease. These include endothelial dysfunction characterized by oxidative stress, leukocyte and platelet recruitment, and diminished arteriolar vasodilation. Neutrophils represent the primary cell type that is recruited, and these in turn propagate the response by contributing to the ROS generation and supporting platelet adhesion. However, hypercholesterolemia also promotes the activation of T lymphocytes and the production of IFN-γ, possibly because of IL-12 released from other cells. The IFN-γ release is a key component of the oxidative stress and neutrophil adhesion responses, although it is unlikely that such actions of T lymphocytes necessitate their interaction with the endothelium. Thus, the adaptive immune response may activate the innate system, and these act in concert to invoke/propagate the proinflammatory and prothrombogenic phenotype in the microvasculature throughout the body. The fact that the communication between these two immune systems may be primarily through soluble mediators supports the hypothesis that this entire feedback system may lead to a low-grade systemic inflammation, which feeds the development of disease in large vessels where many of the same cell types and mediators are important. Several pharmaceutical agents developed to combat specific risk factors of cardiovascular disease, such as statins and AT1-receptor antagonists, are now known also to downregulate or block both innate and adaptive immune components. Taken together with the growing appreciation of the interactions between these two systems, the future development of therapies may encompass specific targeting of key events in the communication network between the innate and adaptive immune systems.

PERSPECTIVES

The exact signaling pathways through which the inflammatory and thrombogenic responses are initiated in the microvasculature during hypercholesterolemia remain unclear; however, because of the prolonged nature of the process, it is likely that many transcription-dependent events are involved. Hypercholesterolemia leads to an oxidative stress in microvessel; thus it is conceivable that the activation of redox-sensitive transcription factors such as NF-κB participate in the observed responses to elevated cholesterol levels in the microvasculature. As discussed earlier, IFN-γ–mediated superoxide production from NAD(P)H oxidase contributes to an oxidative stress (74). This oxidative stress may activate NF-κB–dependent transcription of adhesion molecules and inflammatory cytokines (2). Interestingly, IFN-γ itself may promote NAD(P)H oxidase activation by increasing the gene expression of gp91phox, a membrane component of the NAD(P)H oxidase complex (1), perhaps via JAK2-dependent gene transcription (41). NAD(P)H oxidase also may participate in platelet P-selectin expression and platelet–leukocyte aggregate formation. sCD40L, which is elevated in hypercholesterolemic humans (25), stimulates these responses by promoting reactive oxygen and nitrogen species generation via a PI3 kinase and p38 MAPK–dependent pathway (11). Conversely, activated platelets release sCD40L via gp91phox-mediated superoxide release (65); therefore these events may represent a self-propagating pathway that promotes the recruitment of platelets and platelet–leukocyte aggregates to different tissues. Furthermore, the CD40-CD40L dyad may be an important component of the T cell– and IFN-γ–mediated responses (3), indicating the existence of several common pathways between the innate and adaptive immune responses during hypercholesterolemia.

ACKNOWLEDGMENTS

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ABBREVIATIONS

ADMA, asymmetric dimethylarginine; AT1a, the angiotensin II type 1a receptor; eNOS, endothelial nitric oxide synthase; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon-gamma; IL-12, interleukin-12; NO, nitric oxide; oxLDL, oxidized low-density lipoprotein; PSGL-1, P-selectin glycoprotein ligand-1; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-alpha.

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