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Dedicated to Rita Levi-Montalcini

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NEUROIMMUNE HYPOTHESIS OF ATHEROSCLEROSIS

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Although "many roads lead to atheroma ", the prevailing hypothesis at present is the Russell Ross' response-to-injury hypothesis, which sates that atherosclerosis is an inflammatory disease that involves several aspects of wound healing. It is noteworthy that, emphasized by the current studies of neurotrophic factors and nerve-immune cell interactions, neuroimmune mechanisms are increasingly implicated in the pathogenesis of a number of inflammatory diseases. Here we highlight the possibility that neuroimmune mechanisms, including the participation of neurotrophic factors and immune cells, may also be involved in the process of atherogenesis. ***Biomed Rev 1999; 10: 37-44.***

INTRODUCTION

NGF's classical activity: ***a neuroeffector link***

Life at the neuronal level requires trophic support. Work initiated by the discovery of nerve growth factor (NGF) in the early 1950's and later embodied in the neurotrophic theory (1) has brought increasing insight into the bidirectional link between nerves and innervated effector (target) tissues (1-3). In auto-nomic nervous system, NGF-driven differentiation and survival of sympathetic and sensory neurons is well studied (1). NGF is a member of the neurotrophin family of proteins, including brain-derived neurotrophic factor (BDNF), neuro-trophin-3 (NT-3), NT-4/5, NT-6, and NT-7 (3,4). Beginning with the original studies of Levi-Montalcini (1), it is known that the largest amount of NGF is secreted by the convoluted tubular cells of the male mouse submandibular gland. This organ contains NGF as an oligomeric macromolecule (7S) composed of alpha, beta and gamma subunits. The homodimer consisted of beta-subunits and with molecular weight of 26.5 kD is a nerve growth-promoting factor, namely, beta-NGF (2.5S NGF) (1), which further in the text will be referred to as NGF. The gamma-NGF is a member of the kallikrein family of serine proteases, cleaving, for example, plasminogen into plasmin (5), a crucial factor for the conversion of latent transforming growth factor-beta (TGF-J3) into active TGF-(3 (6), a key suppressor of atherogenesis (6,7). Biological actions of NGF are mediated by the initial ligation of two different cell surface receptors: (;') the low-affinity p75 NGF receptor (p75NGFR), also named p75 neurotrophin receptor, and («) the high-affinity receptor ty-rosine kinase (Trk), TrkA (3) (receptozyme).

NGF's moonlighting: a neuroimmune link

During some 25 years after its discovery, there have been few reasons given to indicate that NGF acts on noneuronal cells. Thus, in 1977, it was remarkable to discover that treatment of newborn rats with NGF caused a systemic increase in the num-

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Table 1. Nerve growth factor and nonneuronal cells in atherogenesis

Immune cells	Other cells
Mast cells*	Endothelial cells*
Basophils	Smooth muscle cells*
Lymphocytes*	Fibroblasts
Macrophages	Platelets*
Dendritic cells	Adipocytes
Neutrophils	

* These cells are also source of BDNF. Refs 8-21.

ber of mast cells (MC) (8). Today, there is compelling evidence that NGF, in addition to its neurotrophic function, enhances survival and activity of a large number of nonneuronal cells, and is secreted not only by directly innervated cells but also by MC, lymphocytes and other cell types, implicated in the process of atherogenesis (Table 1). Also, atherogenic cytokines derived from immune cells increase NGF secretion in a variety of cells (18,22), whereas NGF induces release of various mediators from MC (23,24). Moreover, beyond their importance in differentiation and activation of immune cells, interleukin-6 (IL-6) and leukemia inhibitory factor (LIF) (25) and stem cell factor (SCF) (MC growth factor, *c-kit* ligand) (26) are able to enhance nerve growth. Indeed, there is at present considerable evidence that the sharing of ligands (growth factors, cytokines, and neurotransmitters) and their receptors constitute a molecular information network between neuronal, immune and effector (NIE) cells in different organs (25,27-29), including blood vessels (30-32). Historically, RamonyCajal (cited in 33) and Hamburger and Levi-Montalcini (34) firstly envisaged neuroimmune interactions and also neuronal programmed cell death (apoptosis) by their studies on the involvement of macrophages in neuronal injury and development.

ATHEROGENESIS: HOT JUST FOR INTIMA

The artery wall consists of intima, media, and adventitia, the latter gradually transits into periadventitial tissue, that is, the artery-associated adipose tissue (AAAT). An artery affected by atherosclerosis displays intimal and adventitial lesions associated with medial atrophy.

Although "many roads lead to atheroma" (6) (Table 2), the prevailing hypothesis at present is the Russell Ross' response-to-injury hypothesis, which sates that atherosclerosis is an inflammatory disease that involves several aspects of wound healing (35). The response-to-injury hypothesis proposes endothelial dysfunction, lymphocyte and monocyte extravasation into the intima, and vascular smooth muscle cell (VSMC) proliferation (35) and oversecretion of matrix molecules (35,36) as key events in the generation and development of athero-

***Table** 2. Current hypotheses of atherosclerosis*

Response-to-injury
Response-to-retention
Response-to-oxidation
Infectious
Hemorheologic-hemodynamic
Adventitial vasa vasorum hypoperfusion
Adventitial inflammation
Neuroimmune

Refs 35-43.

sclerotic plaques. Because advanced intimal lesions lead to luminal loss, resulting in infarction, the intima is considered by many authors the most important vascular area involved in atherogenesis (35,37-41). Recently, growing evidence, however, rises the possibility of alternative, adventitial (42,43) and AAAT (44,45) pathways of the vascular injury response, suggesting that atherogenesis is not just for intima.

Our review will focus on possible role for the neurotrophins NGF and BDNF and the immune cells MC, macrophage and lymphocytes in neuroimmune mechanisms in atherosclerosis.

NEUROTROPHINS, IMMUNE CELLS, AND ATHEROSCLEROSIS

Within the artery wall, VSMC comprise the primary target of the sympathetic neurons and, respectively, serve as the main source of NGF (17). Hence much of our insight into the possible role of NGF in atherogenesis arises from studies usually dealing with VSMC (46-50). Recent evidence shows that VSMC besides their classical role in providing neurotrophic support *via* secretion of NGF (17), BDNF and NT-3 (51) are also able to respond to these neurotrophins (49,52). Moreover, immune cells, like VSMC, secrete a plethora of mediators with neurotrophic, proinflammatory, fibrogenic and angiogenic effects that play an important role in tissue remodeling (9,23-25,27,53-56).

In vascular pathology, considerable evidence exists for the involvement of NGF and perivascular sympathetic innervation in hypertension (57,58). More recently, two publications deal with giant cell arteritis (31) and Kawasaki diseases (59). Indeed, an increasing body of evidence supports the hypothesis that the cell biology of atherosclerosis (35,39,43-45,60), including acute coronary syndromes (61,62), shares many similarities with inflammatory-fibroproliferative diseases (35,63,64), and also with cancer (65). It is noteworthy therefore that, emphasized by the current studies of NGF and immune cells (66,67) and nerve-immune cell interactions (25,27-30,32), neuroimmune mechanisms are implicated in the pathogenesis of a large number of these diseases (68, their Table, this volume *ofBiomedi-cal Reviews).* Specifically, while multiple growth factors and

also immune cells (6,35,53,60) that are potential sources of NGF (8-12) are identified in developing lesions of atherosclerosis, as well as an essential nonneuronal function of neurotro-phins implicated in cardiovascular tissue development (51,69), the role of neurotrophins in atherosclerosis has only recently emerged (45-50). Intriguingly, NGF shares a striking structural homology with proinsulin and exerts certain insulin-like effects on lipid metabolism in adipocytes (71,72) and both BDNF and NGF exert hypoglycemic effect acting on pancreatic beta cells, which also secrete NGF (73,74). Furthermore, atherogen-esis-associated growth factors, such as LIF, SCF, TGF-p, hepa-tocyte growth factor, vascular endothelial growth factor, and bone morphogenetic proteins (25,26,75-78) and even the paradigmic antherogenic factor platelet-derived growth factor (PDGF) (35) exert neurotrophic activities (79), and NGF uses similar intracellular signaling pathways as PDGF in VSMC (50).

Given the key role of inflammation and fibrosis in the initiation and development of atherosclerotic lesions (35,37-40,43-45,60-64), what role, for example, might NGF and MC play in the process of atherogenesis? First, NGF influences certain atherogenesis-associated functions of NIE cells including survival of peri vascular nerves (17), proliferation and activity of MC (8-10), and apoptosis and migration of VSMC (46,50,52). Second, atherogenesis related molecules, such as PDGF, IL-1(3, and angiotensin II (47), and also thrombin (48) increase NGF secretion in cultured VSMC, whereas MC-derived mediators modulate the growth of VSMC (80). Third, local and/or systemic levels of NGF (68,81,82) and the number of MC (23,25,30,32,61,68) increase in response to inflammatory stimuli, and, importantly, exogenous administration of NGF inhibits such inflammatory reactions, whereas histamine receptor antagonists inhibit atherosclerotic intimal lesions (83). Fourth, there is an inverse relation between the density of peri vascular sympathetic nerves and the development of atherosclerosis (84). Finally, NGF upregulates low density lipoprotein receptor (LDLR)-related protein (85), a member of the LDLR gene family whose malfunction is causally related to atherosclerosis, while another neurotrophic factor, LIF, decreases serum cholesterol levels and upregulates LDLR, and thereby inhibits the development of atherosclerosis (86,87). Effects of NGF as potentially related to atherogenesis are summarized in Table 3.

Altogether, these results taken in conjunction with the involvement of MC in LDL metabolism in atherosclerotic lesions (95) suggest a complex arrangement between lipoproteins, NGF, and immune cells and the process of atherogenesis. For example, in human coronary atherosclerosis, VSMC, like in culture conditions (49,52), express BDNF, NT-3, and NT-4/5, and their TrkB and TrkC receptors (52), while the level of NGF is significantly reduced and the adventitial p75NGFR immunore-activity increased (70). Further, the number of MC (39,70) and lymphocyte aggregates (39), as well as their links to perivascu-lar nerves (96-98), is increased at the adventitia in atherosclerotic compared with control coronary.arteries. Most likely, the

Table 3. *NGF potentials in atherogenesis*

Promotion of survival of sympathetic neurons
Stimulation of vascular smooth muscle cell migration
Stimulation of vascular smooth muscle cell apoptosis
Stimulation of mast cell proliferation
Stimulation of release of mast cell mediators
Stimulation of lymphocyte proliferation
Stimulation of neutrophil apoptosis
Stimulation of tissue repair
Stimulation of angiogenesis
Conversion of plasminogen to plasmin (gamma-NGF)
Regulation of adipogenesis
Increase in serum triglyceride and free fatty acid levels
Upregulation of LDL receptor-related protein
Upregulation of caveolin-1, -2
Activation of antioxidant enzymes
Activation of matrix metalloproteinase
Inhibition of vascular permeability
Inhibition of major histocompatibility class n expression
Inhibition of synaptic norepinephrine release

Refs 1,8,9,14,21,68,85,88-94.

increased presence of these immonocytes, which are potential sources of NGF (9,11,29,70), is not able to compensate the neurotrophic deficit that may result from VSMC atrophy (17,99). This deficit may lead to degenerative changes in perivascular nerves (100-102). Nothworthy, *(f)* denervation results in a significant decrease in NGF content in vascular wall, particularly in the adventitia (102), and *(if)* apolipoprotein E-deficient mice that spontaneously develop atherosclerosis (103) also develop neu-ronal degeneration (104). Clearly, the importance of neuroimmune and neuroeffector interactions in atherogenesis requires further evaluation.

NGF: protective or damaging to the artery ?

There are conflicting opinions about the role of NGF in atherogenesis. While some authors suggest that an elevated NGF amount may provide damaging effect (47), there are certain reasons to suggest that NGF might exert a vasculoprotectivc effect. First of all, brief myocardial ischemia induces a dysfunction of sympathetic cardiac innervation that is accompanied by a rapid increase in NGF release, whereas exogenous administration of NGF protects against such neuronal dysfunction, thus suggesting that the endogeneous NGF release is insufficient for complete neural protection (81). Second, as indicated in the Introduction, the process of atherogenesis involves several aspects of chronic inflammation and wound healing (35). It is noteworthy therefore that p75NGFR-deficient mice develop skin ulcers (105), while the administration of NGF promotes healing of skin (82,106) and corneal (107) ulcers, and inhibits experimental inflammation more potently than indometha-

cin and betamethasone (108). Significantly, patients with severe coronary atherosclerosis (7) and restenosis (6,7) do indeed have reduced levels of both active TGF-J3 and estrogen (7) and also mutation of type II TGF [3 receptor (67) compared to patients with normal coronary arteries, whereas one factor crucial for the activation of VSMC-secreted TGF-J3 is plasmin (6) that can be generated upon the action of gamma-NGF (5; also see 109,110 for NGF-TGF-(3- estrogen interactions). Future studies may provide the answer of whether TGF-(3-induced increase in NGF secretion by VSMC is damaging (47) or protective to the artery. Yet, the principle question remains: if NGF is causally related to atherogenesis, is it low or high levels of NGF that are associated with a possible vasculoprotective (atheroprotective) effect? Genetically-modified mice deficient in NGF or neurotrophin receptors (105,111) fed with high cholesterol diet may help to answer this question. Another pressing question is whether gamma-NGF (5) is expressed in atherosclerotic vascular wall?

CONCLUSION AMD PERSPECTIVES

From the evidence presented here the following main conclusion may be drawn: the neurotrophic theory and its currently expanded neuroimmune framework may be set into the context of neuroimmune hypothesis of atherosclerosis. Of course, many pressing questions remain to be answered, and future studies scheduled, in order to evaluate, for example, *(i)* plasma levels of NGF in patients with angina pectoris and with myocardial infarction, *(ii)* MC/basophils, lymphocytes, and platelets as potential peripheral markers for alterations in neurotrophins in atherosclerosis, and *(Hi)* potential involvement of AAAT in human atherosclerosis (44,45,112-120), as well as in Watanabe heritable hyperlipidemic rabbits and spontaneously hypertensive rats, and, more specifically, in neurotrophin-deficient mice and in MC-deficient mice (121) fed with cholesterol-supplemented diet.

In effect, a continued pursuit of the possible interactive role of neurotrophic factors and immune cells in atherogenesis may help to answer many questions which have continuously arisen, and hence provide new insight into atherosclerosis. If the neuroimmune hypothesis of atherogenesis proposed herein is proved, a new, neurotrophin-directed (57; 122, this volume *ofBiomedical Reviews)* and immune cell-directed therapeutic approach in atherosclerosis may then be designed. Indeed, "the submerged areas of the NGF iceberg loom very large", Rita Levi-Montalcini stated in her Nobel prize lecture reviewing 35 years of research on NGF (123). Blessedly, she is continuing to contribute.

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