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Linking Immunity to Atherosclerosis: Implications for Vascular Pharmacology - A tribute to Göran K. Hansson

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Abstract

For the past decade, we have deepened our understanding of the pathogenesis of atherosclerosis, a chronic arterial disease that causes cardiac and cerebral infarction and peripheral vascular disorders. Because of this extended understanding, more effective strategies for prevention and treatment of this disease are emerging. One of the fundamental mechanisms that lead to progress or regression in atherosclerosis, thus influencing its life-threatening complications, occurs through functional changes in vascular immunity and inflammation. This review briefly summarized the discoveries in basic and translational sciences in this area and recent advances in clinical medicine against atherosclerotic vascular diseases.

Keywords

Atherosclerosis; immunity; inflammation; macrophages; lymphocytes; antibody; immunotherapy

Introduction

Atherosclerosis is a chronic arterial disease that causes myocardial and cerebral infarctions, leading causes of death in developed societies. The incidence of atherosclerosis-based diseases remains high in developed societies, and it is rapidly increasing also in many developing countries along with economic development. Through the history, countless efforts have been devoted to delineate the pathogenesis of atherosclerosis and to seek for effective strategies of preventing and treating this life-threatening disorder. For the past decades, lipid deposition (Brown and Goldstein, 1983; Goldstein et al., 1983) and smooth muscle cell proliferation in response to endothelial injury (Ross, 1976, 1999) were dominant theories to explain the development of atherosclerosis. However, in the mid 1980s the

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This article is dedicated to our dear friend and mentor Göran K. Hansson on the occasion of his 60th birthday for his contributions to the research field of vascular immunology and atherosclerosis over 30 years.

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discovery of cellular immunity in the human atherosclerotic plaque created a paradigm shift in the understanding of atherogenesis. It has become well established that the arterial disease process is governed by immune-mediated inflammatory mechanisms (Hansson and Hermansson, 2011; Libby et al., 2011). As the body of evidence increases, anti-inflammatory and immunomodulatory strategies are now becoming emerging treatments to target the root causes of the disease. Dr. Göran K Hansson with his research team in Sweden has been a true pioneer in this research field. Over the years, his innovative contributions have broadly ranged from the molecular characterization of the immune response in human and experimental atherosclerosis to its physiological relevance and potential clinical applications. Göran K Hansson's combination of enthusiasm and extraordinary scientific skill with genuine scientific intuition had the power to turn wonderful ideas into reality. Also to be noted is his role as a great inspirer and tutor of a large group of researchers, among whom the authors of the present tribute article written on the occasion of his 60th birthday. While over the years Dr. Hansson has himself been the author of several excellent reviews on immune mechanisms in atherosclerosis (Hansson, 1993; Hansson and Hermansson, 2011; Hansson et al., 2006; Libby et al., 2011), our intention here is to provide a short list of research highlights, focusing on his unique contributions to the field and illustrating how he introduced and developed the concept of immunity in atherosclerosis.

Evidence for active immune reactions in atherosclerosis

The inflammatory characteristics of atherosclerotic lesions were first described in 1858 by Rudolf Virchow, a German pathologist who pointed out an inflammation of the inner arterial coat – the intima – as the starting point of atherogenesis (Virchow, 1858). In the early phases of atherosclerosis, namely the fatty streak, monocytes and macrophages infiltrate the arterial intima where they take up lipoprotein particles, particularly those chemically modified by oxidation. Subsequently, lipids accumulate inside the cells and transform them into lipid-laden “foam cells”, a hallmark of atherosclerotic lesions. Interestingly, there are T lymphocytes residing adjacent to macrophages in the lesions. In 1985, Dr. Hansson and his group provided the first evidence of immune activation in human atherosclerotic lesions by using monoclonal antibodies to cell-specific surface molecules on leukocytes and to HLA-DR, a Major Histocompatibility Complex (MHC) class II protein involved in antigen presentation and T cell activation (Jonasson et al., 1985). Many HLA-DR-positive cells are actually smooth muscle cells that normally do not express this antigen (Fig. 1). Staining of cryostat-sectioned plaque tissue revealed that T cells were abundantly present, particularly in the fibrous cap, where they constituted up to 20% of the cells (Hansson et al., 1986; Jonasson et al., 1986). As expected, the central lipid core of the plaque was dominated by macrophages. However, a more surprising finding was the expression of HLA-DR on smooth muscle cells in lesions, but not in normal arterial tissue, indicating an immune-mediated phenotypic shift of smooth muscle cells in atherosclerosis (Jonasson et al., 1986; Jonasson et al., 1985). This was further confirmed in vitro when both conditioned medium from activated T cells and recombinant interferon (IFN)- γ were capable of inducing MHC class II proteins on arterial smooth muscle cells. In addition, such expression was induced in vivo as a response to balloon catheter de-endothelialization of rat carotid arteries (Hansson et al., 1988; Jonasson et al., 1988a, b). When cyclosporin A, a drug that inhibits T cell proliferation, was administered to the rats directly after balloon injury, the expression of MHC class II protein on smooth muscle cells was inhibited, and so was the proliferation of smooth muscle cells in the intima (Jonasson et al., 1988b). When monoclonal antibodies to T cell-specific antigens and immune activation markers, such as HLA-DR and interleukin-2 receptor, were used for double-staining of cryostat sections or of cells isolated from carotid lesions, a substantial portion of T cells appeared to be in a state of late or chronic activation (Hansson et al., 1989; Stemme et al., 1992). The next logical step was to identify the culprit antigen. T cell clones were isolated and cultured from human atherosclerotic lesions and

thereafter exposed to potential antigens. Interestingly, some of the clones responded to oxidized low density lipoprotein (LDL) cholesterol by proliferation and secretion of IFN- γ , thus indicating the presence of an immune response to modified lipoproteins in the plaque (Stemme et al., 1995).

T cell receptors for cellular immunity in atherosclerosis

If an antigen-mediated immune response occurs in atherosclerosis, one would expect to find the expansion of CD4⁺ T cell clones carrying one unique T cell receptor (TCR) for antigen recognition in the lesions. In the '90s, the genetically hypercholesterolemic apolipoprotein E-null (apoE^{-/-}) mouse was introduced as a model of human atherosclerosis, allowing studies of the underlying pathogenic mechanisms. In this model, Dr. Hansson and his coworkers analyzed T cell receptor (TCR) mRNA in lesions at different stages of disease development. They found that the TCR expression was highly restricted and strongly skewed within both fatty streaks and fibrofatty plaques, thus supporting the presence of oligoclonal expansions of T cells (Paulsson et al., 2000). The data obviously help address the question: What extent are the findings relevant for the human disease? However, a perturbed TCR repertoire was also detected in patients with coronary artery disease, in particular patients with unstable angina, the majority of whom exhibited oligoclonal expansion of T cells (Caligiuri et al., 2000). Moreover, T cells from patients with unstable angina, but not from patients with stable angina or healthy individuals, proliferated in response to autologous proteins from the coronary culprit lesions and/or oxidized LDL (Caligiuri et al., 2000). This finding highlighted the intriguing possibility of a culprit self antigen, such as oxidized LDL. Soon after, the research group could demonstrate an autoreactive immune response against oxidized LDL in the mouse model. By exposing newborn apoE^{-/-} mice to high doses of oxidized LDL, T cell tolerance was induced, concomitantly with a reduction in the formation of atherosclerotic lesions (Nicoletti et al., 2000). In further studies, apoE^{-/-} mice were immunized with homologous plaque homogenates or homologous malondialdehyde LDL. Both immunization strategies reduced the development of lesions and were associated with specific T cell-dependent elevations of IgG antibodies (Zhou et al., 2001).

Regulation of vascular cell function by activated T cells and their cytokines

There are complex interplays between immune and non-immune cells, mainly smooth muscle cells and endothelial cells, in atherosclerosis (Hansson and Hermansson, 2011). Macrophages and T cells activated inside or outside atherosclerotic lesions can elaborate cytokines and growth factors that play pivotal roles in proinflammatory signal transduction. In the early '90s, Dr. Hansson and his research team showed that, in response to stimulation of the T cell cytokine IFN- γ , human macrophages express low levels of type A scavenger receptors and consequently accumulate less lipids (Geng and Hansson, 1992; Geng et al., 1995). The exposure to this cytokine also activates and induces endothelial cells to express high levels of adhesion proteins, which in turn attract more mononuclear cells into the vascular lesions (Geng and Hansson, 1995). Macrophages stimulated with IFN- γ become biologically activated and possess an increased capacity of cytokine expression, free radical production, migration, and phagocytosis, all of which play critical roles in atherogenesis (Fazio and Linton, 2001; Geng, 1997; Hansson, 1994; Harvey and Ramji, 2005; Leon and Zuckerman, 2005; Libby and Aikawa, 1998). The coexistence of activated macrophages and T cells in atherosclerotic lesions offers a unique environment where cytokines released from the immune cells regulate vascular cell function. Dr. Hansson's research team found that tumor necrosis factor (TNF)- α and IFN- γ can synergistically induce expression of inducible nitric oxide synthase (iNOS) leading to synthesis of the gaseous signalling molecule nitric oxide (NO) in a large quantity in a variety of cell types, including vascular smooth muscle

cells (Geng et al., 1992; Geng et al., 1994a; Hansson et al., 1994). The Hansson's team cloned the full-length cDNA of iNOS from smooth muscle cells (Geng et al., 1994b; Hansson et al., 1994), and characterized the biological activity of iNOS in the regulation of mitochondrial function (Geng et al., 1992; Geng et al., 1994b). Further studies by this group demonstrated that overproduction of NO can prompt the formation of nitrosyl complexes with the iron-containing enzymes of the Krebs's cycle and respiratory chain, which may consequently lead to dysfunctional mitochondria and turn the smooth muscle energy metabolism from aerobic to anaerobic (Geng et al., 1992). The mitochondrial dysfunction induced by the cytokine stimulation and NO release may trigger vascular cell apoptosis, resulting in the weakening of smooth muscle layers and destabilizing atherosclerotic plaques (Geng et al., 1996).

Impacts of immune deficiency on atherogenesis

The Hansson's group has also documented the evidence that CD4⁺ T cell activation represents a prominent feature in both early and late atherosclerotic lesions of apoE^{-/-} mice (Zhou et al., 1996). These findings raised the hypothesis of a direct link between cholesterol accumulation and the local immune response in atherosclerotic lesions. Indeed, in 1998 the group published the first thrilling evidence that immune-based therapy may be a way to treat the disease. Immunoglobulin treatment was found to reduce the formation of lesions in both early and late phases of the disease in apoE^{-/-} mice, in parallel exerting effects on both T cell activation and antibody production (Nicoletti et al., 1998). The pivotal role of CD4⁺ T lymphocytes as disease-promoting cells in atherosclerosis was then further elucidated by elegant experiments in which immunodeficient, atherosclerosis-prone mice were generated by crossing apoE^{-/-} mice with the *scid/scid* mouse strain that lacks T and B cells. The immunodeficient mice exhibited a markedly reduced development of fatty streak lesions. However, when CD4⁺ T cells from atherosclerotic donors were transferred into immunodeficient recipients, lesions increased by > 160 %. This was associated with homing of CD4⁺ T cells into the arterial lesions, elevations in systemic IFN- γ levels and increased expression of MHC class II protein in the lesions (Zhou et al., 2000).

So far, a substantial body of evidence pointed to a proatherogenic role for Th1 cells, while findings from immunization studies suggested that protective immunity may also occur. It is postulated that T cells play the role of bad guys and B cells the role of good guys in atherogenesis. Further experiments elegantly supported this hypothesis by showing that removal of the spleen dramatically aggravated atherosclerosis in apoE^{-/-} mice, and that the transfer of spleen cells from apoE^{-/-} mice reduced disease development in young apoE^{-/-} mice. Moreover, when donor spleen cells were separated into B and T cells, it could be demonstrated that the reduction in lesion formation was due to B cells (Caligiuri et al., 2002).

The concept that Th1 cells acts as a "bad guy" is supported by breeding interleukin (IL)-18^{-/-} mice with apoE^{-/-} mice. IL-18 is a proinflammatory cytokine that induces the production of IFN- γ , thereby acting for Th1 differentiation. IL-18 deficient mice exhibits a marked reduction in aortic lesion formation (Elhage et al., 2003). Dr. Hansson's team tested the hypothesis that the Th1 response dependent on a specific antigen, such as oxidized LDL, using the immunodeficient apoE^{-/-} *scid/scid* mouse model, but this time the immunodeficient mice received CD4⁺ T cells from mice that had been immunized with either oxidized LDL, a nonrelevant antigen (keyhole limpet hemocyanin), or adjuvant alone. For the first time, it was possible to demonstrate that cell-mediated immunity to oxidized LDL accelerated atherosclerosis. The transfer of CD4⁺ T cells from mice immunized with oxidized LDL caused a nearly doubled increase in lesion size compared with T cells from mice immunized with the nonrelevant antigen (Zhou et al., 2006).

The role of a proinflammatory T cell response in atherosclerosis was further explored by studying the role of T cell regulation. Prominent among the negative regulators is transforming growth factor (TGF)- β , which has been consistently shown to suppress T cell activation. The group generated a mouse model with disrupted TGF- β signalling in T cells by crossing an apoE^{-/-} mouse with a mouse that lacks functional TGF- β receptors. This mouse developed dramatically increased atherosclerosis, including enhanced expression of IFN- γ and morphologic characteristics of vulnerable plaques, such as reduced content of mature collagen fibers (Ovchinnikova et al., 2009; Robertson et al., 2003). Besides supporting the proatherogenic role of T cells, the data strongly indicated that TGF- β acted as an atheroprotective cytokine by inhibiting T cell activation.

Immunotherapy for atherosclerosis

In spite of the evidence linking changes in immunity to the pathogenesis of atherosclerosis, up to now there has been no clinically available immunotherapy for atherosclerosis. However, exploitation of immunoregulatory agents and vaccination against atherosclerosis has obtained encouraging experimental data. Through the efforts of Dr. Hansson's research team and of other investigators, several immunotherapeutic studies using animal models of atherosclerosis have shown that immunotherapeutic approaches with anti-oxidized LDL vaccination or antibodies may reduce atherosclerosis and increase plaque stability (Nilsson et al., 2005). For instance, immunization of hypercholesterolemic animals with low-density lipoprotein preparations reduces atherosclerosis, suggesting that vaccination may represent a useful strategy for disease prevention or modulation. The presentation of antigens to T cells responsible for the anti-atherosclerotic effects may occur through dendritic cells, which are known to be the most potent antigen-presenting cells. In a recent study of mice transgenic for human apolipoprotein B100 [ApoB100] and deficient for the low-density lipoprotein receptor, the intravenous injection with dendritic cells that had been pulsed with the low-density lipoprotein protein ApoB100 in combination with the immunosuppressive cytokine interleukin-10 have been shown by Dr. Hansson's team to reduce proliferation of effector T cells, inhibit IFN- γ production, and increase the de novo generation of regulatory T cells. A significant (70%) reduction of atherosclerotic lesions was found in the aorta, with decreased CD4⁺ T-cell infiltration and signs of reduced systemic inflammation (Hermansson et al., 2011). Moreover, immunization with a peptide of apoB-100 (p210) fused to the B subunit of cholera toxin (CTB), which binds to a ganglioside on mucosal epithelia for 12 weeks, caused a 35% reduction in aortic lesion size in atherosclerosis-prone apoE^{-/-} mice (Klingenberg et al., 2010). The treatment also induced regulatory T cells while suppressing effector T cells as well as a peptide-specific antibody response. Thus, the nasal administration of an apoB-100 peptide fused to CTB attenuates atherosclerosis and induces regulatory Tr1 cells that inhibit T effector responses to apoB-100, paving the road to a novel preventive strategy for atherosclerosis.

Conclusions

Taken together, altered immunity plays a major role in the development of atherosclerosis. Atherosclerotic plaques contain almost all the major cellular components of the innate and adapted immune systems, such as lymphocytes, macrophages and dendritic cells, which recognize and react with atherogenic antigens, e.g., oxidized LDL (Fig. 2). The pioneering work by Dr. Hansson and his colleagues has opened up a new research field in vascular immunology and atherosclerosis that offers tremendous opportunities in vascular pharmacology. Today, many basic scientists and clinicians around the world work together in delineating the pathogenesis, risk factors and therapeutic targets for the immunoregulation of atherosclerosis. For the past decades, characteristic changes in both innate and adapted immunity impacting on atherosclerotic lesion formation have been identified. Novel

therapeutic tools, particularly, anti-atherosclerotic vaccination, are under development. All of these obviously now warrant further investigations, especially in large animal models and humans.

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Figure 1. Immunofluorescence of human HLA-DR antigens at the edge of a human carotid atherosclerotic plaque. Strong green immunofluorescent staining of HLA-DR was found in the majority of vascular cells in the plaque tissue (Jonasson et al. J Clin Invest. 1985 Jul; 76:125–31, with permission).

Figure 2. Schematic demonstration of atherogenic low density lipoprotein (LDL) and immune cell infiltration in atherosclerotic lesions

During the development of atherosclerosis, monocytes infiltrate the arterial intima where they differentiate into macrophages and take up modified LDL particles. T cells also enter the lesions and become activated when they encounter antigen-presenting cells, such as dendritic cells and macrophages.