Immune and inflammatory mechanisms in the development of atherosclerosis

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Investigations over the past 10 years have shown that the immune system is involved in the development of atherosclerosis. There is evidence for an inflammatory process in the vessel wall and also for an involvement of specific immunological mechanisms in atherogenesis.

The data can be summarised as follows: (a) immunoglobulins as well as T lymphocytes and macrophages are found in large numbers in the atherosclerotic plaque; (b) B lymphocytes and plasma cells can often be detected in the adventitia adjacent to advanced plaques; (c) antibodies to antigens present in plaques can be detected in atherosclerotic individuals; (d) immunocompetent cells are recruited to the arterial intima early in the development of atherosclerosis-like lesions in laboratory animals; and (e) T cell depletion can significantly modify the development of vascular lesions in animal models.

From all these studies it is clear that components of the immune system are involved in atherosclerosis—but they do not elicit the disease process. Immune mechanisms seem to be potent modulators of the atherosclerotic process. It is, however, important to realise the complexity of these mechanisms. The immune system is, together with the nervous system, the most complicated organ system in the human body. Its organisation in interconnecting networks with counterbalancing factors makes it unrealistic to attempt to reduce its involvement in atherosclerosis into a simplistic “good” or “bad” function.

Intraplaque cellular immune responses in atherosclerosis

Immunohistochemical analysis using monoclonal antibodies for specific cell types has shown that the lipid-rich core region of advanced human atherosclerotic plaques is dominated by macrophages.¹⁻⁶ In fact the cholesterol-laden foam cell that is the hallmark of atherosclerosis is a macrophage derived from blood monocytes.¹⁻⁴ The fibrous cap that surrounds the lipid core is dominated by vascular smooth muscle cells, but it also contains substantial numbers of both T lymphocytes and macrophages.^{3,6} An analysis of the cell surface protein composition of such cells shows that they differ from the corresponding cells in the blood and that many of them are in an activated state.⁷⁻⁹ Finally, recent studies using monoclonal antibodies and cDNA probes showed that several cytokines—that is, immune cell-derived, hormone-like substances—are produced in the plaque.^{5,8,10,11}

Other studies showed that monocyte-

derived macrophages and T lymphocytes are present from a very early stage of atherosclerosis and are detectable in the fatty streak.^{6,12} Among the lymphocytes both CD4 and CD8 T cells can be detected but there are very few if any B cells or plasma cells within the plaque.

The presence of activated T lymphocytes in the atherosclerotic plaque suggests a local immune response and it has been postulated that such a response may be directed against local antigens in the plaque. Recent molecular genetic studies¹³ have, however, demonstrated that these T cells are heterogeneous in terms of their immunological specificities. It is therefore possible that only a small proportion of plaque T cells respond to local antigens: these cells probably elicit a process that brings in other T cells by immunologically non-specific mechanisms.

Adventitial inflammatory responses

Immunoglobulins are present in high concentrations in the atherosclerotic plaque.¹⁴ They can be detected both extracellularly and in the cytoplasm of injured vascular cells.¹⁵ Very few B lymphocytes are, however, present in the plaque.^{3,16} The immunoglobulins found in the plaque are therefore probably synthesised at other sites and enter the plaque in the same way as any other plasma protein.

An intense adventitial B cell infiltration is, however, present in the inflammatory periaortitis or periarteritis that may develop around advanced atherosclerotic plaques.¹⁷ The periaortic lesion is an adventitial inflammatory infiltrate that contains large amounts of B lymphocytes, plasma cells, and immunoglobulins together with oxidised lipids. It has been proposed that it represents an autoimmune response to the oxidised lipids that are generated during the atherosclerotic process.¹⁷ Since these adventitial lesions appear late in the course of the disease, it seems unlikely that the immune response mounted in them is of major pathogenetic importance. If the autoantibodies that are presumed to develop can be detected in patients' sera, they might, however, be useful as markers of advanced atherosclerosis.

Autoimmune responses in patients with cardiovascular disease

Autoantibodies have been implicated in the pathogenesis of atherosclerosis for a long time and certainly long before the cellular immune component of the plaque was identified 10

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years ago. On the whole, the search for autoantibodies has been disappointing but the recent identification of autoantibodies to oxidised lipoproteins could shed new light on these autoimmune responses.

Oxidised low density lipoprotein (LDL) particles are strong antigens and it has been shown that antibodies develop to oxidation products such as malondialdehyde-coupled lysine and 4-hydroxy-nonenal.¹⁸ Such products of lipid oxidation have been found in plaques,¹⁹ and antibodies to them have been detected in humans. A recent epidemiological study showed that antibodies to oxidised LDL predict atherosclerosis that is in the progressive phase: so the autoantibody test seems to be a useful marker for active disease.²⁰

The pathogenetic mechanism by which auto-antibody to oxidised LDL acts is less clear. It is possible that the autoantibodies enhance the elimination of oxidised lipoproteins, because antigen-antibody complexes are taken up both by Fc receptors for IgG and by the scavenger receptor for modified LDL.²¹ It is, however, also possible that antigen-antibody complexes could aggravate the disease—for example, by activating complement. This would be expected to attract and activate more monocyte/macrophages and thus enhance the inflammatory response in the vessel wall.

Animal experiments show that immunocompetent cells are early participants in atherosclerosis

Studies of human plaque specimens have shown that T lymphocytes and macrophages are present in all types of lesions, from fatty streaks to advanced plaques, and it is therefore likely that they are involved throughout the pathogenesis of atherosclerosis. Direct evidence for this has been obtained in animal studies, which have made it possible to deduce a pathogenetic sequence for the process.

Most studies have been performed in cholesterol-fed New Zealand White rabbits. A few weeks after the introduction of cholesterol supplement to the diet, arterial endothelial cells started to express surface proteins that bind monocytes and lymphocytes.²² Such adhesion molecules are not normally present on the endothelial surface, but their expression may be induced by certain lipids such as lyso-phosphatidylcholine, which could be derived from the lipoproteins in these hyperlipaemic rabbits. Soon after the expression of the adhesion molecules, monocytes and lymphocytes started to adhere to the endothelial surface and enter the intima. After 1½–2 months of hyperlipidaemia, a fatty-streak-like intimal lesion had formed which consisted almost exclusively of cholesterol-laden macrophages with interspersed T lymphocytes.^{23 24} With continuing hyperlipidaemia, this fatty streak lesion gradually transformed into a fibrofatty atherosclerotic plaque that exhibited intimal smooth muscle proliferation as well as macrophage and T cell accumula-

tion.^{23 24} It seems likely that different mechanisms control these two phases of lesion development.

Immunologically derived cytokines regulate vascular functions

Studies on immune interactions with blood vessels have underlined the regulatory role of immunologically derived cytokines. Several monocyte-derived cytokines promote growth of vascular smooth muscle and endothelial cells. Interleukin-1 (IL1) is particularly active in this respect^{25 26} and seems to act by inducing an autocrine growth factor production that drives the cell to divide.²⁷ Interestingly, IL1 has been found in atherosclerotic plaques^{10 11} and it is possible that it stimulates smooth muscle proliferation during the formation of the fibrous plaque. T cells may have an opposite role since one of their major secretory products is interferon-gamma. This cytokine is a potent growth inhibitor both for endothelial²⁸ and smooth muscle cells.²⁹ Interferon-gamma is produced by activated T cells, which are present in plaques. Injections of recombinant interferon-gamma significantly reduced restenosis after balloon catheter angioplasty in the rat carotid artery.³⁰ In vivo elimination of the interferon-gamma producing T lymphocytes, on the other hand, increased the size of restenosis lesions.³¹ This demonstrates that T lymphocytes deliver growth-inhibitory interferon-gamma (and possibly also other growth-inhibitory substances) in the vessel wall. On the basis of these studies, we have proposed that recombinant interferon-gamma could be used to inhibit smooth muscle proliferation and the formation of arterial stenosis after angioplasty and vascular surgery.

Cytokines derived from macrophages and lymphocytes may also regulate cellular metabolism. An interesting example is the control of cholesterol metabolism in macrophages. The scavenger receptor for oxidised lipoproteins constitutes a major pathway for cholesterol uptake by macrophages. In contrast to the receptor for native LDL, the scavenger receptor is not downregulated by a high intracellular cholesterol content, and the macrophage may therefore continue to express scavenger receptors and take up more lipoproteins even if it already contains large amounts of cholesterol. It has, however, recently been found that scavenger receptor expression is under cytokine control. Both interferon-gamma and tumour necrosis factor (which is produced both by macrophages and T lymphocytes) can turn off the gene for the scavenger receptor.^{32 33} This inhibits the transformation of the macrophage into a foam cell. This implies that immune-derived cytokines could be directly involved in the cholesterol metabolism of the plaque. Immune activation would inhibit scavenger receptor expression and intracellular cholesterol accumulation. The figure shows a hypothetical view of the cytokine network in the vessel wall.

Figure Some of the regulatory effects of cytokines in the vessel wall during atherogenesis. IL1, interleukin-1; TNF α/β , the α and β forms of tumour necrosis factor; IFN γ , interferon-gamma; PDGF, platelet-derived growth factor; ScR, scavenger receptor for modified lipoproteins; O_2^- , free oxygen radicals; iNOS, inducible form of the nitric oxide-synthesising enzyme, NO synthase; α -actin, α isoform of actin; CAMs, cell adhesion molecules.

Conclusion

In vivo and in vitro studies of cytokine effects on vascular cells showed that the cytokines of the immune system can modulate vascular tissue responses and thus influence the atherosclerotic process. Analyses of human atherosclerotic tissue specimens showed the cellular basis for immune responses in the plaque. Assays of patient sera identified autoantibody responses that seem to be associated with atherosclerosis, and experimental immunisation protocols showed that immune responses can enhance or perturb the atherosclerotic process.

It is therefore clear that components of the immune system are involved in the pathogenesis of atherosclerosis. The complexity of the immune system and the multiple effects of its signal substances mean that it is unclear how the immune involvement affects the various stages of disease processes. The immunological specificity of the response is also still unknown, although recent data suggest that autoimmune reactions against oxidised lipoproteins could be important in atherosclerosis. Further experimental and epidemiological studies will obviously be necessary to elucidate the role of the immune system in atherogenesis.

Finally, it is possible that monitoring of immune responses could be useful for the diagnosis and evaluation of the atherosclerotic process and that interference with

cytokine networks could be used to inhibit or reverse pathological processes in the vessel wall. These exciting possibilities should stimulate further research.

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