Inflammation in cardiovascular disease

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This conference, which was held at the Royal College of Physicians on 22 September 1999, was organised by Professor Dorian Haskard, Sir John McMichael Professor of Cardiovascular Medicine at the National Heart and Lung Institute, Hammersmith Hospital, London.

Inflammation has been associated with cardiovascular disease in several epidemiological studies. Its involvement is indicated by the presence of monocyte-macrophages and lymphocytes in pathological specimens. The endothelium is now recognised as more than a passive barrier between lumen and plaque, whilst the putative role of the smooth muscle cell has changed from villain to protector in recent years. The macrophage, controlled in part by T-cells, may be the primary destabiliser in acute coronary syndromes (ACS). The mechanism of benefit of the statins in particular has recently become clearer. They appear to reduce inflammation within the plaque, suggesting that inflammation may be crucial not only in chronic atheromatous disease but also in ACS. The advent of new technologies has now started to allow the detailed investigation of the genetics of inflammatory response in relation to atherogenesis and its complications. As well as in coronary disease, inflammation may well be involved in the dilatation of abdominal aortic aneurysms (AAA) and the cachexia of heart failure.

The conference focused in large part on the role of inflammation in atherosclerosis, where there is firm evidence for its pivotal role. A glimpse into inflammation in other cardiovascular syndromes was also given.

The epidemiological evidence

The risk of death from coronary disease increases with raised levels of acute phase reactants, which act as markers of inflammation. The most studied inflammatory markers with respect to ischaemic heart disease (IHD) are C-reactive protein (CRP), serum amyloid A (SAA), the leucocyte count (WCC) and serum albumin. An overview of the epidemiological studies suggests that comparing the highest quartile of marker level to the lowest gives odds ratios (OR) of 1.8 for CRP, 1.6 for SAA, 1.6 for WCC, and 1.5 for albumin (low levels being associated with risk in this case). If inflammation were important, then treatment that saves lives might be expected to reduce inflammatory indices. Indeed, treatment with aspirin is most effective in those with a greater degree of inflammation, whilst pravastatin treatment reduces CRP in line with fewer coronary event rates.

What is the contributing influence of infections to the inflammation associated with IHD? Myocardial infarction (MI) is more common after a viral infection and avian herpes virus certainly can cause atheromatous lesions in chickens. A summary of all the trials of Helicobacter pylori (HP) suggests an OR for cardiovascular events of only 1.16, which is not statistically significant. Looking at CAG-A positive HP only, the type associated with increased risk of gastro-intestinal complications, there is still no dramatic increase in the risk of IHD. Although initial studies have suggested a link between Chlamydia pneumoniae (CP) infection and CAD, this has not been substantiated by...
prospective population studies once confounding factors are corrected for. This suggests that CP is just a coloniser of plaques and not a cause of IHD. Data on the eradication of this infection as a means of reducing recurrent cardiovascular event rates have been conflicting, the initial positive study by Gupta et al3 being backed by one large trial and refuted by another. No good prospective data exist for cytomegalovirus (CMV) infection and a link to IHD in the absence of transplantation. The conclusion from epidemiological data is that inflammation is an important predictor of long term cardiovascular mortality, but the role of infection in its pathogenesis is unclear.

The pathological evidence

In a mature plaque, the lipid core has no collagen fibres or matrix, but the fibrous cap overlying this has a lattice-like collagen matrix – shown well by immunohistochemistry (IHC) – within which sit smooth muscle cells. At the edge of the plaque is a vulnerable zone that is often the site of rupture. IHC shows this area to have a less well-organised collagen mesh and to be rich in macrophages. The process of monocyte-macrophage accumulation requires adhesion, migration (eg in response to MCP-1), scavenging of oxidised low density cholesterol (LDL) (via scavenger receptors) and eventually death within the plaque and the development of a lipid core. Gene knockout mice show reduced atheroma production when this pathway is interrupted. These macrophages are active since they also stain for matrix metalloproteinases (MMPs), in particular MMP-9.

IHC also demonstrates T-cells in the shoulders of plaques. They may be involved in several pathways, including acting as cytotoxic cells to kill foam cells, modulating smooth muscle function via γ-interferon (γ-IFN) production, or activating macrophages via the CD40-CD40 ligand interaction.

The inflammatory process makes the plaque unstable. Death occurs when thrombosis causes abrupt occlusion of an artery. Thrombosis may occur on a plaque fissure (deep type II injury), or on superficial erosions (type I injury). The cause of superficial erosions is not clear. Local production of MMP-2 may break down type IV collagen in the basement membrane, and so loosen endothelial cell adherence.

The endothelium

Atherosclerosis was initially viewed as a disease of lipid accumulation in the vessel wall, with the endothelium being affected secondary to the underlying necrosis. Now it is clear that the endothelium is intimately involved in the process, with endothelial dysfunction being one of the earliest signs of the initiation of the process of atherosclerosis. The endothelium plays an active part in leucocyte recruitment into the plaque. Acute activation via a number of pathways (including IL-1, TNF-α, complement activation, viral infection) produces up-regulation of chemokines (eg IL-8, MCP-1, fractalkine) and adhesion molecules such as the selectins (eg E-selectins and P-selectin) and integrin ligands (eg ICAM-1, VCAM-1). The adhesion cascade involves slowing down of passing leucocytes via selectin binding, followed by the process of firmer adhesion via β1- and β2-integrins binding their ligands on leucocytes. Regulation of these factors is at the gene expression level, and involves the NFκB pathway. The pro-inflammatory endothelial response is balanced by the up-regulation of cytoprotective mechanisms controlling apoptosis, anti-oxidant properties and complement. Further protection for endothelial cells may be provided by high density lipoprotein (HDL), which can directly prevent the up-regulation of adhesion molecule expression and simultaneously promote expression of prostacyclin. Future attempts to normalise endothelial function may use inhibitors of NFκB, or treatment with HDL. This may lead to plaque stabilisation via reduction in inflammatory cell traffic.

The smooth muscle cell-macrophage interaction

In 1976, Russell Ross summarised the pathogenesis of atherosclerosis as a process of mechanical injury, following which there was platelet aggregation, release of chemo-attractants for smooth muscle cells, and the migration of these cells from the media to the intima. There they underwent a morphological change from a contractile phenotype to a synthetic one. These smooth muscle cells, taking up lipid, produced foam cells. Thus smooth muscle cells were seen as the ‘culprits’.

However, the smooth muscle cell may, in fact, be the guardian of plaque stability. The regenerative and repair function may be dominant, with the smooth muscle cell supporting the collagen mesh and producing the intercellular matrix. Studies of plaques that have ruptured showed reduced collagen and matrix and fewer smooth muscle cells, whilst macrophage and T-cell numbers were increased, especially at the site of rupture.

Apoptotic markers are increased in smooth muscle cells from the fibrous cap and these cells do not proliferate as well in vitro as those from the media. The macrophage may well be the ‘villain’. In vitro, macrophages adhere to smooth muscle cells and may induce their apoptosis via contact-dependent and soluble mechanisms. The macrophage may also be involved in degrading the matrix through expression of matrix metalloproteinases such as MMP-1, -3 and -9. One of the best ways to stimulate smooth muscle proliferation, and so stabilise a plaque, is to perform angioplasty. Although re-stenosis may be a problem, these modified plaques rarely become unstable, due to a thicker fibrous cap being produced.

Professor Peter Libby (Harvard Medical School, Boston, USA), in a keynote lecture, summarised the work of his laboratory within the field. In particular, he highlighted the study of a rabbit model of atherosclerosis. These studies have now shown that lowering the amount of cholesterol in the diet, following a period of high fat intake, reduced the number of macrophages present and lowered MMP activity
and tissue factor expression within coronary plaques. Since profound dietary manipulation is not possible in man, lipid lowering medications offer the best alternative for quieting the plaque and have had dramatic effects on mortality in primary and secondary prevention trials.

The future

A lowering of primary heart rates and gene levels. The promoter and enhancer regions suggest that the gene for IL-6, a key inflammatory component, is active in the genome. In vitro work on the GC polymorphism of IL-6 promoter region suggested that the G allele showed enhanced ability to promote protein synthesis at rest and, particularly, upon stimulation. The in vitro correlate of this was that the GG genotype patients had higher serum IL-6 levels. In a group of young myocardial infarction patients, the GG genotype also occurred more frequently. This does not prove that IL-6 has a pivotal role, but it does suggest that alteration in the ability to mount an inflammatory response may affect long term risk of coronary artery disease.

The use of microarray technology to describe patterns of gene expression in atherosclerosis will allow a more detailed understanding of the mechanisms controlling the response to injury in the arterial wall, and may in due course be helpful in establishing genetic risk profiles.

Other cardiovascular syndromes

Factors other than the ejection fraction seem to be important in determining features such as exercise capacity and degree of cachexia in patients with heart failure (HF). TNF levels are raised in some HF patients. Myocytes have the ability to produce TNF, and giving TNFα in vitro produced a receptor-dependent reduction in contractility. TNFα has a biologically plausible role since it can lead to wasting, anorexia, endothelial dysfunction and apoptosis, all of which are seen in heart failure. TNF receptor levels are also elevated in HF.

Although it is possible that ischaemia due to hypoxia is causing the inflammatory stimulus, there is evidence to suggest that endotoxin translocation through an oedematous gut may be important. After an episode of oedema, endotoxin levels are elevated in HF patients compared to controls and non-oedematous HF patients. Diuretic treatment reduces these levels slowly (over four months) to baseline. The endotoxin levels are reflected in the soluble CD14 receptor levels, a marker of binding of endotoxin to its LPS-binding protein. The soluble CD14 receptor levels correlate with TNFα, ICAM-1 and ESR levels, whilst cachetic patients also have higher levels. Cachexia may be a response to chronic inflammation.

Investigation of the role of the inflammatory response in HF may lead to new strategies for therapy. Anti-TNFα therapy has been used in rheumatoid arthritis and inflammatory bowel disease, and may have a role in heart failure. The unexplained mechanism whereby ACE inhibitors reduce coronary event rates, while actually being given for IV dysfunction, may be by reduction of inflammation, since these drugs can lower IL-6 levels.

The pathogenesis of abdominal aortic aneurysms may be different from occlusive atheromatous disease of large vessels. Dilatation of the aorta occurs due to proteolysis weakening the connective tissue structure. This process is driven by inflammation in the adventitia rather than the media, and may involve B-cells as well as macrophages and T-cells. Indomethacin, when added to a culture of macrophages and smooth muscle cells, allows the survival of smooth muscle cells that would otherwise die. It inhibits PGE2 production: indeed, the transferable smooth muscle growth inhibitor produced by macrophages may be PGE2.

In a non-randomised trial, patients with abdominal aortic aneurysms who were given non-steroidal anti-inflammatory drugs appeared to have less progressive dilatation. Further work to identify patients at risk of continued dilatation and rupture is needed.

Conclusion

Investigation into the role of inflammation in cardiovascular syndromes has led to a greater understanding of the mechanisms involved, so improving the ability to develop new treatments and assess coronary risk. The macrophage, under T-cell control, now appears to be the culprit cell in plaque destabilisation in atherosclerotic vessels. The conference provided an opportunity to hear some of the leading researchers in the field present their own findings and put them in context with the rapidly changing ideas about the pathogenesis of both atherosclerosis and non-ischaemic cardiovascular disease.

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