Morphology of the endothelium over atherosclerotic plaques in human coronary arteries

MICHAEL J DAVIES,* NEVILLE WOOLF,† PETER M ROWLES,† JOHN PEPPER‡

From the *British Heart Foundation Cardiovascular Pathology Unit, St George's Hospital Medical School;* †*Bland-Sutton Institute of Pathology, Department of Histopathology, University College and Middlesex School of Medicine; and* ‡*South West Thames Regional Cardiothoracic Unit, St George's Hospital, London*

SUMMARY   The right coronary arteries of six hearts removed from patients with atherosclerosis, who were undergoing cardiac transplantation, were perfused with 2% buffered glutaraldehyde for 20 minutes before preparation for scanning electron microscopy. Perfusion was started within five minutes of explantation. In two patients the artery was angiographically normal, in one it was irregular in outline, and three had focal segments with significant stenosis. None of the patients had concentrations of plasma lipids above 5·5 mmol/l. The endothelial surface showed widespread focal abnormalities ranging from adhesion and migration of monocytes to loss of individual endothelial cells. Larger areas of endothelial denudation with exposure of underlying collagen were also seen consistently. Loss of endothelial cells was associated with accumulation of monocytes, on and deep to the surface, as well as adhesion of platelets to the exposed subendothelial tissue.

These results accord with the endothelial damage and platelet adhesion seen in hyperlipidaemic animals fed a high lipid diet.

Lesions resembling those seen in human atherosclerosis were induced by diets rich in fat that caused hyperlipidaemia in a wide range of animals including pigs,[1] rodents,[2] rabbits,[3] and primates.[4-6] Hyperlipidaemia caused by inherited abnormalities of lipid metabolism in the rabbit also led to the development of atherosclerosis.[7 8] These animal models permitted the sequence of changes in the vessel wall to be followed serially, and they consistently showed that the first morphological change in the development of atherosclerosis was adhesion of monocytes to an intact endothelial surface followed by their migration into the intima,[2] a process that begins within 12 days of the start of a high lipid diet in primates.[4] By 12 weeks sufficient foam cells appeared within the intima to form fatty streaks and most of these lipid filled cells were believed to be macrophages.[3 9 10]

Demonstrable morphological changes were preceded by an increased rate of replication of endothelial cells[11] associated with enhanced permeability to Evans Blue and it was at such sites that monocyte adhesion occurred.[12] It has been suggested that the early increase in the turnover of endothelial cells is associated with increased desquamation of endothelial cells[13] but not with exposure of the subendothelial collagen. The mechanism by which monocytes react with intact endothelial cells is unclear. On one hand, hyperlipidaemia may produce functional changes in the endothelial and smooth muscle cells—for example, increasing the expression of factors that are chemotactic for monocytes.[14] On the other hand, hyperlipidaema or hyperfibrinogenaemia may enhance the capacity of circulating monocytes to adhere to endothelial surfaces.[15-17]

The appearance of fibro-lipid plaques in animal models of atherosclerosis was associated with a considerable increase in the adhesion of monocytes to the endothelial surface and morphological evidence suggesting that lipid filled macrophages were both entering and leaving the intima.[5 18] At this stage, usually 3–4 months after the onset of hyperlipidaemia, smooth muscle migration into the intima was also well established. The presence of large aggregates of lipid filled macrophages then became associated with focal defects in the endothelial lining that exposed collagen, and in some models platelet adhesion was seen in such areas.[5 19]

Requests for reprints to Professor Michael J Davies, British Heart Foundation Cardiovascular Pathology Unit, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE.

Animal models have therefore given considerable insights into the injury of the arterial wall caused by hyperlipidaemia. All animal models, however, have limitations[20] and we cannot be certain that the sequence of cellular change is the same in humans who are not clinically hyperlipidaemic but who have atherosclerosis.

It is difficult to obtain suitable material in which to examine the endothelium over human atherosclerotic plaques. After death deposition of a superficial layer of polymerised fibrin on the inner surface of the vessel makes it impossible to see the underlying endothelium clearly by scanning electron microscopy. Endarterectomy specimens are a source of atherosclerotic plaques from living patients but damage during sugical removal leads to considerable artefactual change in the endothelium. Many of the difficulties in assessing the integrity of the endothelium over atherosclerotic lesions in human beings can be overcome by examination of explanted hearts removed during cardiac transplantation. We report the results of a preliminary study of six such hearts.

Patients and methods

Hearts were obtained from six patients undergoing cardiac transplantation. All six had varying degrees of coronary atherosclerosis. Five were male and one was female, and all were less than sixty. Plasma total cholesterol concentrations ranged from 2·0 to 5·5 mmol/l; plasma triglyceride concentrations were not raised. In two of the patients the right coronary artery was angiographically normal; in one it had an irregular outline, and in three there were segments showing severe stenosis. In all cases the hearts were removed at operation after cross clamping the aorta; intracoronary perfusion of the recipient heart was not started during the operation. The patients were anticoagulated with heparin as part of the surgical procedure. The orifice of the right coronary artery was cannulated less than five minutes after removal of the heart from the patient and the artery was perfused with 2% buffered glutaraldehyde at a pressure of 130 mm Hg at 4°C for 20 minutes. Because fresh tissue was needed for other studies a similar technique was not used on the left coronary artery. The right coronary artery, from its origin to the origin of the posterior descending coronary artery, was dissected free from the underlying heart and stored in buffered glutaraldehyde. The coronary artery was then divided into segments that were 1–1·5 cm long. Small portions of vessel wall (0·2 cm long) were removed from the ends of each segment and processed for routine microscopy. The remaining part of each segment was processed routinely for scanning electron microscopy and critically point dried before

being carefully bisected longitudinally and coated with gold-palladium. The coated samples were examined in an ISI DS130 scanning electron microscope, operating at a voltage of between 10 and 20 kV.

Results

Where examination of the intima at low magnifications showed no atherosclerotic lesions the endothelial surface was intact and the cells were aligned with their long axes in the direction of blood flow (fig 1). Endothelial cells over atherosclerotic lesions of all types, however, were irregularly arranged and varied in size and shape (fig 2). Simple fatty streaks had an intact endothelial covering (fig 3).

In all cases leucocytes adhered to the plasma membrane of the endothelial cells in many areas, both over diffuse intimal thickening and localised plaques (fig 4). The surface of the underlying endothelial cell beneath adherent cells was often indented
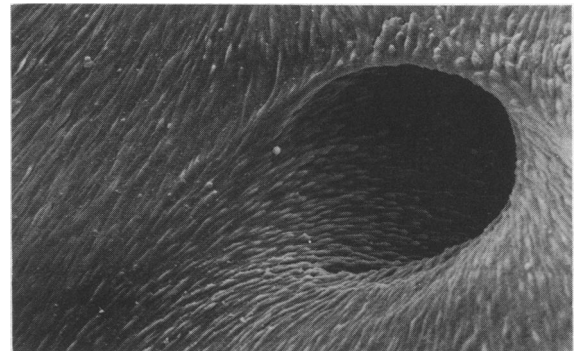

Fig 1   *Normal intact endothelium at a point of arterial branching. The long axes of the cells are aligned in the direction of flow (original magnification, × 278).*
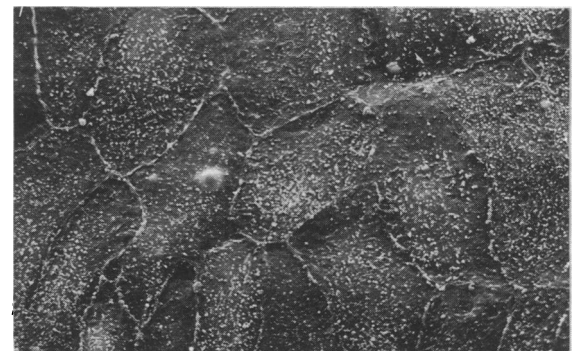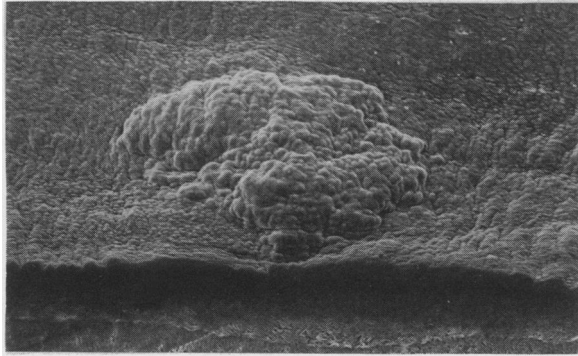

Fig 2   *Intact endothelium over plaque surface showing variation in shape and size of cells and loss of normal orientation. The plasma membranes of the cells have short stubby surface processes (original magnification, × 1480).*

Fig 3   *Micrograph showing a small and complete fatty streak. There is no defect in endothelial integrity but the cells are strikingly raised above the surface of the normal surrounding endothelium because of the subendothelial accumulation of foam cells (original magnification, × 106).*
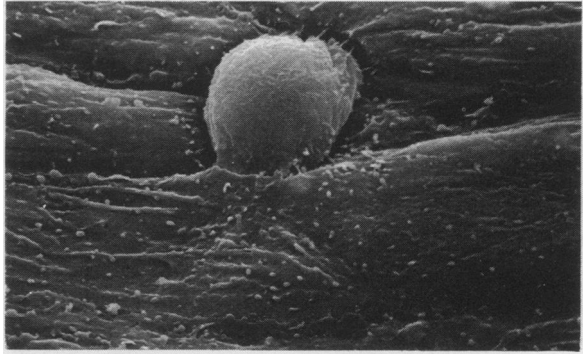
Fig 6   *Leucocyte, part of which is inserted under the edge of an intact endothelial cell (original magnification, × 3870).*
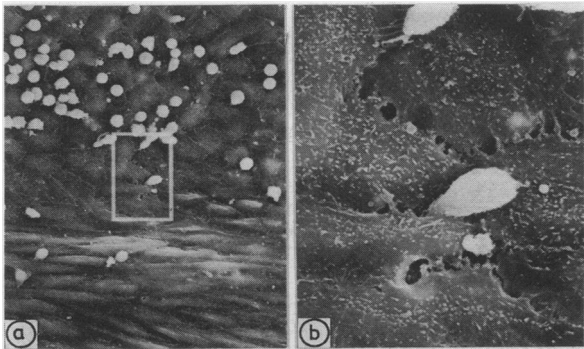
Fig 4(a)/(b)   *Leucocytes adherent to an intact endothelial surface over an atherosclerotic plaque. An adjacent area with endothelial cells showing more normal alignment is not associated with leucocyte adhesion (original magnification, × 407). The higher magnification shows a leucocyte, probably monocytic, in transit between the endothelial cells (original magnification, × 2035).*
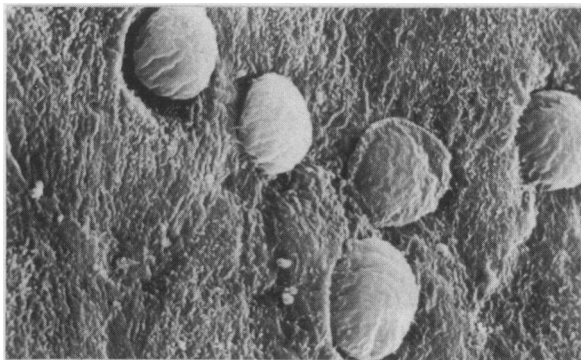

Fig 5   *Leucocyte adherence to intact endothelial cells. The adherent cells appear to settle within small indentation in the plasma mambrane (original magnification, × 2200).*

(fig 5). Leucocytes seemed to be in transit through the endothelium (fig 6); some were strikingly elongated at one pole and attached to the subendothelium via a small but distinct gap in the endothelium (fig 7a and b). Many of the migrating leucocytes had a ruffled plasma membrane suggesting a monocytic origin. Where gaps in the endothelium were larger cells with similar morphological appearances could be seen in the most superficial portions of the intima just beneath the endothelial surface (figs 8 and 9).
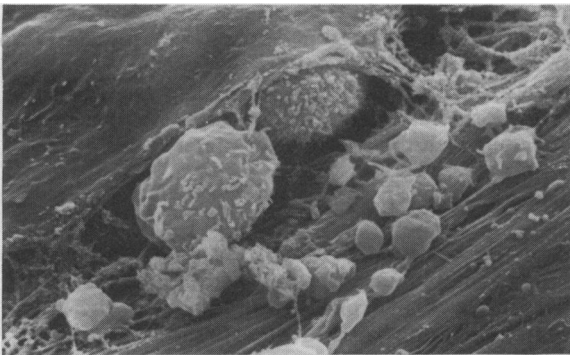
In all six hearts there were defects in the endothelial surface over plaques; and the more severe the extent of intimal disease in any individual patient the more severe and extensive was this change. But endothelial damage was not, however, directly related to the presence or absence of stenosis; it was often found in segments of artery in which there was no significant obstruction. Exposure of subendothelial collagenous tissue took various forms. In some areas loss of a single endothelial cell caused a clearly demarcated defect in the surface; such defects were associated with the presence of adherent platelets (fig 10). Larger gaps in the endothelial surface were seen where groups of endothelial cells had been lost (fig 11). Endothelial denudation was often but not always associated with the presence of platelets (fig 12).
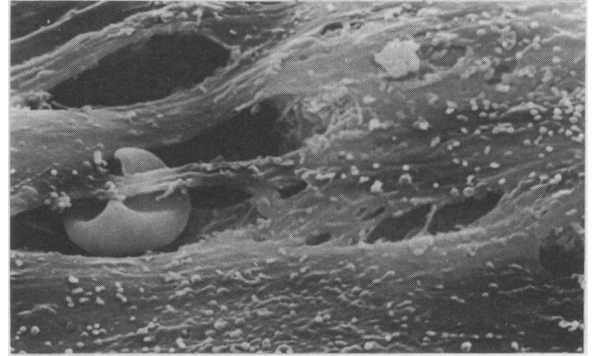
Discussion

All of the patients in this study were undergoing cardiac transplantation because they had advanced atherosclerosis. Morphological damage to the endothelium was common and widespread. Such a study cannot give information on the possible role of endothelial dysfunction in the early stages of atheroma, but it showed that in common with many animal models endothelial denudation in man is a late phenomenon. In this context "late" refers to the
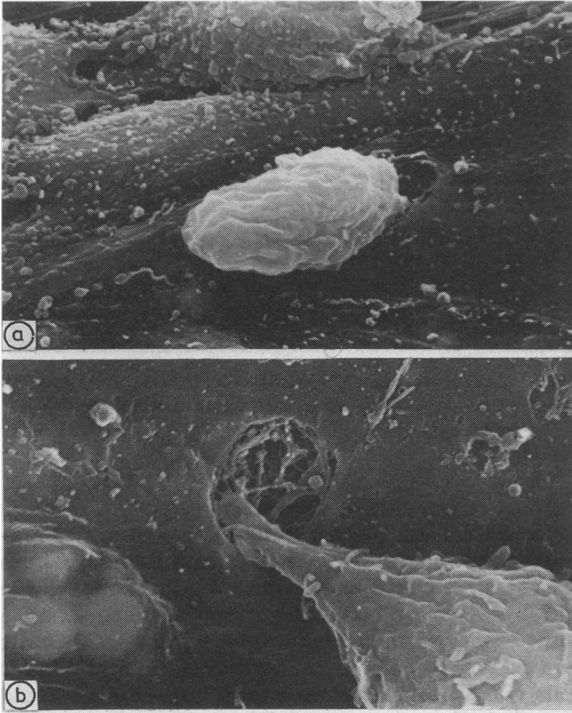
Fig 7   *Leucocyte migration. In (a) the cell is closely applied to a small defect in the endothelial surface (original magnification, × 4430). In (b) the cell is attached to a defect in the endothelium by a strand of cytoplasm. An adjacent area of intact endothelium shows a lipid containing cell immediately beneath the surface (original magnification, × 9800).*

Fig 9   *Endothelium in which there is a defect in the surface that exposes the underlying connective tissue, but no platelet adherence has occurred. A red cell is trapped under a strand of endothelial cytoplasm (original magnification, × 2690).*
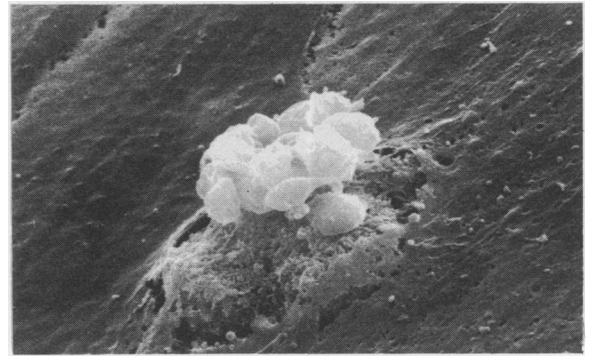
Fig 10   *Loss of a single endothelial cell. The exposed surface is covered by platelets some of which have changed shape (original magnification, × 3570).*
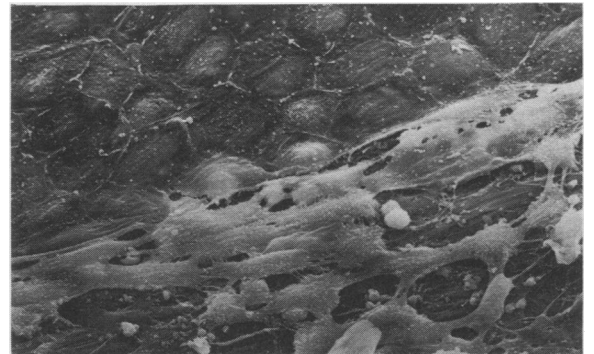

Fig 8   *Fenestrations in the endothelial surface covering two macrophages which are immediately beneath the surface. There are platelets adhering to the exposed subendothelial tissue (original magnification, × 5300).*

Fig 11   *Large area of endothelial damage with exposure of the underlying connective tissue but minimal platelet adherence. An immediately adjacent area of endothelium is intact but of irregular pattern (original magnification, × 880).*
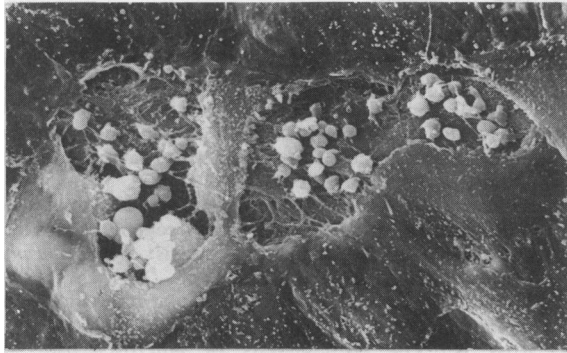
Fig 12   *Endothelial damage and denudation with platelets adhering to the exposed subendothelial tissue (original magnification, × 1340).*

overall degree of intimal involvement and not the degree of stenosis caused by a particular plaque. Endothelial damage was present in many segments without significant obstruction, which shows that angiography is an insensitive method for the detection of diffuse non-stenosing intimal disease.

All the morphological changes noted in this study of human atherosclerosis have been seen in the lesions that develop as a consequence of hyperlipidaemia, whether diet related or inherited, in animal models. Our study showed that monocyte/ endothelium/platelet reactions were also present in human atherosclerotic lesions and gave clear evidence of both monocytic adhesion to an intact endothelial surface and focal endothelial denudation over more advanced plaques in the coronary arteries of patients who were not grossly hyperlipidaemic. We do not believe that the endothelial defects can be the result of artefact because many of them were covered by a layer of platelets, most of which had changed shape. Such a process is unlikely to have occurred during the preparation of the tissue samples. If the endothelial changes were purely secondary to the stress of awaiting cardiac surgery, with the inevitable increase in endogenous catecholamine release, we would not have expected to see a selective effect on the atheromatous lesion. All of the patients had morphologically intact endothelium in other areas.

A morphological study cannot establish the direction of migration of individual monocytes. Over early lesions, however, we saw portions of monocyte cytoplasm within the junctions between intact endothelial cells, which suggests that the monocytes were entering the intima. In more advanced atheromatous lesions macrophages were associated with larger defects in the endothelial surface. In these areas endothelial cells were stretched over underlying cells

that bulged toward the lumen. Such major defects in the endothelium may well allow lipid filled monocytes to be released into the lumen. All the morphological changes seen in animals and taken to indicate emigration of lipid filled cells from the intima[18] were also seen in our study of human material. The association of denuding endothelial damage with macrophages lying immediately beneath the surface also accords with a direct cytotoxic effect on the endothelium. Morphological studies cannot, however, elucidate the mechanism of such an effect; one of many possibilities is the generation of reactive oxygen species.[19]

The finding of endothelial denudation and platelet interaction with the vessel wall in animal models has given rise to the hypothesis that growth factors derived from platelets cause smooth muscle proliferation and plaque growth.[21] The observed abnormal vasomotor responses of atheromatous arteries in both animal models and man[22-24] have also been explained by endothelial damage and platelet deposition.[25 26] The present study of human endothelium over atheromatous plaques indicates that these hypotheses are tenable in man.

The results reported here are preliminary. The degree of endothelial damage needs to be measured with regard to individual factors such as smoking and blood lipid levels as well as local factors such as the degree of stenosis and arterial geometry.

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