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# Expression of Intercellular Adhesion Molecule-1 in Atherosclerotic Plaques

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***Immunohistochemistry of human atherosclerotic arteries demonstrates expression of the intercellular adhesion molecule-1 (ICAM-1) on endothelial cells, macrophages, and smooth muscle cells of the plaques. Normal arterial endothelial cells and intimal smooth muscle outside plaques give weaker or negative reactions; these differ from the strong endothelial expression in small vessels. Quantitative color-image analysis of the endothelial layer shows increased expression of ICAM-1 in all subtypes of atherosclerotic lesions, except fibrous plaques. Endothelial expression of ICAM-1 may be involved in the recruitment of monocytes to the lesion, as suggested by its role in the entry of leukocytes, including monocytes, into foci of inflammation. Collaboration with other mechanisms, particularly chemoattractant factors, may be important for this effect. ICAM-1 enhanced monocyte recruitment is a potential mechanism for the growth of an atherosclerotic plaque. (Am J Pathol 1992, 140:665–673)***

Despite the universal occurrence of atherosclerosis in the Western world, the pathogenesis of disease remains incompletely understood. Virchow was the first to suggest that it is a modified form of chronic inflammation induced by lipid, and many have followed in this path.[1–5]

The active stages of the lesion of atherosclerosis are characterized by the extensive infiltration of blood-derived macrophages through the endothelium into the arterial intima.[4–7] By contrast, non-atheromatous areas contain little or no macrophage infiltrate.

Research has shown that endothelial cells can be induced to express adhesion molecules that facilitate the binding of leukocytes *in vitro*.[8] Increased expression of these molecules on vessels in sites of inflammation suggest that they have a role in the transendothelial passage of leukocytes *in vivo*.[9–11]

The intercellular adhesion molecule 1 (ICAM-1) is expressed on the endothelium and promotes the adhesion of monocytes, neutrophils, and lymphocytes.[9,12,14,15] Similarly the vascular cell adhesion molecule (VCAM-1) binds monocytes and T lymphocytes,[13] whereas the endothelial leukocyte adhesion molecule 1 (ELAM-1) enhances polymorph, monocyte, and T-lymphocyte interactions.[9,14,15] ICAM-1 interacts with leukocytes principally by binding to the surface membrane beta-2 integrin molecule, LFA-1,[12] but also by binding another beta-2 integrin, MAC-1.[16] A second endothelial adhesion molecule, named ICAM-2, exists which binds LFA-1, but its expression appears to be constant and not regulated.[17] A 140 kD granule membrane protein (GMP140) is present in endothelial cells and platelets that can bind neutrophils and monocytes. This molecule is rapidly translocated to the surface membrane on cell activation.[10,18]

Adhesion molecule expression is induced *in vitro* by the action of cytokines on endothelial cells. A basal level of ICAM-1 expression exists on resting small vessels; however, ELAM-1 and VCAM-1 are normally absent. Interleukin 1 IL-1 and tumor necrosis factor TNF induce ICAM-1, VCAM-1, and ELAM-1, whereas interferon gamma (IFN-g) induces ICAM-1 alone.[8,9,13,14] *In vivo*, these cytokines may be produced by several cell types; in chronic inflammation activated macrophages are likely to be an important source of IL-1 and TNF.[19,20] T lymphocytes produce IFN-g, together with IL4 and other cytokines which modulate the effects of the IFN-g.[21,22] Recently IL-1 mRNA and TNF protein have been detected in macrophages and smooth muscle cells of atherosclerotic plaques.[24,25]

In view of the importance of transendothelial monocyte entry to the genesis of an atherosclerotic plaque, the expression of adhesion molecules in the cells of the en-

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dothelial layer is a matter of great interest. Recently a molecule resembling VCAM-1 has been found in the endothelium of rabbit atherosclerotic plaques.[23] There is no information on other adhesion molecules. We report on the distribution of ICAM-1 in human atherosclerosis and compare it with the normal artery.

## *Materials and Methods*

Monoclonal antibodies (MCA) were raised against cytokine- stimulated human endothelial vein monolayers,[26] and selected for specific reactivity against stimulated cells. Two clones of mouse $IgG_1$ class were produced, designated 6.5B5[26] and 8.4A6. Both were shown to be specific for reaction with ICAM-1 by studies on L-cell transfectants. An additional $IgG_1$ anti-ICAM-1 clone, 84H10, was obtained from Immunotech, Marseille, France. In our previous immunohistochemical studies, the 6.5B5 antibody gave staining in most small vessels of tonsil and skin, whereas inflamed skin showed increased reactivity,[11] in keeping with previous reports.[8,9] All antibodies and conjugates were titrated to determine the optimal concentrations for use. The concentration of mouse immunoglobulin in the monoclonal antibody containing culture supernatants was determined by a sandwich ELISA assay with plates coated with rabbit anti-mouse immunoglobulin 10 μg/ml (Dako). The samples were incubated diluted 1/10–1/100 for 2 hours, and the reaction developed with peroxidase conjugated rabbit anti-mouse Ig 1/200 (Dako), followed by o-phenylenediamine dihydrochloride 0.3 mg/ml (Sigma) and hydrogen peroxide as substrates. The plates were read in a MR700 ELISA reader (Dynatech) at 490 nM. A standard curve was produced by dilutions of the $IgG_1$ mouse myeloma MOPC21 (Sigma).

Thirteen aortic and 15 coronary artery specimens were obtained from 21 cadavers undergoing postmortem examination at Guy's Hospital. The time interval from death to the obtaining of the specimens ranged up to 3 days; previous studies have shown good preservation of cell antigens to that time.[27] The subjects were of both sexes (age 49–93 yr) and 12 patients had died from atherosclerosis and its complications. In addition, six specimens were obtained from the aorta and femoral arteries of patients who had surgery for aneurysmal or occlusive atherosclerosis. Segments of the left anterior descending coronary artery and pieces of the other arteries were removed and snapfrozen. The cadaveric blocks (the main study) contained a total of 46 atherosclerotic lesions that were suitable for analysis. A further similar series was used for the doublestaining experiments. In all cases, care was taken with the handling of the arteries so as to minimize the disruption of the fragile endothelial layer. Serial cryostat sections (5 μm) were cut onto silane-coated slides,[28] fixed in acetone and stored at −70°. They were stained immunohistochemically by the indirect and avidin-biotin complex (ABC) immunoperoxidase techniques, with diaminobenzidine as chromagen. All reagents were from Dako Ltd. (High Wycombe, UK) unless stated otherwise. With the ABC technique, either whole IgG or $F(ab')_2$ fragments were used as the biotinylated anti-mouse Ig antibodies in the second layer, with the same results. On staining, care was taken to avoid the drying of sections. With these precautions, artifactual staining of section edges (particularly endothelium) was minimized. All sections of the series were stained together in a single experiment for each antibody tested.

The cells of the lesions were characterized in sequential sections by the use of the following antibodies: −EBM11 (pan-macrophage), HHF35 (muscle actin),[29] UCHT1 (CD3, anti-T lymphocyte), To15 (CD22, anti-B lymphocyte) and rabbit anti-von Willebrand factor (vWF) (endothelial cells). Inflamed tonsil was used as a positive control in all experiments, and as a negative control, all sections were stained with 2 μg/ml of MOPC21 (Sigma), which lacks relevant antigenic specificity.

To confirm the specificity of staining to cell types, double immunoenzyme reactions were carried out. The cell-marking antibodies used were HAM56 (a mouse IgM class pan-macrophage antibody[6]), 1A4 (a mouse $IgG_{2a}$ class antibody to smooth muscle alpha actin, Sigma, Poole, UK), and rabbit anti vWF. Biotinylated anti-mouse $IgG_1$ (Seralab, Crawley Down, Sussex, UK) and ABC-peroxidase were used to stain 6.5B5 or 84H10 brown. These were combined with alkaline phosphatase conjugated anti-mouse IgM (Sigma), anti-mouse $IgG_{2a}$ (Seralab), or swine anti-rabbit Ig followed by BCIP/ nitro-blue tetrazolium (Sigma)[30] to give a deep blue reaction with the cell markers. This method allowed for the dehydration and permanent mounting of the slides. All conjugates were absorbed with human liver powder and human serum to reduce background staining. Double-stained cells showed mixtures of blue and brown tones. For the optimal demonstration of double staining, the cell-marking antibodies were used at submaximal concentrations so that the dense blue alkaline phosphatase reaction product did not obscure the brown produced by the ICAM-1 reaction.

The lesions were classified into subtypes, on the basis of morphology and the reactions of the cell-specific antibodies: fatty streaks (16); intermediate lesions (a stage between fatty streaks and fibro-fatty plaques found in the aorta)[31] (5); fibro-fatty plaques (16); complicated, advanced lesions (2); fibrous plaques (7). Nonlesional areas of the arterial intimas without eccentric thickening or significant macrophage infiltrates served as controls.

ICAM-1 staining in the endothelial region of the arteries was analyzed quantitatively by use of a color image analyzer (Sight Systems, Newbury, Berks; software by Foster-Findlay Associates, Newcastle on Tyne, UK). This

could detect the peroxidase reaction product by measurement of pixels in terms of hue, saturation, and intensity (HSI) values (Figure 5). By this method the percentage of a defined area that was stained could be measured.[32] The thresholds were adjusted so that all but the weakest staining was detected. The arterial image to be measured was displayed on a color monitor, using the microscope X40 objective. Staining of the innermost intima was measured by setting the area for analysis as a strip of 10 pixel (4.5 micron) width, which approximated to the average endothelial thickness in the section. This strip was traced manually along the intimal edge of the arterial image. To aid the sampling of lesions, color print montages were made from low-power photomicrographs of the specimens; lesions were identified, and areas with intact endothelia as determined by staining for vWF were marked out. Between one and six measurements were made in such areas from each lesion so that the majority of the intact endothelium of the lesions was measured. Nonlesional areas were similarly identified and measured.

## Results

### Characterization of the Lesions

The macrophage, as visualized by EBM11 staining, was the most useful cell type for the characterization of lesions, as large numbers were present in all lesion types, except fibrous plaques. Few macrophages were present outside lesions. The actin antibody HHF35 stained the smooth muscle cells of plaques and thickened intima. The endothelium was identified by intense staining with anti-vWF; artifactual loss was noted in some specimens. T lymphocytes, as detected by UCHT1, were present in most lesions, with the greatest numbers in fatty streaks. No B lymphocytes were seen. These reactions, together with cell morphology, and double staining, provided the means of recognizing the cell types present in the serially cut ICAM-1 stained slides.

### ICAM-1 Expression

Normal or diffusely thickened arteries showed little staining in the intima or media (Figure 1 A, B). Occasionally weak or patchy reactions were seen in the endothelial cells, and the medial muscle sometimes stained weakly. Small vessels in the adventitia were strongly positive, resembling those in control tonsils. Thus the level of ICAM-1 expression within these arteries was much lower than in small vessels.

In the plaques, however, there was a massive increase in reactivity for ICAM-1 (Figure 1 C, D, E). Macrophages were well stained (Figure 1D), and intimal smooth muscle cells, particularly near macrophages and in the subendothelial region, were also strongly reactive. Staining of medial smooth muscle adjacent to plaques was occasionally seen. ICAM-1 reactivity was demonstrated by both the indirect and ABC techniques although the intensity of reaction was greater with the latter. All three anti-ICAM-1 antibodies gave similar results, but 6.5B5 gave the most intense staining and a low background.

The intimal cells of plaques adjacent to the lumen often had their intensity of reaction for ICAM-1 much increased above that of the normal artery (Figures 1E, 2A, 5). Caution is required in interpreting such cells as endothelial cells since artifactual loss of the endothelium can occur. However, the specificity of the reaction to endothelial cells was confirmed by double staining for ICAM-1 and vWF. Figure 2A shows the endothelium with individual cells of normal appearance staining for both ICAM-1 and vWF; this endothelium lay at the border (shoulder) region of a plaque, a region often showing the maximum ICAM-1 expression. Figure 2B shows the well-demarcated staining with vWF of an endothelium over a plaque, which was used to confirm the endothelial integrity of the region seen in Figure 5. Although some weaker vWF staining is present in the subendothelial matrix, the endothelial layer is clearly defined.

In some lesions, however, there was strong reactivity in the region of the endothelial layer that did not correspond with the normal morphology of endothelial cells. Spindle-shaped cells lying parallel to the endothelial surface were frequent, and some of these cells were macrophages, as shown by EBM11 staining (Figure 4).

Smooth muscle cell staining by ICAM-1 was confirmed by double staining with 1A4 anti-actin and 84H10 anti-ICAM-1 (Figure 3).

ICAM-1 reactivity in nonmacrophage cells was also demonstrated by double staining with 84H10 combined with HAM56 anti-macrophage antibody, when cells of smooth muscle morphology were stained with 84H10, but were clearly distinguished from macrophages strongly reactive with HAM56 (not shown).

Staining of the surgical specimens showed similar ICAM-1 expression to the autopsy—derived material described earlier, and did not show any superiority in general morphology.

Quantitative image analysis (Figures 5, 6) was performed on the innermost layer of cells in areas with the endothelium intact as indicated by vWF staining (Materials and Methods). The image analysis demonstrated that positive staining for ICAM-1 in these innermost cells was found in increased frequency and intensity in all atherosclerosis lesion subtypes except fibrous plaques, when compared to normal areas of arteries (Figure 6). These differences were significant by the chi-squared test. Fatty

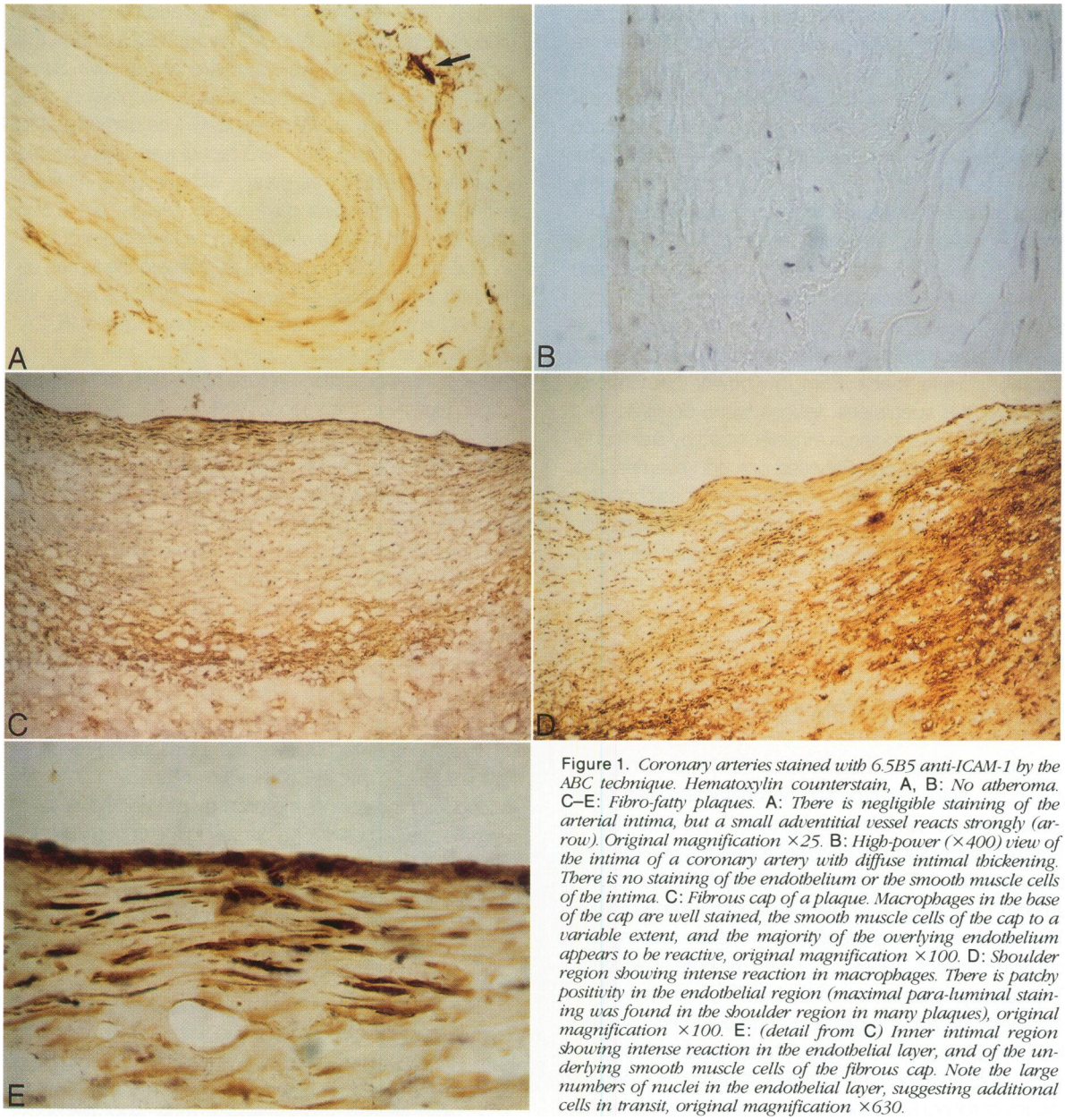


Figure 1. *Coronary arteries stained with 6.5B5 anti-ICAM-1 by the ABC technique. Hematoxylin counterstain,* A, B: *No atheroma.* C–E: *Fibro-fatty plaques.* A: *There is negligible staining of the arterial intima, but a small adventitial vessel reacts strongly (arrow). Original magnification ×25.* B: *High-power (×400) view of the intima of a coronary artery with diffuse intimal thickening. There is no staining of the endothelium or the smooth muscle cells of the intima.* C: *Fibrous cap of a plaque. Macrophages in the base of the cap are well stained, the smooth muscle cells of the cap to a variable extent, and the majority of the overlying endothelium appears to be reactive, original magnification ×100.* D: *Shoulder region showing intense reaction in macrophages. There is patchy positivity in the endothelial region (maximal para-luminal staining was found in the shoulder region in many plaques), original magnification ×100.* E: *(detail from* C*) Inner intimal region showing intense reaction in the endothelial layer, and of the underlying smooth muscle cells of the fibrous cap. Note the large numbers of nuclei in the endothelial layer, suggesting additional cells in transit, original magnification ×630.*

streaks gave the strongest reactions, some with more than 50% of the inner cell layer stained.

Cells that were judged to be T lymphocytes from morphology and UCHT1 staining of serial sections demonstrated little or no ICAM-1 reactivity, as compared with macrophages and smooth muscle cells.

With the control $IgG_1$ class monoclonal MOPC21 at 2 μg/ml in the ABC technique, some traces of staining was seen in the endothelium and in macrophages, but these were much less than the staining with the test antibodies. ELISA assay of the monoclonal antibody concentrations showed that 6.5B5 was used at 0.3 μg/ml, 8.4A6 at 1.4 μg/ml, and 84H10 at 0.9 μg/ml. Therefore there was a considerable margin of safety in the control experiment.

## Discussion

This work has shown that the endothelium of arteries differs from small vessels in having a much lower level of normal ICAM-1 expression. However a unique pattern of extensive strong immunoreactivity is seen in atherosclerotic plaques with the antibodies reactive with ICAM-1. The results with control immunoglobulin confirm the

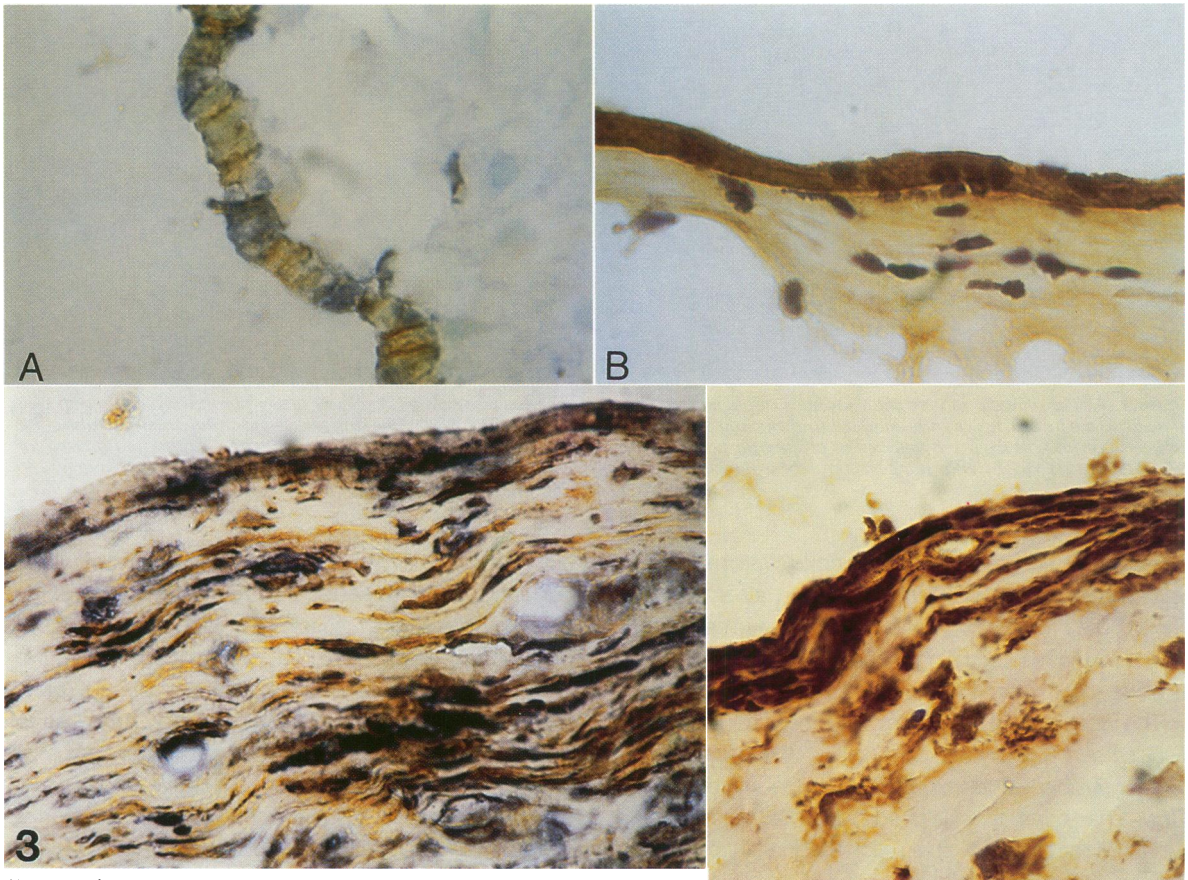


**Figure 2.** A: *Endothelium with normal morphology overlying the margin of a large fibro-fatty plaque in a coronary artery, double stained with rabbit anti-von Willebrand factor (blue) and 84H10 anti ICAM-1 (brown). The anti-vWF staining was reduced in intensity so as to facilitate the visualisation of the ICAM-1 reaction. Original magnification ×1000.* B: *Endothelium over a fibro-fatty plaque of a coronary artery stained with rabbit anti-von-Willebrand factor. Immunoperoxidase, original magnification ×1000.*

**Figure 3.** *Dense fibrous cap of a large heavily lipid-laden aortic fibro-fatty plaque. Section double stained with 1A4 anti actin (dark blue/black) and 84H10 anti-ICAM (brown). Most smooth muscle cells are double stained, original magnification ×1000.*

**Figure 4.** *High power (×630) view of the endothelial region of an aortic fatty streak, stained with EBM11 to show macrophages. The spindle-shaped cells were strongly positive for ICAM-1 in a serial section. Note the morphologic similarity to smooth muscle cells at this position; further away from the endothelium, macrophages are larger and more foamy.*

specificity of this staining reaction. Much of the ICAM-1 staining is of macrophages, which inevitably express the molecule, but it is the reactions seen in the other cells of the plaque that are of great interest.

Expression of ICAM-1 in the endothelial cells is important because of the possible effects on leukocyte adhesion. However, analysis of the plaque endothelial layer is difficult since it shows a complex pattern of ICAM-1 expression, with contributions from endothelial cells, macrophages in passage, and probably from smooth muscle cells close to the lumen. In addition, artifactual loss of endothelial cells can occur. When the expression of ICAM-1 in the inner layer of the intima was quantified by image analysis, all these contributions were likely to have been measured. Nevertheless the total data, which include double staining results, strongly suggest an increased expression in the endothelial cells themselves.

An induction of ICAM-1 on endothelial cells implies a state of activation, and is analogous to that seen in inflammatory reactions.[8–11] However the levels achieved on the arterial endothelial cells of plaques are usually less than in inflamed small vessels.[11]

It is known that ICAM-1 can be induced on many non-endothelial, nonleukocytic cells by cytokines[33] and is found on them in inflammatory diseases, for example on keratinocytes and hepatocytes.[34] However, the expression on smooth muscle cells has not been reported widely; some positivity was previously noted in the vascular smooth muscle of the synovium.[35] The strong reactions in the plaques suggest that the smooth muscle cells

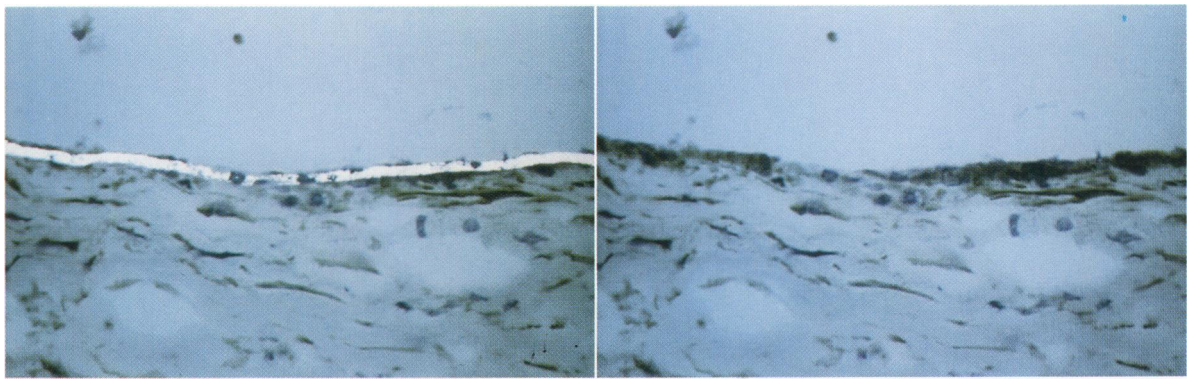

Figure 5. A: *The image analyzer color monitor displaying an image of a peroxidase stained coronary artery fibro-fatty plaque, with cells adjacent to the lumen, apparently endothelium, showing extensive positivity for ICAM-1. Within the strip selected for analysis, white false color has been overlaid on the pixels detected as positive for peroxidase staining by color image analysis. The same area is displayed stained for vWF in Figure 2B.* B: *The same image without the white false color overlay. Images from microscope ×40 objective, width of each image, 230 microns.*

are activated, and concur with the demonstration of other activation antigens on the these cells in atherosclerosis.[36,37] The presence of ICAM-1 on the plaque cells may have a role in concentrating macrophages in the lesion, as all leukocytes express the LFA-1 ligand.[38]

The inducibility of ICAM-1 by cytokines suggests that cytokine secretion by activated cells in the lesions may be responsible for its expression. It is of interest that ICAM-1 has been demonstrated in a human aortic endothelial cell line after TNF stimulation (JM Harlan, personal communication). The recent work by Bareth et al,[24] and our unpublished observations have shown that TNF is present in the smooth muscle cells and macrophages of plaques. Likewise the study by Moyer et al[25] has shown IL-1 mRNA in a similar distribution. The maximal expression of ICAM-1 in fatty streaks, compared to the small amount in fibrous plaques which lack macrophages (Fig. 6), suggest that macrophages could have an important role in its induction. Macrophages in the plaque, particularly those close to the endothelium, synthesise and ex-

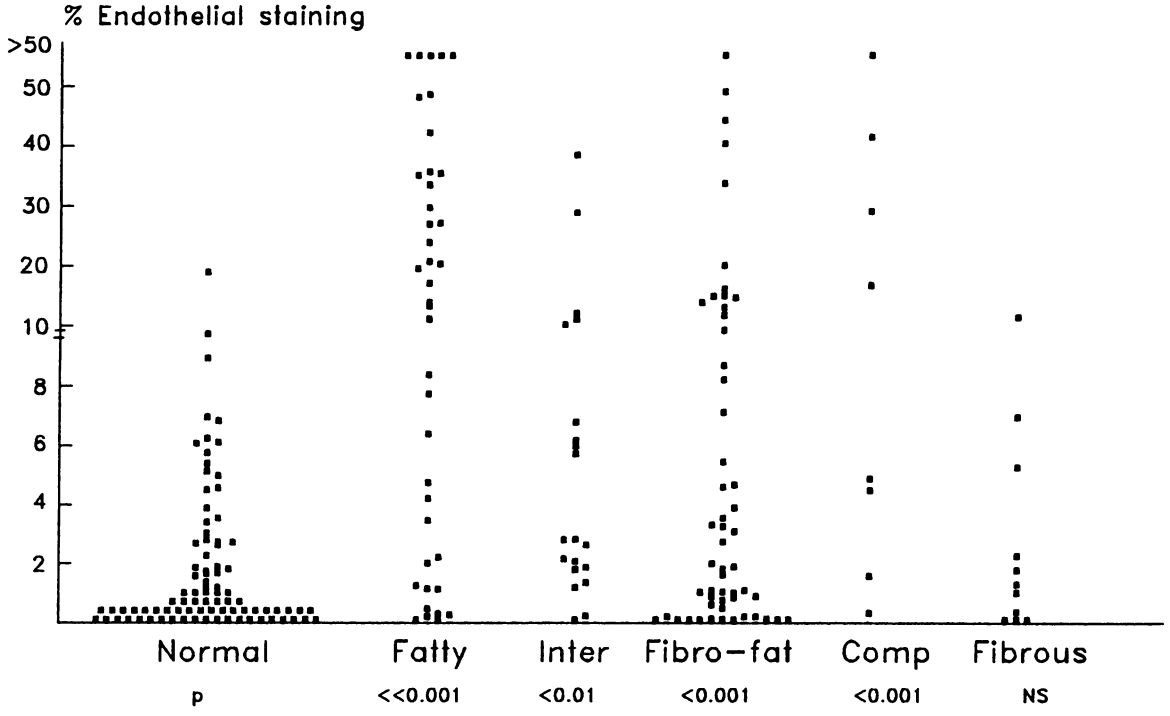


Figure 6. *Percentage of innermost 4.5 micron intimal layer stained by 6.5B5 anti-ICAM-1/ABC method, in sampled areas of atherosclerotic lesions and normal areas of artery. Subtypes of lesions: fatty = fatty streaks, inter = intermediate lesions, fibro-fat = fibro-fatty lesions, comp = complicated plaques, fibrous = fibrous plaques. Total numbers of each subtype are given in Materials and Methods. Probability values (P) are by the chi-squared test, on numbers of observations above 95% of normals. Note the change of Y-axis scale in the middle of the range.*

press a further cytokine, platelet-derived growth factor.[39] This is capable of activating smooth muscle cells; therefore activation events in smooth muscle may be secondary to the presence of macrophages. The expression of ICAM-1 in atherosclerosis could prove to be a useful index of the activity of a lesion.

Although the role of ICAM-1 in the adhesion and transendothelial migration of monocytes remains to be reported in detail, studies on binding to cultured human umbilical vein endothelial cells have shown that beta-2 integrin dependent adherence (presumably to ICAM-1 plus ICAM-2) collaborates with VCAM-1 and ELAM-1 in promoting adhesion.[15] Furthermore monocyte adhesion onto cultured arterial endothelium has been shown to be partially beta-2 integrin dependent.[40] Likewise, U937 cells, which resemble an early stage in the monocyte lineage, bind on ICAM-1 coated wells,[12] and the homotypic (monocyte-monocyte) adhesion of activated monocytes is mediated by ICAM-1—integrin interactions.[41] However, *in vivo,* ICAM-1 expression per se cannot account for cell migration, as small vessels normally express ICAM-1. There is increasing evidence that activation of the LFA-1 ligand on leukocytes may be important in allowing binding,[38] as was found with monocyte homotypic adhesion.[41]

Cooperation between adhesion systems may enhance interactions, as was shown recently with neutrophil binding to ICAM-1 and GMP140 in artificial lipid bilayers.[42] Further work is needed in this field with regard to atherosclerosis. In our preliminary studies, ELAM-1 and VCAM-1 appeared to be less well expressed on the plaque endothelium compared with ICAM-1.

Cooperation with chemotactic factors may also amplify the effects of ICAM-1. Activation of complement occurs in plaques, and the C5a chemotactic factor is generated.[43] Chemotactic factors often activate the target cells, and in the case of neutrophils, C5a activation has been shown to lead to an increase in adhesiveness.[44] Other chemoattractants could be involved: fibronectin, TGF-β and the monocyte chemoattractant protein (MCP-1) are monocyte chemotactic factors derived from macrophages, smooth muscle cells, and endothelial cells.[45–47] Monocyte chemoattractant factors can be induced in endothelial cells by macrophage-derived cytokines, and by the culture of minimally modified LDL with macrophages.[48,49]

In addition to monocytes, T lymphocytes also enter atherosclerotic plaques.[5] As ICAM-1 promotes the adhesion of T cells,[12] these may be recruited without regard to their immune specificity.

Current evidence suggests that the entry of leukocytes into the intima is sufficient to induce many of the major features of an atherosclerotic lesion. Invading leukocytes can induce smooth muscle proliferation.[50] Macrophages accumulate in the lesions, take up lipid to become large foam cells, and add to the bulk of the lesion.[3–7] They can also produce ICAM-1—inducing cytokines, as described earlier.

Monocyte recruitment mechanisms therefore have a potentially significant involvement in the growth of the lesion. It seems likely that they may depend on cellular activity within the plaque, and that this has important consequences, particularly on the morphology of the lesion. In all but its latest stages, atherosclerosis is a strikingly focal disease of the arterial intima. This applies to both human and experimental animal atherosclerosis. However one hypothesis that has been widely considered, the clonal growth of smooth muscle cells, has not proven to be an adequate explanation.[51] Athough the general localization of the plaques may depend on endothelial injury by hemodynamic factors, it seems unlikely that these forces alone could give rise to the well-demarcated lesions that occur. An alternative view is that the lesions expand from initiating stages by a locally acting self-perpetuating mechanism.[2] The induction of ICAM-1 on the arterial endothelium, which is seen in the early stages of atherosclerosis, may contribute to the recruitment of more monocytes to the lesion, probably by operating in concert with other factors. The hypothesis can therefore be advanced that a young lesion is capable of enhancing its own development, resulting in the sharply localized plaque. Increased numbers of monocytes have been shown to adhere to atheromatous plaques,[4,52] and many were seen within the endothelium in this study. The essentially all or none response of the vascular wall to atherosclerosis is a typical response produced by a self-perpetuating positive feedback system.

A potential exists for synergistic action between cytokine-activated endothelium and thrombogenic mechanisms in the generation of a plaque. Thrombin can stimulate GMP140 expression[53] and endothelial cell adhesiveness for mononuclear cells,[54] whereas cytokine-activated endothelium is able to produce tissue procoagulant factor and platelet-activating factor.[55,56] As our results suggest that the endothelium of plaques is activated, a thrombogenic capacity for endothelium in this state could be of particular significance.

The stark differences between atheromatous and unaffected areas of the arterial intima imply that the local mechanisms involved must be powerful. It is proposed that these are the local activation of a cytokine network, cell activation, the induction of adhesion molecules and chemoattractants, and a regenerative accumulation of monocytes. These form a current explanation for the long-standing view of a similarity of atherosclerosis to inflammation since inflammatory mechanisms are similar. If they prove to have the major role that is suggested, their

interruption could offer a new therapeutic approach to this important disease.

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## References

1. Virchow R: Der ateromatose prozess der arterien. Wien Med Wochenschr 1856, 6:825
2. Poston RN, Davies DF: Immunity and inflammation in the pathogenesis of atherosclerosis. Atherosclerosis 1974, 19:353–367
3. Schwartz CJ, Valente AJ, Sprague EA, Kelley JL, Sueneram CA, Rozek MM: Atherosclerosis as an inflammatory process. The roles of the monocyte—macrophage. Ann NY Aced Sci 1985, 454:115–120
4. Munro JM, Cotran RS: The pathogenesis of atherosclerosis: atherogenesis and inflammation. Lab Invest 1988, 58:249–261
5. Hansson GK, Jonasson L, Seifert PS, Stemme S: Immune mechanisms in atherosclerosis. Arteriosclerosis 1989, 9:567–578
6. Gown AM, Tsukada T, Ross R: Human atherosclerosis II. The immunocytochemical composition of human atherosclerotic lesions. Am J Pathol 1986, 125:191–207
7. Poston RN, Hussain IF, Matyszak M, Adams CWM: The heterogeneity of macrophages in atherosclerosis revealed by immunohistochemistry. 8th International Symposium on Atherosclerosis 1988, CIC, Rome, p 743
8. Pober JS: Cytokine-mediated activation of vascular endothelium. Physiology and pathology. Am J Pathol 1988, 133:426–433
9. Munro JM, Pober JS, Cotran RS: Tumor necrosis factor and interferon gamma induce distinct patterns of endothelial activation and associated leucocyte accumulation in skin of Papio Anubis. Am J Pathol 1989, 135:121–133
10. Carlos TM, Harlan JM: Membrane proteins involved in phagocyte adherence to endothelium. Immunol Rev 1990, 114:5–28
11. Norris P, Poston RN, Thomas DS, Thornhill M, Hawk J, Haskard DO: The expression of the endothelial leukocyte adhesion molecule -1 (ELAM-1), intercellular adhesion molecule -1 (ICAM-1), and vascular cell adhesion molecule -1 (VCAM-1) in experimental cutaneous inflammation: a comparison of ultraviolet B erythema and delayed hypersensitivity. J Invest Dermatol 1991, 96:763–770
12. Makgoba MW, Sanders ME, Luce GEG, Dustin ML, Springer TA, Clark EA, Mannoni P, Shaw S: ICAM-1 a ligand for LFA-1 dependent adhesion of B,T and myeloid cells. Nature 1988, 331:86–88
13. Elices MJ, Osborn L, Takada Y, Crouse C, Luhowsky S, Hemler ME, Lobb RR: VCAM-1 on activated endothelium interacts with the leucocyte integrin VLA-4 at a site distinct from the VLA-4/ fibronectin binding site. Cell 1990, 60:577–584
14. Graber N, Gopal TV, Wilson D, Beall LD, Polte T, Newman W: T cells bind to cytokine-activated endothelial cells via a novel inducible sialoglycoprotein and endothelial leukocyte adhesion molecule-1. J Immunol 1990, 145:819–830
15. Carlos T, Kovach N, Schwartz B, Rosa M, Newman B, Wayner E, Benjamin C, Osborn L, Lobb R, Harlan J: Human monocytes bind to two cytokine-induced adhesive ligands on cultured human endothelial cells: endothelial leucocyte adhesion molecule -1 and vascular cell adhesion molecule-1. Blood 1991, 77:2266–2271
16. Diamond MS, Staunton DE, Marlin SD, Springer TA. Binding of the integrin MAC-1 (CD11b/CD18) to the third immunoglobulin-like domain of ICAM-1 (CD54) and its regulation by glycosylation. Cell 1991, 65:961–971
17. Staunton DE, Dustin ML, Springer TA. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. Nature 1989, 339:61–64
18. Geng J-G, Bevilacqua MP, Moore KL, McIntyre TM, Prescott SM, Kim JM, Bliss GA, Zimmerman GA, McEver RP: Rapid neutrophil adhesion to activated endothelium mediated by GMP140. Nature 1990, 343:757–760
19. Bayne EK, Rupp EA, Limjuco G, Chin J, Schmidt JA: Immunohistochemical detection of interleukin 1 within stimulated human monocytes. J Exp Med 1986, 163:1267–1280
20. Chensue SW, Remick DG, Shmyr-Forsch C, Beals TF, Kunkel SL: Immunohistochemical demonstration of cytoplasmic and membrane associated tumor necrosis factor in murine macrophages. Am J Pathol 1988, 564–572
21. Thornhill MH, Haskard DO: IL4 regulates endothelial cell activation by IL-1, tumor necrosis factor or IFN-γ. J Immunol 1990, 145:865–72
22. Doukas J, Pober JS: Lymphocyte-mediated activation of cultured endothelial cells. J Immunol 1990, 145:1088–1098
23. Cybulsky MI, Gimbrone MA: Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 1991, 251:788–791
24. Barath P, Fishbein MC, Cao J, Berenson J, Helfant RH, Forrester JS: Tumor necrosis expression in human vascular intimal smooth muscle cells detected by *in situ* hybridization. Am J Pathol 1990, 137:503–509
25. Moyer CF, Sajuthi D, Tulli H, Williams JK: Synthesis of IL-1 alpha and IL-1 beta by arterial cells in atherosclerosis. Am J Pathol 1991, 138:951–960.
26. Wellicome SM, Thornhill MH, Pitzalis C, Thomas DS, Lanchbury JSS, Panayi GS, Haskard DO: A monoclonal antibody that detects a novel antigen on endothelial cells that is induced by TNF, IL1 or LPS. J Immunol 1990, 144:2558–65
27. Pallesen G, Knudsen LM: Leucocyte antigens in human post-mortem tissues: their preservation and loss as demonstrated by monoclonal antibody immunohistological staining. Histopathology 1985, 9:791–804

28. Maddox PH, Jenkins D: 3-Aminopropyltriethoxysilane (APES): a new advance in section adhesion. J Clin Pathol 1987, 40:1256–7
29. Tsukada T, McNutt MA, Ross R, Gown AM: HHF35, a muscle actin specific monoclonal antibody. II Reactivity in normal, reactive and neoplastic human tissues. Am J Pathol 1987, 127:389–402
30. De Jong ASH, van Kessel-Van Vark M, Raap AK: Sensitivity of various visualization methods for peroxidase and alkaline phosphatase activity in immunoenzyme histochemistry. Histchem J 1985, 17:1119–1130
31. Small DM: Summary of concepts concerning the arterial wall and its atherosclerosis lesion. Atherosclerosis V. Edited by Gotto AM et al. Berlin, Springer-Verlag, 1980, pp 514–523
32. Poston RN, Gall NP: Hue-saturation-intensity color image analysis for the quantitation of immunoperoxidase staining. Micro 90. Edited by Elder HY, Adam Hilger. Philadelphia & NY, Bristol, 1990, pp 525–528
33. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA: Induction by IL 1 and interferon γ: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 1986, 137:245–254
34. Editorial. Adhesion molecules in diagnosis and treatment of inflammatory diseases. Lancet 1990, 336:1351–1352
35. Koch AE, Burrows JC, Haines GK, Carlos TM, Harlan JM, Leibowich SJ. Immunolocalization of endothelial and leucocyte adhesion molecules in human rheumatoid and osteoarthritic synovial tissues. Lab Invest 1991, 64:313–320
36. Ju Printseva O, Pecio MM, Tjurmin AV, Faerman AL, Danilov SM, Repin VS, Smirnov VN: A 90-kd surface antigen from a population of smooth muscle cells from atherosclerotic lesions. Am J Pathol 1989, 134:305–13
37. Seifert PS, Hansson GK: Decay-accelerating factor is expressed on vascular smooth muscle cells in human atherosclerotic lesions. J Clin Invest 1989, 84:597–604
38. Figdor CG, van Kooyk Y, Keizer GD: On the mode of action of LFA-1. Immunol Today 1990, 11:277–280
39. Ross R, Masuda J, Raines EW, Gown AM, Katsuda S, Sasahara M, Malden LT, Masuko H, Sato H: Localization of PDGF-B protein in macrophages all phases of atherosclerosis. Science 1990, 248:1009–1012
40. Beekhuizen H, Corsel-van Tilburg AJ, van Furth R: Characterization of monocyte adherence to human macrovascular and microvascular endothelial cells. J Immunol 1990, 145:510–518
41. Lauener RP, Geha RS, Vercelli D: Engagement of the monocyte surface antigen CD14 induces lymphocyte function-associated antigen-1 -dependent homotypic adhesion. J Immunol 1990, 145:1390–1394
42. Laurence MB, Springer TA. Leukocytes roll on a selectin at physiologic flow rates: distinction from and perquisite for adhesion through integrins. Cell 1991, 65:859–873
43. Seifert PS, Hugo F, Tranum-Jensen J, Zahringer U, Muhly M, Bhakdi S: Isolation and characterisation of a complement activating lipid extracted from atherosclerotic lesions. J Exp Med 1990, 172:547–556
44. Goldstein IM: Complement: biologically active products. Inflammation: basic principles and clinical correlates. Edited by Gallin JI, Goldstein IM, Synderman R. New York, Raven Press, 1988, pp 55–74
45. Denholm EM, Wolber FM, Phan SH: Secretion of monocyte chemotactic activity by alveolar macrophages. Am J Pathol 1989, 135:571–580
46. Wahl SM, McCartney-Francis N, Mergenhagen SE: Inflammatory and immunomodulatory roles of TGF. Immunol Today 1989, 10:258–261
47. Leonard EJ, Yoshimura T: Human monocyte chemoattractant protein-1 (MCP-1). Immunol Today 1990, 11:97–101
48. Rollins BJ, Yoshimura T, Leonard EJ, Pober JS: Cytokine-activated human endothelial cells synthesize and secrete a monocyte chemoattractant, MCP-1/JE. Am J Pathol 1990, 136:1229–1233
49. Berliner JA, Territo MC, Sevanian A, Ramin S, Kim JA, Bamshad B, Esterson M, Fogelman AM: Minimally modified low density lipoprotein stimulates monocyte-endothelial interactions. J Clin Invest 1990, 85:1260–1266
50. Prescott MF, McBride CK, Court M: Development of intimal lesions after leucocyte migration into the vascular wall. Am J Pathol 1989, 135:835–846
51. Thomas WA, Kim DN: Atherosclerosis as a hyperplastic and/or neoplastic process. Lab Invest 1983, 48:245–255
52. Bylock AL, Gerrity RG: Visualization of monocyte recruitment into atherosclerotic arteries using fluorescent labelling. Atherosclerosis 1988, 71:17–25
53. McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF: GMP140 a platelet alpha granule membrane protein, is also synthesised by vascular endothelial cells and is located in the Weibel-Palade bodies. J Clin Invest 1989, 84:92–99
54. Saegusa Y, Cavender D, Ziff M: Stimulation of mononuclear cell binding to human endothelial cell monolayers by thrombin. J Immunol 1988, 141:4140–4145
55. Bevilacqua MP, Pober JS, Majeau GR, Fiers W, Cotran RS, Gimbrone MA: Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium. Characterization and comparison with interleukin 1. Proc Natl Aced Sci USA 1986, 83:4533–4537
56. Bussolino F, Breviario LB, Aglietta M, Sanavio F, Bosia A, Dejana E: Studies on the mechanism of interleukin 1 stimulation of platelet activating factor synthesis in human endothelial cells in culture. Biochim Biophys Acta 1987, 927:43–54