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# Various Cell Types in Human Atherosclerotic Lesions Express ICAM-1

## *Further Immunocytochemical and Immunochemical Studies Employing Monoclonal Antibody 10F3*

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***The specificity of monoclonal antibody 10F3, generated to smooth muscle cells isolated from fetal human aorta, has been further explored in a series of biological, biochemical, and immunocytochemical studies. In the first assay, it was found that 10F3 could inhibit aggregation of phytohemagglutinin (PHA)-induced lymphocytes in a manner comparable to that of antibody RR1/1, an anti-intercellular adhesion molecule 1 (ICAM-1) monoclonal antibody. In immunoprecipitation experiments followed by one-dimensional gel electrophoresis, both 10F3 and RR1/1 immunoprecipitated 90 kd proteins, with results suggesting that the two antibodies recognized different epitopes of the same molecule. A series of immunocytochemical studies on human atherosclerotic lesions was performed; using single-labeling techniques, 10F3-positive cells were found in the vessel wall and in lesions of virtually all specimens of fatty streaks and fibrous plaques. Using double-labeling techniques, 10F3-positive macrophages and 10F3-positive smooth muscle cells were found; however, there were also a significant number of non-smooth muscle, nonmacrophage 10F3-positive cells. These studies demonstrate that 10F3 identifies ICAM-1, and that this protein is expressed on a variety of cell types in human atherosclerotic lesions. ICAM-1 may represent a developmentally regulated protein that is expressed in fetal but not adult mesenchymal cells, but can be re-expressed in pathologic processes such as atherosclerosis. (Am J Pathol 1992, 140:889–896)***

Previous investigations of the cellular composition of the atherosclerotic lesions have shown that smooth muscle cells predominate over other cell types.[1,2] Nonetheless, smooth muscle cells in these lesions are heterogeneous with respect to phenotype; some are in the so-called "synthetically" active state[3,4] and others in a contractile state. In addition, recent studies have demonstrated heterogeneity in the smooth muscle cell phenotype with respect to cytoskeletal protein expression.[5,6] Furthermore, smooth muscle cells involved in pathologic processes of vessel walls may express certain features of immature smooth muscle cells.[7,8] As part of a program to further probe smooth muscle cell heterogeneity, we have generated the monoclonal antibody 10F3, which identifies a cell-surface 90 kd protein of human mesenchymal cells present on smooth muscle cells of 7–8 week old fetal aorta. It was shown that the 10F3-defined antigen is lost during differentiation and maturation of blood vessels (by all cells other then endothelium), but is then re-expressed upon the development of atherosclerosis by a subpopulation of intimal cells.[9]

Springer and coworkers[10–13] have generated monoclonal antibody RR1/1 which is specific for the ICAM-1 molecule, a member of the cellular adhesion molecule family, which binds specifically to the leukocyte activation antigen LFA-1. ICAM-1 has subsequently been shown to be a member of the immunoglobulin supergene family, and is responsible for the natural adherence of leukocytes to connective tissue cells.[10–12] In this study, we describe the reactivity of antibody 10F3 to the same ICAM-1 molecule (90 kd) identified by antibody RR1/1; in addition, using double immunohistochemical staining, we describe the extent of expression of ICAM-1 human atherosclerotic lesions. ICAM-1 is demonstrated to be ex-

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pressed by smooth muscle cells, macrophages, as well as unidentified mesenchymal cells in the atherosclerotic vessel wall.

## Materials and Methods

### Antibodies

Monoclonal antibody 10F3 was generated in this laboratory as previously described,[9] and was raised to smooth muscle cells isolated from fetal human aorta. Monoclonal antibody RR1/1, which is specific to the ICAM-1 90 kd molecule,[10] was a gift of Dr. T. Springer. Monoclonal HAM56, a macrophage-specific monoclonal antibody and antibody HHF35, an antibody specific for muscle actin isoforms, have been previously described.[1,18] Finally, antibodies to CD45 were obtained from Sigma (St. Louis, MO).

### Aggregation Assay

A quantitative aggregation assay was carried out as previously described.[9] Briefly, peripheral blood lymphocytes were obtained from normal donors and $4 \times 10^5$ PHA-induced immunoblasts (obtained according to methods previously described[14]) in 100 ml RPMI 1640 with 10% fetal calf serum (Flow laboratories) were added to 100 ml of 10F3 hybridoma supernatant in 12 × 75 mm plastic tubes. Simultaneously, 15 mg/ml of PHA (Sigma) were added to the tubes and were incubated for 1 hour at 37° in a shaker at 175 rpm. After 1 hour, the tubes were vortexed for 3 seconds and the number of nonaggregated cells was determined using a hemocytometer. The total number of cells were similarly determined after addition of 10 ml EDTA. The percent aggregation was determined by applying the following formula:

$$\text{percent aggregation} = \left(1 - \frac{\text{number of free cells}}{\text{number of total cells}}\right) \times 100$$

An irrelevant monoclonal antibody of the same isotype, 1A9, obtained in the same hybridization procedure as 10F3, was used as a negative control.

### Radioimmunoprecipitation and SDS-PAGE

The putative antigen was immunoprecipitated with 10F3 and RR1/1 monoclonal antibodies from the U937 cell line, or sixth-passage fetal smooth muscle cells, which had been previously labeled with $^{14}C$ leucine for 20 to 24 hours; immunoprecipitation was carried out according to previously described methods.[9] Cells were harvested with a rubber policeman and lysed in a solution containing phenylmethylsulfonylfluoride (Merck) and 0.05% NP-40. Briefly, the lysates were cleared by centrifugation at 2000 g (10 minutes at 4°C). Appropriate antibodies were added at a dilution of 10 mg/ml to 0.5 ml of cell lysate. After 2-hour incubation, rabbit anti-mouse antibody (8 μl) was incubated for 15 hours at 4°C. The precipitate thus formed was pelleted by centrifugation at 600 g, and rinsed with 0.05% Tween-20 and phosphate-buffered saline (PBS), pH 7.4. After a final wash in PBS, the material was separated into aliquots; 20-ml samples of these solubilized precipitates were applied to a 7 to 22% gradient SDS polyacrylamide gel electrophoresis (SDS-PAGE) as previously described.[15] A combination of low molecular weight kit standards (Pharmacia), plus betagolactosidase (Sigma) and ferritin (Serva) were run in parallel lanes as molecular weight standards. Gels were stained with Coomassie blue, dried, and put in contact with x-ray film for 1-week exposures. After the first immunoprecipitation reaction, samples were incubated with a second monoclonal antibody, repeating the procedure outlined earlier.

### Immunohistochemical Procedures

#### *Single-labeling Studies*

For immunohistochemical studies of human arteries, 12 autopsy samples (age, 25–61 years, Table 1) were obtained within 2–4 hours postmortem and stored in liquid nitrogen. Frozen sections (4–5 μm) were cut, mounted onto glass slides, dried, and fixed for 10 minutes in acetone and washed in PBS. Sections were then incubated for 30 to 60 minutes at room temperature with 1:1000 dilution of purified antibody RR1/1 or undiluted supernatant fluid of hybridoma clone 10F3. After additional washing steps, sections were incubated for 30 minutes with goat anti-mouse IgG (Sigma), containing 3% normal human serum. After washes, the sections were then incubated for 30 minutes with monoclonal peroxidase anti-peroxidase (PAP) complex prepared as previously described[16] by adding horseradish peroxidase (Sigma type VI) to the supernatant anti-peroxidase hybridoma clone AP-FC-2B4[17] at 50 μg/ml. Sections were then incubated for 5 minutes in Tris buffer pH 7.4 containing 0.05%, 3,3′ diaminobenzidine (Sigma) and 0.01% hydrogen peroxide (Merck, Rahway, NJ). Sections were counterstained with hematoxylin. Control sections were treated with an irrelevant antibody, MPC-11, or with secondary antibodies and PAP complex only.

**Table 1.** *Summary of Cases Studied by Immunohistochemistry*

| Age/Sex | Specimen | Site | Nature of lesion | 10F3+ cells clusters | Localization of 10F3+ cells |
|---|---|---|---|---|---|
| 33M | 4-1 | thor | fs | mf | intima |
| 33M | 4-2 | thor | fs | mf | intima |
| 60F | 7-1 | thor | fpl | mf, smc | intima |
| 60F | 7-2 | thor | fpl | ni | b int |
| 25M | 8-1 | thor | fs | mf | intima |
| 25M | 8-2 | thor | gn | not present | — |
| 55M | 9-1 | thor | fpl | ni | b int |
| 61M | 11-1 | thor | fpl | mf | intima |
| 61M | 11-2 | thor | fpl | mf | intima |
| 61M | 11-2 | thor | fpl | smc | media |
| 42F | 1-1 | abd | gn | not present | |
| 42F | 1-2 | abd | fs | mf | s int |
| 47M | 2-1 | abd | fs, fpl | mf, smc | intima |
| 47M | 2-2 | abd | fs, fpl | mf, smc | s int |
| 47M | 2-3 | abd | fs, fpl | ni | b int |
| 47M | 2-4 | abd | fs, fpl | mf, smc | intima |
| 25M | 3-1 | abd | fs | smc | b int |
| 25M | 3-2 | abd | fs | smc | media |
| 25M | 3-2 | abd | fs | mf | b int |
| 25M | 3-3 | abd | fs | ni | b int |
| 25M | 3-3 | abd | fd | mf | intima |
| 25M | 3-4 | abd | fs | smc | media |
| 25M | 3-4 | abd | fs | mf | intima |
| 52M | 5-1 | abd | fs | mf | intima |
| 46M | 6-1 | abd | fpl | mf, smc | intima |
| 46M | 6-2 | abd | fpl | mf, smc | intima |
| 40M | 10-1 | abd | fpl | mf, smc | intima |

abd = abdominal aorta, mf = macrophages, thor = thoracic aorta, smc = smooth muscle cells, ni = non-identified, gn = grossly normal, s int = subendothelial, fs = fatty streak, b int = basal intima, fpl = fibrous plaque.

#### *Double Immunohistochemical Staining*

Arterial sections (fatty streaks, fibrous plaques) were sequentially labeled with monoclonal antibody 10F3 as well as cell type specific antibodies HAM56 or HHF35, as previously described.[9] Briefly, fixed cryostat sections were stained with 10F3 antibody by the PAP technique as described earlier. After incubation in 3,3′ diaminobenzidine solution, sections were washed in PBS and incubated with antibody HHF35 supernatant (1:50) or HAM56 supernatant (1:50) as described earlier except that peroxidase activity in the last step was detected by a 10-minute incubation in 0.05 M Tris-HCL buffer (ph 7.6) containing 0.3 mg/ml of 4 chloro-1-naphthol (Sigma) and 0.03% $H_2O_2$. After final washes in PBS, sections were mounted in glycerol. Control sections were treated identically, although the 10F3 antibody or HHF35 antibodies were omitted.

### *Competitive Binding of 10F3 and RR1/1 Antibodies*

U937 cells ($4 \times 10^5$ per 2.5 ml) were washed in DMEM containing 10% fetal calf serum, 10% human serum, and 0.02% sodium azide; 50 μl of antibodies as indicated in Figure 2 (in the same solution, in concentrations as indicated in Figure 2) were added to cells and incubated for 30 minutes at room temperature. The cells were washed, and 90 ng of $^{111}$In-10F3 antibody (specific activity, 1,100 cpm/ng) were added and incubated for 30 minutes at room temperature. Cells were then washed four times in pH 7.4 PBS containing 0.02% sodium azide. Radioactivity was counted using a Beckman gamma counter. Data were normalized to binding of $^{111}$In-10F3 antibodies in the absence of the nonlabeled antibodies, and were represented as percent of binding of the labeled antibodies. A replicate set of experiments was also performed at 4°C.

## *Results*

### *Aggregation Assay*

An aggregation assay similar to that originally described for monoclonal antibody RR1/1 was used with monoclonal antibody 10F3. As shown in Figure 1, in three separate aggregation assay tests, PHA induced blasts isolated from three different individuals were employed; 10F3 effected inhibition of aggregation ranging from 25% to 35%.

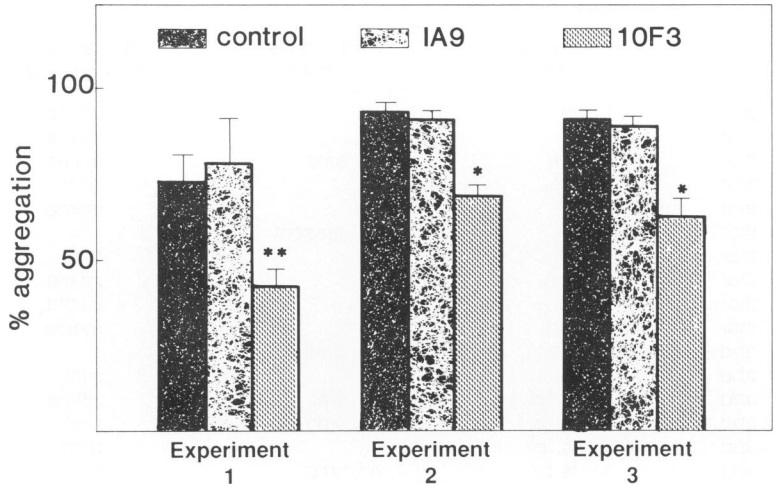


**Figure 1.** *Inhibition of PHA-induced aggregation with anti-ICAM-1 monoclonal antibodies. Results of three separate experiments are depicted; in each case, PHA induced immunoblasts were incubated with one of three monoclonal antibodies: 10F3, 1A9, and negative control. In each experiment, aggregation was inhibited only by antibody 10F3.*

## *Competitive Binding of 10F3 on RR1/1 Antibodies*

Monoclonal antibodies 10F3 and RR1/1 yielded similar inhibition curves when tested against $^{111}$In labeled antibody 10F3, in contrast to the negative control antibody 1A9 (Figure 2). Identical results were obtained at room temperature and at 4°C (data not shown).

## *Cross Immunoprecipitation of RR1/1 and 10F3 Monoclonal Antibodies*

Using radioimmunoprecipitation methods, both RR1/1 and 10F3 monoclonal antibodies immunoprecipitated 90 kd antigens from the U937 cell line (Figure 3). As demonstrated in Figure 3, a second immunoprecipitation step with the same antibody yielded a negative result, but when RR1/1 antibody followed immunoprecipitation with monoclonal antibody 10F3, a 90 kd band was detected (Figure 3).

## *Immunohistochemistry*

Samples from 24 specimens obtained from 11 individuals at autopsy were used, with clinical data and results summarized in Table 1. 10F3-positive cells were present in the vessel wall and or lesions of virtually all the specimens of fatty streaks and fibrous plaques. Using double-labeling procedures, 10F3-positive and HHF35-positive smooth muscle cells were identified in 10 of 24 specimens examined; 10F3-positive macrophages, as identified by double-labeling experiments using the HAM56 monoclonal antibody, were identified in 15 of 24 specimens (Table 1). In virtually all cases in which there were significant numbers of macrophages, a subset of these cells were demonstrated to be 10F3-positive. A subset of the smooth muscle containing lesions were demonstrated to coexpress 10F3. 10F3-positive cell populations were often represented by clusters of cells (Figures 4–6). Cell counting experiments in the double-labeling studies indicated that the fraction of 10F3-positive cells that were also HHF35-positive ranged from 6.3% to 35.6% (mean, 20.4%), whereas the fraction of 10F3-positive cells that

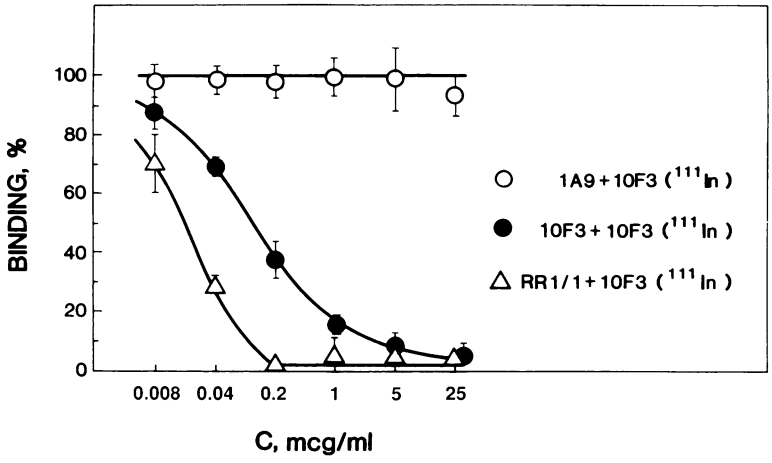


**Figure 2.** *Competitive binding of 10F3 and RR1/1 antibodies toU937 cells. Ordinate refers to percent binding relative to $^{111}$In-labeled 10F3 antibody alone. Both antibodies 10F3 and RR1/1 compete with 10F3 for binding to U937 cells. 1A9 represents irrelevant negative control monoclonal antibody.*

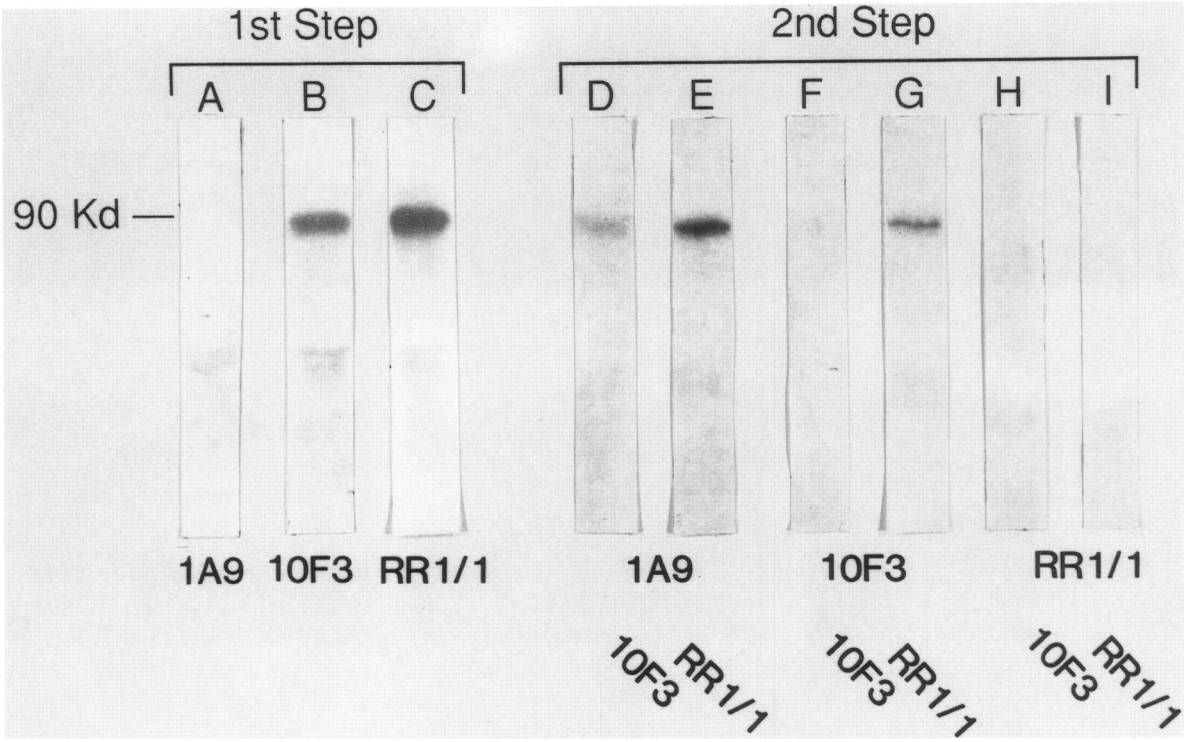


**Figure 3.** *Cross immunoprecipitation of RR1/1 and 10F3 monoclonal antibodies of U937 lysates followed by SDS-PAGE. Left panel: Following first step of immunoprecipitation, both 10F3 and RR1/1 antibodies immunoprecipitate a 90 kd band. 1A9 is negative (irrelevant) antibody control. Right panel: Following second step of cross immunoprecipitation procedure, Lanes A, B: Both 10F3 and RR1/1 immunoprecipitate 90 kd band following use of irrelevant antibody 1A9. Lanes C, D: RR1/1 immunoprecipitates an additional 90 kd band after immunoprecipitation with 10F3. Lanes E, F: No further bands are immunoprecipitated by 10F3 following immunoprecipitation by RR1/1.*

were also HAM56-positive ranged from 7.7% to 47.5% (mean, 25.6%). Thus, this total of HAM56-positive and HHF35-positive cells did not account for the total 10F3-positive cell population, i.e., there were significant numbers of 10F3-positive cells not identified as differentiated smooth muscle cells or macrophages. 10F3-positive smooth muscle cells were also present in the media subjacent to atherosclerotic plaques in several cases (Figure 7). Analysis of lymphocyte composition of these lesions demonstrated that there was no association between the amount of lymphocyte infiltration and the presence of a 10F3-positive cell population (Figure 6c). Results were identical when the 10F3 antibody was replaced by the RR1/1 antibody (data not shown).

## Discussion

As has been described previously,[9] the 10F3 monoclonal antibody, recognizing a 90 kd antigen, was obtained by immunizing mice with cultured smooth muscle cells originally isolated from human fetal aorta. In previous immunohistochemical studies, we have demonstrated that the 10F3-defined antigen is present on mesenchymal cells of human fetal tissue, in particular the smooth muscle cells of 7-week-old fetal aorta. In normal adult aorta, the 10F3-defined antigen is present only on the surface of endothelial cells but not on smooth muscle cells in the media or intima. However, a subpopulation of 10F3-positive cells was found within the intima of atherosclerotic lesions. In the present study, we extend these previous observations, demonstrating the identity of the 10F3 defined antigen as ICAM-1, a specificity with which it shares with the RR1/1 monoclonal antibody described by Springer.[10,11] Identity of the antigen was demonstrated by a functional assay, in which 10F3 was shown, along with RR1/1, to inhibit homotypic adhesion of PHA stimulated lymphoblasts. The percent inhibition, up to 35%, is within the range of inhibition (36–50%) found by Springer and colleagues using a similar assay with antibody RR1/1. In straight-binding competition experiments, it was found that 10F3 and RR1/1 could compete for binding sites on the surface of the myelomonocytic cell line U937. However, the data suggest that RR1/1 is more efficient in blocking binding of 10F3 than is 10F3 itself. Possible explanations of this include differences in binding capacity of the two antibodies, steric hindrance, and the presence of complex postbinding phenomena, although the the replication of these results at 4°C and room temperature argues against the latter possibility.

The cross-immunoprecipitation experiments suggested that both antibodies recognize the same 90 kd molecule as isolated from U937 cells. In these experiments, cross-immunoprecipitation sequentially with

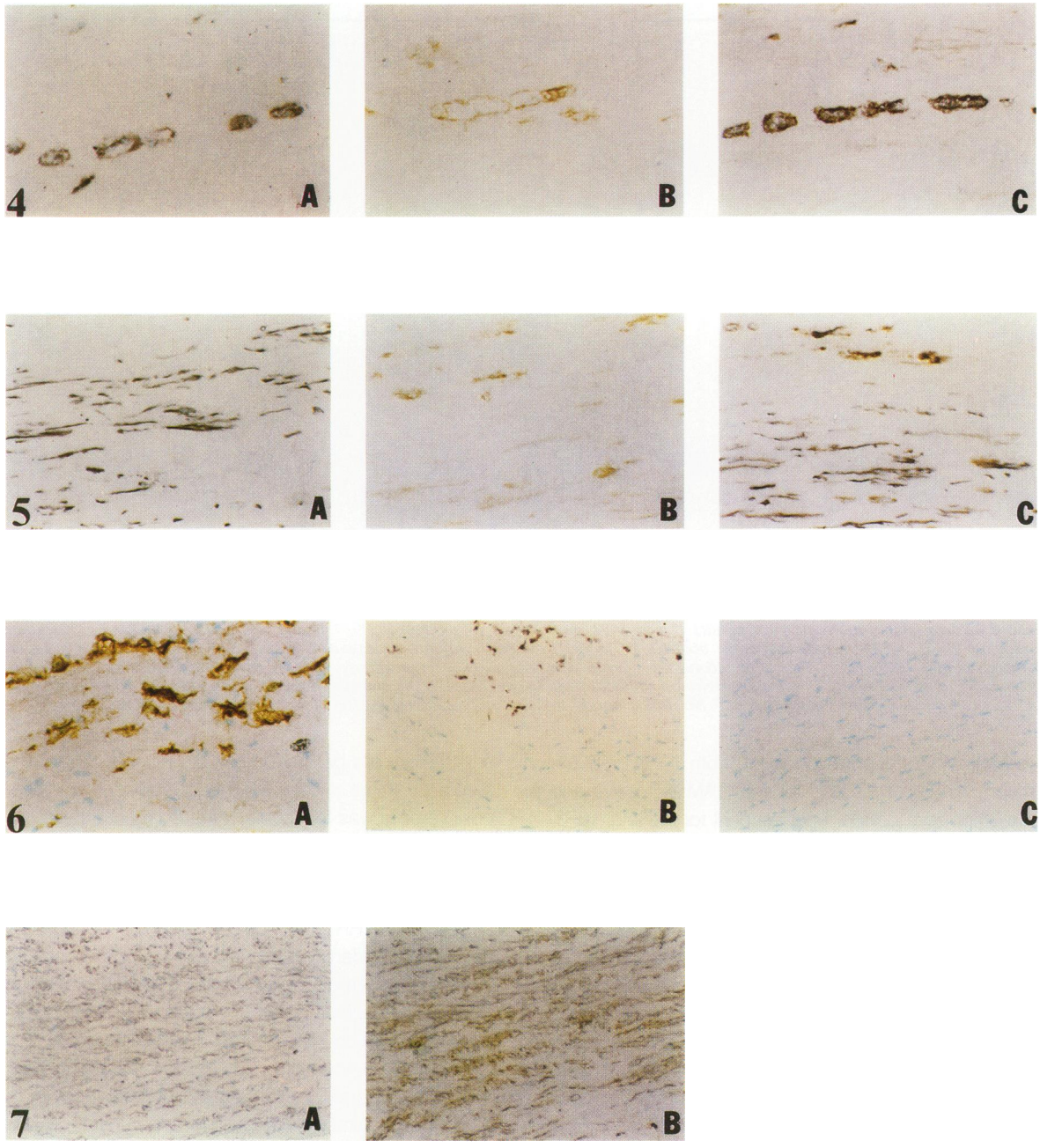


**Figure 4.** *ICAM-1 expression by macrophages in human atherosclerotic plaque.* **A**: *immunostained section with antibody HAM56 showing blue-black reaction product on positive cells.* **B**: *Adjacent immunostained section with antibody 10F3, showing brown reaction product on positive cells.* **C**: *Adjacent double immunostained section showing blue-black reaction product (HAM56) and brown reaction product (10F3), in overlapping cell population, with ICAM-1 expressed on the surface of the macrophages (arrows) (all PAP preparations; original magnification ×400.)*

**Figure 5.** *ICAM-1 expression by smooth muscle cells in human atherosclerotic plaque.* **A**: *Immunostained section with antibody HHF35 showing blue-black reaction product on positive cells.* **B**: *Adjacent immunostained section with antibody 10F3, showing brown reaction product on positive cells.* **C**: *Adjacent double immunostained section showing blue-black reaction product (HHF35) and brown reaction product (10F3), in overlapping cell population (arrows) (all PAP preparations; original magnification ×400.)*

**Figure 6.** *Sequential sections demonstrating distribution of HAM56 macrophages* **(A)** *and CD45-positive lymphocytes* **(B)** *in comparable regions of human atherosclerotic lesion;* **(C)** *negative control with irrelevant primary antibody. Note that the number of HAM56-positive macrophages exceeds the number of CD45-positive lymphocytes (all PAP preparations; original magnification ×400.)*

**Figure 7.** *ICAM-1 expression by smooth muscle cells in the media subjacent to atherosclerotic plaque.* **A**: *Single-label immunoperoxidase preparation showing uniform expression of muscle actins with antibody HHF35.* **B**: *Adjacent section with double labeling immunoperoxidase study; brown reaction product represents 10F3-defined ICAM-1 expression and blue-black reaction product represents HHF35-positive smooth muscle cells. Note HHF35 positivity by only a subset of ICAM-1 positive cells (all PAP preparations; original magnification ×200.)*

RR1/1 and 10F3 antibodies yielded negative results, but when preformed in the reverse order (10F3 followed by RR1/1) a 90 kd band persisted. One possible explanation of this lies in the glycoprotein nature of the ICAM-1 molecule. On the basis of results of chemical deglycosylation experiments, it has been suggested that heterogeneity of the ICAM-1 molecule is a function of variable glycosylation of a common polypeptide core, with the latter having a molecular weight of 73 kd which is then converted to a mature form of the molecule with a molecular weight of 97 kd. It has also been shown that the RR1/1 antibody appears to bind to a protein epitope of ICAM-1, because RR1/1 can immunoprecipitate ICAM-1 from cells labeled in the presence of tunicamycin.[11] Nonetheless, it remains to be rigorously demonstrated to which portion of the molecule (protein or carbohydrate residue) the epitope recognized by 10F3 belongs. Definitive data in this regard might be forthcoming from future transfection experiments utilizing cloned ICAM-1 cDNAs.

In addition, we have extended previous studies by applying the 10F3 monoclonal antibody to sections of human vascular tissue, including early and advanced lesions of human atherosclerosis. ICAM-1 has been previously demonstrated[11–13] to be expressed by a subset of hematopoietic cells such as tissue macrophages and stimulated lymphocytic cells. Given the known composition of human atherosclerotic lesions, which includes various hematopoietic cells such as macrophages and lymphocytes, we applied the 10F3 antibody to sections of human atherosclerotic lesions, including fatty streaks and fibrous plaques.

Our immunocytochemical studies demonstrated ICAM-1 expression in both major cell types present in atherosclerotic lesions: macrophages and smooth muscle cells. Expression of ICAM-1 was noted both in early lesions (fatty streaks) and more advanced lesions (fibrous plaques). In the majority of cases, the vast majority of the HAM56 defined macrophages were ICAM-1 positive, whereas the ICAM-1 positive smooth muscle cells represented only a subpopulation of the HHF35-positive smooth muscle cells present in the lesions.

In addition, we have shown that, by using double-labeling studies, a subset of the 10F3-positive cells are not attributable to either HAM56 positive macrophages or HHF35 positive smooth muscle cells. We propose that these represent a subset of nondifferentiated cells present in the vessel wall; in some cases in which quantitative studies were performed, this subset of 10F3-positive cells amounted to approximately 50% of the antigen-positive cells. Although it is possible that some lymphocytes do account for this 10F3-positive subpopulation, immunostaining with antibodies to CD45 an adjacent sections reveals a dearth of lymphocytes in the region of these 10F3-positive "null" cells.

Other authors have also described the presence of undefined cell types in human atherosclerotic lesions, i.e., cells that do not mark with cell-type specific antibodies such as HHF35 (smooth muscle cells), HAM56 (macrophages), or antibodies to CD45 (lymphocytes) or factor VIII related antigen (endothelium). Such null cells have been referred to as mesenchymal-appearing intimal cells,[19] stellate cells,[20] or synthetic-state smooth muscle cells.[3,8] However, the origin and exact nature of this null cell population remains unclear.

These studies also suggest further phenotypic distinctions between intimal and medial smooth muscle cells. Recently, it has been shown[21] that expression of certain smooth muscle specific set of skeletal proteins (in particularly metavinculin and 150 kd caldesmon) are significantly decreased in the intimal smooth muscle cells relative to the medial smooth muscle cells. This smooth muscle heterogeneity may be a reflection of a subpopulation of phenotypically modulated smooth muscle cells in the vessel wall. Specifically, a subpopulation of these cells possessing smooth muscle-like features may actually represent myofibroblast-like or nonprofessional smooth muscle cells, as has recently been described.[22] These latter cells may be responsible for the predominant cells that accumulate in lesions such as atherosclerosis and represent those cells expressing ICAM-1.

ICAM-1 has been demonstrated to represent a "counter receptor" for the lymphocyte activation antigen LFA-1.[12,13] Inflammatory mediators such as interferon gamma (g-TNF), IL1, and tumor necrosis factor cause strong induction of ICAM-1 expression in a wide variety of cell types and markedly increase binding of monocytes to mesenchymal cells through their cell surface antigen LFA-1.[13] As activated macrophages are known to be sources of many of these lymphokines, the presence of macrophages in human atherosclerotic lesions suggests that the local environment could be responsible for the regulation of expression of ICAM-1 by smooth muscle cells. Alternatively, since it has been demonstrated that smooth muscle cells can be a source of TNF expression,[23] it is also positive that smooth muscle cell modification of ICAM-1 expression can occur through an autocrine mechanism. Recent experiments in our laboratory (data not shown) indicated that TNF, but not IL-1, can induce expression of ICAM-1 by smooth muscle cells *in vitro*. The latter hypothesis may also be supported by the expression of ICAM-1 by clusters of smooth muscle cells without contiguous macrophage populations in the vascular media. Another lymphokine-induced marker, class II MLA (HLA-DR), has also been described in macrophages as well as a subset of smooth muscle cells in atherosclerotic lesions. Preliminary studies suggest, however, that the distribution of HLA-DR and ICAM-1 in the atherosclerotic lesions studied here is nonoverlapping.

One might further speculate that the ICAM-1 expression by smooth muscle cells represents a further manifestation of vascularization, which is known to be present even in early atherosclerotic lesions.[1] In other contexts, ICAM-1 expression has been demonstrated to be associated with the metastatic potential of malignant cells[24] which is known to be associated with vascularization.[25,26] We have also observed increased ICAM-1 expression in smooth muscle cells in the vascularized lesions of Takayasu's disease (data not shown).

In summary, we have demonstrated the presence of the 10F3-defined ICAM-1 molecule in mesenchymal cells, both smooth muscle cells and macrophages, as well as an undefined population of cells, in the human atherosclerotic vessels. ICAM-1 may represent a developmentally regulated protein that is expressed in fetal mesenchymal cells, lost in normal adult mesenchymal cells, but re-expressed in pathologic processes such as atherosclerosis.

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