

Lipoprotein lipase in atherosclerosis: its presence in smooth muscle cells and absence from macrophages

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Abstract The localization of lipoprotein lipase (LPL) in human atherosclerotic lesions was studied with immunocytochemical techniques. In the fibrous cap and surrounding intima of the plaque, where the smooth muscle cell is the dominating cell type, a high number of cells reacted with anti-LPL. A much lower number of stained cells was seen in the central lipid core region where the macrophages dominate. Further characterization of the LPL-containing cells in tissue sections showed that most of them were smooth muscle cells. Only a minor fraction of the macrophages in the plaque contained the enzyme. The results were confirmed on isolated cells from atherosclerotic tissue. Lipoprotein lipase was also detected in smooth muscle cells of non-atherosclerotic arteries. ■ These findings suggest that the smooth muscle cells are the major source of LPL in the vascular wall. However, the enzyme was not present in some of the smooth muscle cells in the atherosclerotic lesion. This may imply that LPL synthesis is down-regulated in the atherosclerotic plaque.—Jonasson, L., G. Bondjers, and G. K. Hansson. Lipoprotein lipase in atherosclerosis: its presence in smooth muscle cells and absence from macrophages. *J. Lipid Res.* 1987. 28: 437-445.

Supplementary key words lipoprotein lipase • immunocytochemistry • atherosclerotic plaques • monoclonal antibodies

Lipoprotein lipase (LPL) is responsible for the catabolism of chylomicrons and very low density lipoproteins (VLDL), i.e., the triglyceride-rich lipoproteins in the circulation. Its main site of action is assumed to be the surface of the capillary endothelium where the activity of the enzyme results in the formation of free fatty acids and cholesterol-rich remnant particles (for a review, see ref. 1).

Zilversmit (2-5) has proposed a hypothesis implying an atherogenic role for LPL. Some of the cholesterol-rich remnants, formed by the action of LPL, may be incorporated in the intima of larger arteries and LPL could therefore be a factor that regulates the rate of cholesterol deposition in the arterial wall. A prerequisite for this hypothesis is that LPL is synthesized in the arterial wall, and it has been established that enzyme activity is present in extracts of larger arteries (4, 6, 7). Endothelial cells do not synthesize the

enzyme (8, 9) and, therefore, it has been suggested that other cells in the vascular wall synthesize the enzyme which is transported to the endothelial cells (9, 10). Enzyme activity has been detected in both the intimal and medial layers of aorta (5, 6) and LPL activity was recently found in pig aortic smooth muscle cells (10).

During the last years several investigators have also demonstrated that macrophages in culture synthesize LPL (11-14). This finding is particularly interesting since a high frequency of macrophages is found in atherosclerotic lesions (15-17). Available data thus indicate that both major cell types in the atherosclerotic lesion may synthesize LPL. However, these data are derived from cell culture studies and it is unclear whether they are related to the actual *in vivo* situation.

In the present study, the localization of LPL in human atherosclerotic plaques was evaluated with immunocytochemical techniques. The enzyme was mainly found in the fibrous cap of the plaque where more than half of the cells contained LPL. In a further characterization of the LPL-containing cells, we found that most of the smooth muscle cells in the lesion were stained by the antiserum against LPL. In contrast, macrophages, which were abundant in the central part of the plaque, hardly ever reacted with the antiserum against the enzyme. Thus, we suggest that the smooth muscle cell is the major source of LPL in the plaque.

MATERIALS AND METHODS

Arterial tissue

Atherosclerotic arterial biopsies were obtained during surgery from 12 patients (age 58-72 years) with transitory ischemic attacks. Complicated plaques of the internal

Abbreviations: LPL, lipoprotein lipase; FITC, fluorescein isothiocyanate.

carotid artery were removed during endarterectomy. Thirty minutes prior to occlusion of the artery, the patients were given a dose of 2500 IU heparin as part of the standard clinical procedure.

Non-atherosclerotic arterial tissue was obtained from the aortic base during coronary by-pass surgery of four patients (heparinized 300 IU/kg body weight), and uterine arteries were obtained during hysterectomies of four patients (not heparinized).

Immediately after excision, the tissue was immersed in ice-cold Hanks' balanced salt solution (HBSS), embedded in OCT embedding medium (Miles Laboratories, Elkhart, IL), and snap-frozen in n-hexane that was chilled with liquid nitrogen.

Antibodies

Characteristics of the antibodies are given in **Table 1**. An antiserum against bovine milk LPL was a kind gift from Dr. Gunilla Bengtsson-Olivecrona, University of Umeå, Sweden. Four mouse monoclonal antibodies were used: anti-Leu-M3, anti-Leu-4, OKIa 1, and CGA 7, the latter kindly provided by Dr. David Gordon, University of Washington. Biotinylated goat-anti-rabbit IgG and biotinylated horse-anti-mouse IgG were purchased from Vector Laboratories, Burlingame, CA. Fluorescein isothiocyanate (FITC)-conjugated swine-anti-rabbit IgG, FITC-conjugated rabbit-anti-mouse IgG, FITC-conjugated rat-anti-goat IgG, and rhodamine-conjugated swine-anti-rabbit IgG were all obtained from Dako, Copenhagen, Denmark.

Immunofluorescence

Two 8- μ m cryostat sections, 50 μ m apart, from each of 12 carotid lesions were examined. They were fixed for 5 min in ethanol, preincubated with 2% normal serum, and then subjected to indirect immunofluorescence as described elsewhere (24). Two or three microscopic fields were randomly chosen at a magnification of 400 (40 \times objective) in each of the following areas of the plaque: the surrounding intima, the peripheral fibrous cap, and the central lipid core (for details, see ref. 17). The immunoreactive cells were

counted in each field. The sections were then demounted and stained with Mayer's Hemalun in order to visualize the nuclei and determine the total number of cells. Approximately 130 cells were seen in each visual field. Totally, approximately 1000 cells were counted per section. The percentage of stained cells was determined per region.

Double-staining experiments on sections from four of the carotid lesions described above, one aortic and two uterine arteries, were also performed as described previously (24). In brief, the sections were incubated with the monoclonal antibodies CGA-7, anti-Leu-M3, anti-Leu-4, or OKIa 1 followed by biotinylated anti-mouse-IgG, and, as a third step, FITC-labeled avidin (Vector). Thereafter the same sections were incubated with anti-LPL followed by rhodamine-conjugated anti-rabbit-IgG. In these experiments, four sections per plaque were studied. FITC- and rhodamine-labeled cells were counted and compared in different microscopic fields as described above.

The following control experiments were performed on frozen sections, smears of isolated cells, and cultured cells. 1) Preincubation with normal serum was omitted. No difference in the staining pattern of anti-LPL was seen which excluded the possibility that enzyme present in the serum had bound to the tissue or cells. 2) Incubation with anti-LPL was excluded. In double-staining experiments, either the monoclonal antibody or anti-LPL was excluded. 3) An unrelated antibody was used instead of the first antibody. Finally, 4) the second antibody was excluded or unrelated conjugated or biotinylated antibodies were used instead of the second antibody. These controls were all negative.

Histochemistry

Fresh cryostat sections were incubated for 40 min in a substrate solution for detection of alpha-naphthyl acetate esterase as described (25).

Isolation of arterial cells

Femoral artery atherosclerotic tissue was obtained during surgery. The tissue was digested with collagenase and elastase as previously described (24) and cells were har-

TABLE 1. Characteristics of antibodies

| Name | Specificity | Produced in | Reference | Suppliers |
|------------------|--|-------------|-----------|-----------|
| Anti-LPL | bovine milk LPL human LPL | rabbit | 18, 19 | A |
| Anti-Leu-M3 | surface antigen on human monocytes/macrophages | mouse | 20 | B |
| Anti-Leu-4 | human pan-T cell antigen, CD 3 | mouse | 21 | B |
| OKIa 1 | human HLA-DR | mouse | 22 | C |
| CGA 7 | smooth muscle specific actin isoform (cross-species) | mouse | 23 | D |
| Anti-factor VIII | human FVIII/von Willebrand | goat | | E |

Suppliers: A, Dr. G. Bengtsson-Olivecrona, Umeå, Sweden; B, Becton-Dickinson, Oxnard, CA; C, Ortho Diagnostic System, Raritan, NY; D, Dr. D. Gordon, University of Washington; E, Nordic Immunologic Laboratories, Tilburg, the Netherlands.

vested. With a combination of elutriator centrifugation and density gradient centrifugation, two major cell fractions were obtained. In essence, the method was based on that described by Haley, Shio, and Fowler (26) for experimental lesions in rabbits. One of the fractions had a density of 1.04–1.06 g/ml. The cells of this fraction have been shown to react with anti-Leu-M3. The other cell fraction had a density above 1.06 g/ml. The cells of this fraction react with CGA 7 (Bondjers, G., P. Karlsson, G. K. Hansson, L. Jonasson, G. Bjursell, and S-O. Olofsson, unpublished results). Four drops of cells from each of the two fractions were air-dried onto microscopic slides and fixed. They were then double-stained with anti-Leu M3 or CGA7 and anti-LPL as described. Two hundred Leu M3-positive or CGA7-positive cells were counted in each drop and the percentage of LPL-positive leukocytes and smooth muscle cells was determined.

Isolation and culture of macrophages

Human mononuclear cells were obtained from heparinized blood using Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) and were resuspended in culture medium. The medium consisted of RPMI 1640, supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin sulfate, 50 mg/ml ascorbate, and 20% autologous serum. Glass cover-slips were placed in Petri dishes and overlaid with the cell suspension. After incubation at 37°C for 2 hr, the nonadherent cells were removed by gentle but thorough washing. The remaining adherent cells consisted of > 90% monocytes characterized by fluorescence staining with anti-Leu-M3. Two days and 1 week after onset of culture, the macrophages were subjected to double-staining immunofluorescence using anti-Leu-M3 and anti-LPL. The percentage of cells reactive with anti-LPL per total number of anti-Leu-M3-positive cells was determined.

RESULTS

LPL was detected immunocytochemically in human atherosclerotic tissue with an antiserum against bovine milk LPL. As shown previously, this antiserum blocks the activity of human LPL and detects it in human postheparin plasma by immunodiffusion and in human adipose tissue by immunofluorescence (18, 19). The number of LPL-containing cells was determined in the intima surrounding the plaque, in the fibrous cap, and in the lipid core region of the plaque. Results are given in **Table 2**. The highest frequency of LPL-containing cells was seen in the surrounding intima, whereas the proportion of positive cells was slightly decreased in the fibrous cap. The lowest number of LPL-containing cells was found in the lipid core region. The foam cells in this region never contained LPL. Most medial cells reacted with anti-LPL.

TABLE 2. Cells in different regions of the atherosclerotic plaque

| Antibody | Intima | Fibrous Cap | Lipid Core |
|---------------|-------------|-------------|-------------|
| Anti-LPL* | 71.7 ± 13.5 | 52.8 ± 25.3 | 26.4 ± 14.0 |
| Anti-Leu-M3** | 8.6 ± 4.9 | 23.9 ± 11.7 | 60.3 ± 15.2 |
| Anti-Leu-4** | 7.7 ± 9.1 | 17.5 ± 8.7 | 9.3 ± 8.2 |
| OKIa 1* | 29.0 ± 17.2 | 54.9 ± 14.6 | 77.3 ± 9.5 |

Values are given as percent of the total number of cells (mean ± SD).

*These results have been reported (17).

In order to further characterize the LPL-containing cells in the plaque, four different monoclonal antibodies were used: 1) anti-Leu-M3, reacting with an antigen on monocytes/macrophages; 2) anti-Leu-4, reacting with the pan-T cell marker T3; 3) OKIa 1, reacting with the class II MHC antigen HLA-DR; and 4) CGA 7, which is directed against a smooth muscle-specific actin isoform (23).

The reactivity of plaque cells with anti-Leu-M3, anti-Leu-4, and OKIa 1 in the human carotid plaque has been determined previously (17, 24). The frequencies of different cell types in the plaque are given in Table 2. Double-staining with either of these monoclonals and anti-LPL was then performed in order to identify the LPL-containing cells (**Table 3**). Cells stained by both anti-Leu-M3 and anti-LPL were only occasionally seen in the fibrous cap (**Fig. 1a, b**). Anti-Leu-M3-positive cells in the central lipid core never reacted with anti-LPL.

Similarly, the distribution of nonspecific alpha-naphthylacetate esterases in the plaque did not correspond with that of anti-LPL in serial sections, supporting the finding that most macrophages did not contain LPL (data not shown).

Anti-Leu-4-positive T cells in the plaque never reacted with anti-LPL.

Data from double-staining with OKIa 1 and anti-LPL are given in Table 3. No double-stained cells were seen in the intima surrounding the atherosclerotic lesion. A minor fraction of the OKIa 1-positive cells in the fibrous cap contained the enzyme. This fraction increased in the lipid core region, but still a majority of the OKIa 1-positive cells lacked LPL (**Fig. 2a, b**). In a third series of double-staining experiments, sections were incubated with CGA 7 and anti-LPL (Table 3). The two antibodies gave almost identical staining patterns indicating that the majority of CGA 7-positive smooth muscle cells contained LPL (**Fig. 3a, b**).

The data from the sections were confirmed on cells isolated from atherosclerotic tissue. Seventy one percent of all CGA-7-positive cells in the smooth muscle cell-enriched fraction reacted with anti-LPL. In contrast, only 10% of the Leu-M3-positive cells in the macrophage-enriched fraction was stained by anti-LPL.

The majority of the cells in the intima and media of the non-atherosclerotic uterine artery and aorta reacted with anti-LPL. The endothelium of the uterine arteries, stained by anti-von Willebrand factor, was always positive for the enzyme in double-staining immunofluorescence. Whether

TABLE 3. Immunoreactive cells in different regions of the atherosclerotic plaque: double-staining with anti-LPL and monoclonal antibodies

| Stained cells | Intima | Fibrous Cap | Lipid Core |
|--|-------------|-------------|------------|
| Anti-Leu-M3 ⁺ cells stained by anti-LPL | 0 | 6.8 ± 3.8 | 0 |
| OKIa 1 ⁺ cells stained by anti-LPL | 0 | 6.6 ± 6.0 | 21.7 ± 9.7 |
| CGA 7 ⁺ cells stained by anti-LPL | 83.0 ± 11.5 | 73.0 ± 3.8 | 94.3 ± 3.9 |

Values are given as percent of the number of cells stained with each monoclonal antibody (mean ± SD).

LPL was present in the endothelium of aortic biopsies was not possible to evaluate, as the surgical procedure resulted in desquamation of the endothelium. In the intima and adventitia of the aortic segments, as well as in the adventitia of the uterine arteries, occasional cells, stained by anti-Leu-M3 and OKIa 1, were seen. These cells were never stained by anti-LPL in double-staining experiments.

In order to exclude the possibility that the antiserum against bovine LPL failed to react with the enzyme present in human macrophages, a control experiment was performed in which monocyte-derived macrophages in culture were stained with the antiserum. After 2 days in culture, 89% of all Leu-M3-positive cells reacted with anti-LPL, and after 1 week a similar proportion, 87%, of the Leu-M3-positive cells was stained with the antiserum.

DISCUSSION

LPL activity has been demonstrated in arterial tissue (4, 6, 7). This has led to speculations about the role of the enzyme in the atherogenic process. This study was made in order to identify LPL-containing cells in nonatherosclerotic and atherosclerotic human arteries by immunocytochemical techniques. In order to detect the enzyme, we used an antiserum against bovine milk LPL. Its cross-reactivity with the human enzyme is well documented both immunochemically (18) and immunocytochemically (19).

In the present study, the cells in the intima, including the endothelium, and in the media of nonatherosclerotic arteries reacted with anti-LPL. This is in agreement with biochemical studies in which LPL activity has been detected in the aortic wall (4, 6, 7). It has not been possible to demonstrate that endothelial cells in culture synthesize the enzyme (8, 9). On the other hand, LPL activity has been observed in cultured pig aortic smooth muscle cells (10). We have also detected LPL immunocytochemically in cultured human smooth muscle cells derived from a uterine artery (unpublished results). It is therefore most likely that LPL is synthesized by the smooth muscle cells in the vascular wall and then transported to the endothelial cells where it acts bound to their surfaces (9, 10).

A large proportion of the cells in the atherosclerotic lesions reacted with anti-LPL. This finding might support

Zilvermit's suggestion that a high LPL activity could be important for the development of atherosclerotic lesions (4, 5).

After establishing the presence of LPL, we proceeded to characterize the LPL-containing cells, since the human plaque has been shown to be very heterogeneous with respect to cellular composition. In a recent study we have identified the different cell types in human carotid lesions using a panel of monoclonal antibodies (17). The majority of the cells in the intima and in the fibrous cap were smooth muscle cells, whereas macrophages were most abundant in the lipid core where they constituted up to 60% of all cells (see Table 2). In addition, macrophages and T cells were found throughout the lesion (17).

In the present study the origin of the LPL-containing cells in the plaque was determined by double-staining with the following monoclonal antibodies: CGA 7 directed against smooth muscle-specific actin, anti-Leu-M3 against monocytes/macrophages, anti-Leu-4 against T-cells, and OKIa 1 reacting with the class II MHC antigen HLA-DR. We found that the majority of the CGA 7-positive cells reacted with anti-LPL.

In contrast, a very small fraction of the macrophages in the lesion contained LPL. In the central lipid core, where macrophages dominate, none of them was seen to contain LPL. The poor correspondence between the reactivities for anti-Leu-M3 and anti-LPL was confirmed by a histochemical staining in which the distribution of macrophage-type esterases did not correspond with the cellular reactivity for anti-LPL.

The results were also confirmed by studying the two cell fractions isolated from atherosclerotic tissue. In the macrophage-enriched fraction, 10% of the cells contained LPL, whereas more than 70% of the cells in the smooth muscle cell-enriched fraction contained the enzyme. Taken together, these data indicate that smooth muscle cells are the major source of LPL in atherosclerotic lesions.

The lack of LPL in most macrophages of the lesion was a surprising finding since macrophages in cultures have been shown to synthesize the enzyme (11-14). Macrophages become filled with lipid in the atherosclerotic lesion, and it has been suggested that the synthesis of LPL by macrophages would favor their development into foam cells (11, 12). However, the foam cells which were Leu-M3-positive in the carotid lesions never contained LPL as detected with

Fig. 1. Double-staining with anti-LPL (a) and anti-Leu-M3 (b) in the fibrous cap of the plaque. Only two double-stained cells are seen. (Magnification of 1000).

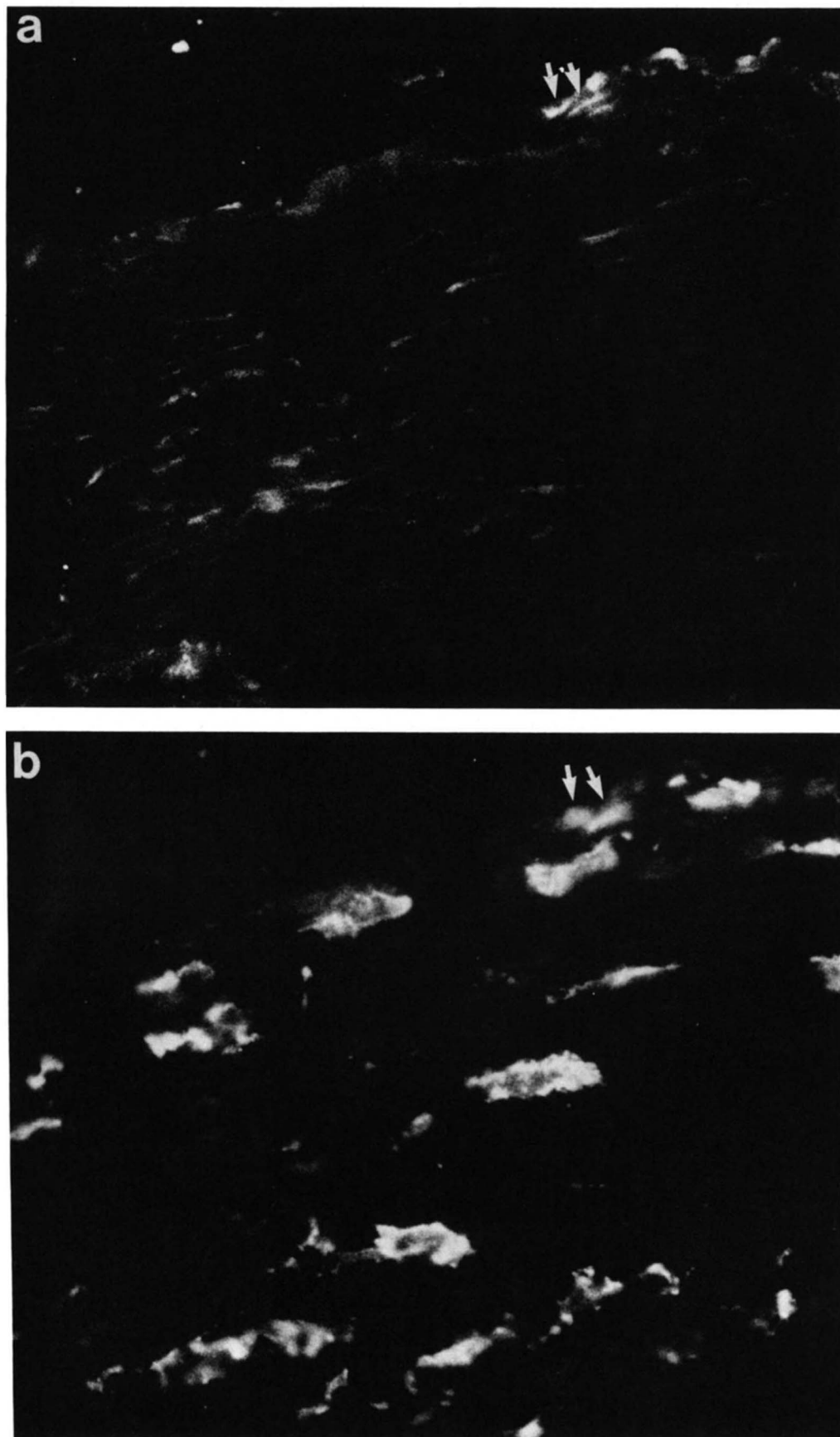


Fig. 2. Double-staining with anti-LPL (a) and OKIa 1 (b). The fibrous cap (fc) and part of the lipid core (lc) is seen. Two double-stained cells are indicated (arrows). (Magnification of 1000).

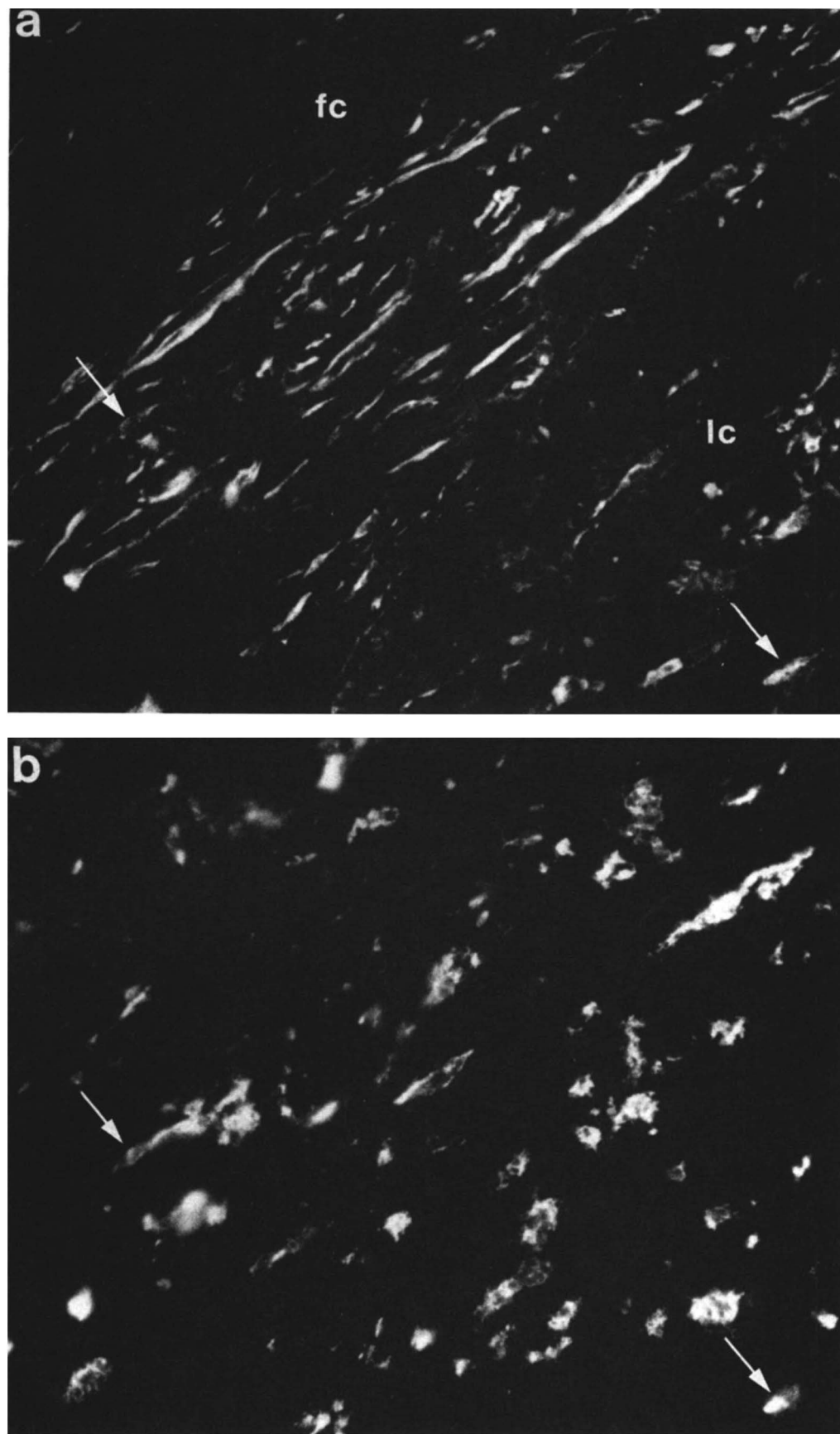
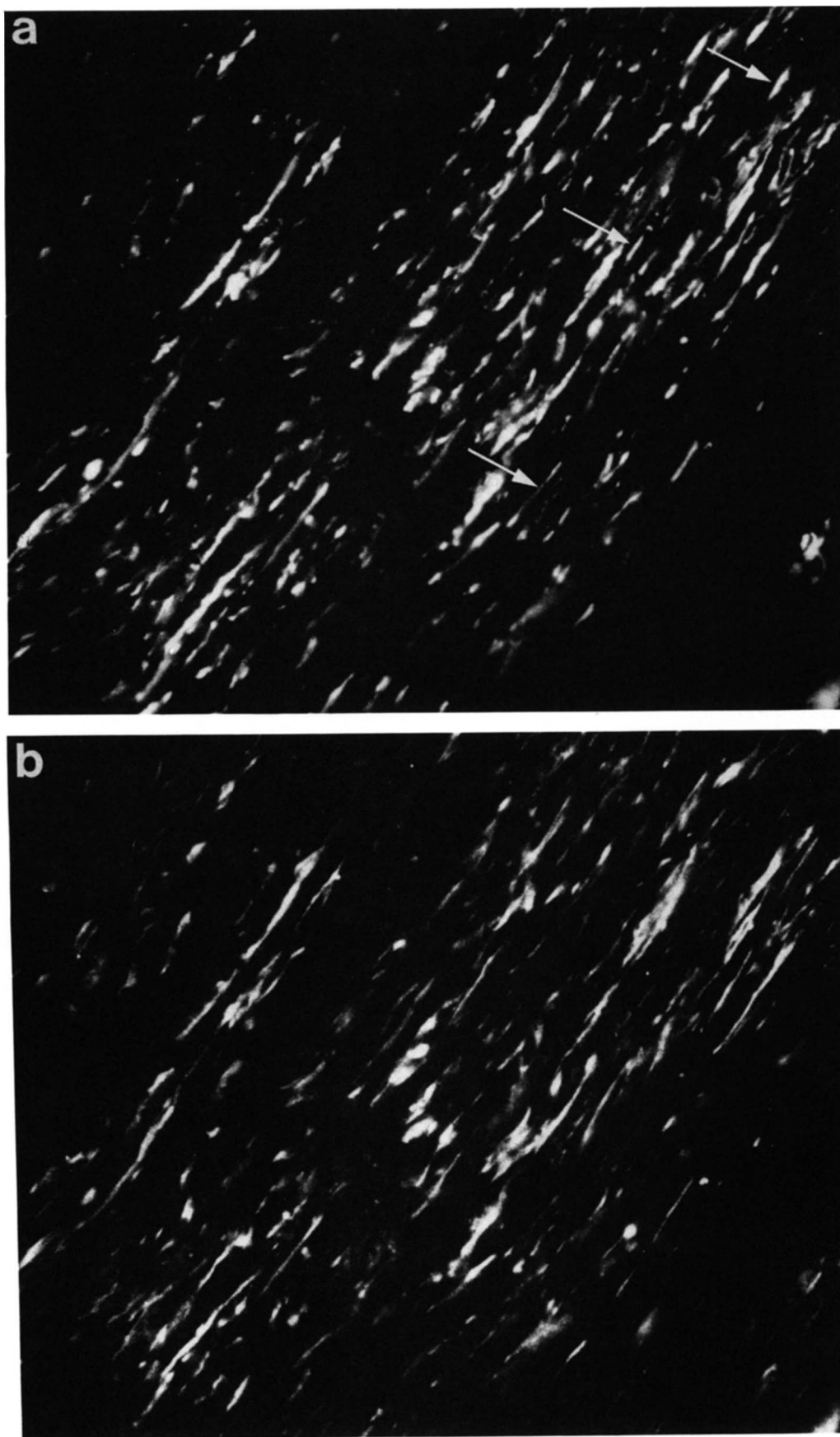


Fig. 3. Double-staining with CGA 7 (a) and anti-LPL, the fibrous cap (fc) region. Most of the CGA 7-positive cells are seen to react with anti-LPL. Two CGA 7-positive smooth muscle cells, which are *not* stained with anti-LPL, are indicated (arrows). (Magnification of 1000).



the antiserum. In this case one must consider the possibility that the enzyme in macrophages might be of another isoform, which is not recognized by the antiserum. Human Leu-M3-positive macrophages in culture were, however, stained by the antiserum, which indicates that the enzyme has the same binding properties in macrophages as in smooth muscle cells.

Other artifacts associated with the double-label immunofluorescence technique (27) were not likely to explain the negative reaction of anti-LPL with the macrophages, since control experiments excluded such possibilities as steric hindrance of binding sites or cross-reactivities between reagents. We therefore conclude that the lack of LPL-staining of the macrophages in the plaque represents an *in vivo* lack of enzyme protein in these cells.

One possibility is that the overload of lipid in the macrophages leads to a loss of protein-synthesizing capacity, including synthesis of LPL. Alternatively, the synthesis of LPL by macrophages might be a phenomenon which occurs only *in vitro* without any relevance to the *in vivo* situation.

Interactions between smooth muscle cells and immunocompetent cells might play a role in the regulation of LPL synthesis. Monokines made by macrophages such as tumor necrosis factor and Interleukin 1 suppress LPL activity in 3T3 cells (28, 29), and recently the T cell lymphokine gamma interferon has been shown to induce hypertriglyceridemia and decreased plasma postheparin lipase activity *in vivo* (30). Gamma interferon also induces the expression of class II MHC antigens on a number of nonlymphoid cells (for a review, see ref. no. 31), including vascular smooth muscle cells (unpublished observations in our laboratory). We have recently shown that many smooth muscle cells in the human carotid lesions express class II antigens, a phenomenon that is never seen on smooth muscle cells in normal arteries (24). The present study shows that very few of the class II antigen-expressing cells in the intima and fibrous cap contain LPL (Table 2). This suggests that not only the HLA-DR-positive macrophages but also many HLA-DR-positive smooth muscle cells in these areas lack the enzyme. Thus, one interesting possibility would be that the expression of HLA-DR and the apparent down-regulation of LPL is mediated by the same factor, *i.e.*, gamma interferon. Such a regulation of enzyme activity could be important in the progress of the atherosclerotic disease.

In summary, this study has shown that the smooth muscle cells are the major source for LPL in the normal as well as in the atherosclerotic artery. Although a fraction of smooth muscle cells, largely HLA-DR positive, in the lesions lacked the enzyme, LPL could still be detected in the majority of smooth muscle cells. In contrast, macrophages, which have been found to synthesize the enzyme *in vitro*, very rarely contained LPL in atherosclerotic lesions. ■

We thank Dr. Gunilla Bengtsson-Olivecrona for the anti-serum against LPL, and Dr. David Gordon for the CGA 7-isoactin antibody. We also thank Dr. Jan Holm for providing the surgical material. This work was supported by the Swedish Medical Research Council (project Nos. 6816 and 4531), the Swedish National Association against Heart and Chest Diseases, King Gustaf V:s and Queen Victoria's foundation, the Göteborg Medical Association and the University of Göteborg.

Manuscript received 21 July 1986 and in revised form 9 December 1986.

REFERENCES

1. Nilsson-Ehle, P., A. S. Garfinkel, and M. C. Schotz. 1980. Lipolytic enzymes and plasma lipoprotein metabolism. *Annu. Rev. Biochem.* **49**: 667-693.
2. Zilversmit, D. B. 1973. A proposal linking atherogenesis to the interaction of endothelial lipoprotein lipase with triglyceride-rich lipoproteins. *Circ. Res.* **33**: 633-638.
3. Zilversmit, D. B. 1976. Role of triglyceride-rich lipoproteins in atherogenesis. *Ann. NY Acad. Sci.* **275**: 138-144.
4. Corey, J. E., and D. B. Zilversmit. 1977. Effects of cholesterol feeding on arterial lipolytic activity in the rabbit. *Atherosclerosis.* **27**: 201-212.
5. Zilversmit, D. B. 1979. Atherogenesis: a postprandial phenomenon. *Circulation.* **60**: 473-485.
6. Dicorleto, P. E., and D. B. Zilversmit. 1975. Lipoprotein lipase activity in bovine aorta. *Proc. Soc. Exp. Biol. Med.* **148**: 1101-1105.
7. Henson, L. C., and M. C. Schotz. 1975. Detection and partial characterization of lipoprotein lipase in bovine aorta. *Biochim. Biophys. Acta.* **409**: 360-366.
8. Howard, B. V. 1977. Uptake of very low density lipoprotein triglyceride by bovine aortic endothelial cells in culture. *J. Lipid Res.* **18**: 561-571.
9. Björntorp, P., G. K. Hansson, L. Jonasson, P. Pettersson, and G. Sypniewska. 1983. Isolation and characterization of endothelial cells from the epididymal fat pad of the rat. *J. Lipid Res.* **24**: 105-112.
10. Vance, J. E., J. C. Khoo, and D. Steinberg. 1982. Lipoprotein lipase in cultured pig aortic smooth muscle cells. *Arteriosclerosis.* **2**: 390-395.
11. Khoo, J. C., E. M. Mahoney and J. L. Witztum. 1981. Secretion of lipoprotein lipase by macrophages in culture. *J. Biol. Chem.* **256**: 7105-7108.
12. Chait, A., P. H. Iverius, and J. D. Brunzell. 1982. Lipoprotein lipase secretion by human monocyte-derived macrophages. *J. Clin. Invest.* **69**: 490-493.
13. Wang-Iversen, P., A. Ungar, J. Blumis, P. R. Bukberg, J.-C. Gibson, and W. V. Brown. 1982. Human monocytes in culture synthesize and secrete lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **104**: 923-928.
14. Mahoney, E. M., J.-C. Khoo, and D. Steinberg. 1982. Lipoprotein lipase secretion by human monocytes and rabbit alveolar macrophages in culture. *Proc. Natl. Acad. Sci. USA.* **79**: 1639-1642.
15. Fowler, S., H. Shio, and N. J. Haley. 1979. Characterization of lipid-laden aortic cells from cholesterol-fed rabbits. Investigation of macrophage-like properties of aortic cells populations. *Lab. Invest.* **41**: 372-378.
16. Schaffner, T., K. Taylor, E. J. Bartucci, K. Fischer-Dzoga, J. H. Beeson, S. Glagov, and R. W. Wissler. 1980. Arterial

- foam cells with distinctive immunomorphologic and histochemical features of macrophages. *Am. J. Pathol.* **100**: 57-78.
17. Jonasson, L., J. Holm, O. Skalli, G. Bondjers, and G. K. Hansson. 1986. The human atherosclerotic plaque: regional accumulations of T cells, macrophages, and smooth muscle cells. *Arteriosclerosis*. **6**: 131-140.
 18. Hernell, O., T. Egelrud, and T. Olivecrona. 1975. Serum-stimulated lipases (lipoprotein lipases). Immunological cross-reaction between the bovine and the human enzymes. *Biochim. Biophys. Acta*. **381**: 233-241.
 19. Jonasson, L., G. K. Hansson, G. Bondjers, G. Bengtsson, and T. Olivecrona. 1984. Immunohistochemical localization of lipoprotein lipase in human adipose tissue. *Atherosclerosis*. **51**: 313-326.
 20. Dimitriu-Bona, A., G. R. Burmester, S. J. Waters, and R. J. Winchester. 1983. Human mononuclear phagocyte differentiation antigens. I. Patterns of antigenic expression on the surface of human monocytes and macrophages defined by monoclonal antibodies. *J. Immunol.* **130**: 145-152.
 21. Ledbetter, J. A., R. L. Evans, M. Lipinski, C. Cunningham-Rundles, R. A. Gord, and L. A. Henzenberg. 1981. Evolutionary conservations of surface molecules that distinguish T lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. *J. Exp. Med.* **153**: 310-323.
 22. Nadler, C. M., P. Stashenko, R. Hardy, J. M. Pesando, E. J. Yunis, and S. F. Schlossman. 1981. Monoclonal antibodies defining serologically distinct HLA-D/DR related Ia-like antigens in man. *Hum. Immunol.* **1**: 77-90.
 23. Gown, A. M., A. M. Vogel, D. Gordon, and P. L. Lu. 1985. A smooth muscle-specific monoclonal antibody recognizes smooth muscle actin isozymes. *J. Cell. Biol.* **100**: 807-813.
 24. Jonasson, L., J. Holm, O. Skalli, G. Gabbiani, and G. K. Hansson. 1985. Expression of class II transplantation antigen on vascular smooth muscle cells in human atherosclerosis. *J. Clin. Invest.* **76**: 125-131.
 25. Hansson, G. K., and G. Bondjers. 1980. Endothelial proliferation and atherogenesis in rabbits with moderate hypercholesterolemia. *Artery*. **7**: 316-329.
 26. Haley, N. J., H. Shio, and S. Fowler. 1977. Characterization of lipid-laden aortic cells from cholesterol-fed rabbits. Resolution of aortic cell populations by metrizamide density gradient centrifugation. *Lab. Invest.* **37**: 287-296.
 27. Pryzwansky, K. B. 1982. Applications of double-label immunofluorescence. In *Techniques in Immunocytochemistry*. Vol. 1. G. R. Bullock and P. Petrousz, editors. Academic Press, Inc., London. 77-90.
 28. Beutler, B. A., I. W. Milsark, and A. Cerami. 1985. Cachectin/tumor necrosis factor: production, distribution and metabolic fate in vivo. *J. Immunol.* **135**: 3972-3977.
 29. Beutler, B. A., and A. Cerami. 1985. Recombinant interleukin 1 suppresses lipoprotein lipase activity in 3T3-L1 cells. *J. Immunol.* **135**: 3969-3971.
 30. Kurzrock, R., M. F. Rohde, J. R. Quesada, S. H. Gianturco, W. A. Bradley, S. A. Sherwin, and J. U. Gutterman. 1986. Recombinant γ interferon induces hypertriglyceridemia and inhibits post-heparin lipase activity in cancer patients. *J. Exp. Med.* **164**: 1093-1101.
 31. Unanue, B. R., and P. M. Allen. 1986. Comment on the finding of Ia expression in non-lymphoid cells. *Lab. Invest.* **55**: 123-125.