Acta Medica et Biologica
Vol. 38, (Suppl.), 31-36, 1990

Macrophage Products and Atherosclerosis

Kentaro SHIMOKADO and **Fujio NUMANO**

Third Department of Internal Medicine Tokyo Medical and Dental University 5-45 Yushima 1-chome, Bunkyo-ku, Tokyo 113

Received October 12, 1989

Summary. **There are many macrophages in the atherosclerotic lesion. Mononuclear cells begin to migrate into the subendothelial space at the early stage of atherogenesis. They take up denatured low density lipoprotein through scavenger receptors and become lipid-laden foam cells. Various cytokines and growth factors produced by macrophages can modulate the growth and many other functions of vascular cells *in vitro*. Those cytokines might be secreted in the arterial wall and play an important role in atherogenesis.**

MACROPHAGES AND ATHEROSCLEROSIS

It is well established that there are many mononuclear cells/macrophages in atherosclerotic lesions. Although the presence of macrophages in athorosclerotic plaques of human aorta was revealed by electron microscopic study in 1960's,[1] more precise evidence was provided with immunocytochemical analysis using polyclonal and monoclonal antibodies against macrophages in 1980's.[2] Owing to those morphological studies, monocyte-macrophages were found in fatty streak, an early lesion of atherosclerosis, as well as advanced lesions such as fibrous plaques and complicated lesions. The majority of foam cells found in these lesions were of monocyte/macrophage origin.[3]

Time-course of accumulation of macrophages was studied using experimental animals in which atherosclerosis was induced by hypercholesterol diet.[4] In porcine, within two weeks after development of hypercholesterolemia, mononuclear cells in the blood stream began to adhere to arterial surface and invade the subendothelial space through interendothelial junction.[5] The same consequence was observed in a non-human primate,[4] a rabbit[6] and pigeons.[7] In the Watanabe heritable hyperlipidemic rabbit, an animal model of familial hypercholesterolemia, mononuclear invasion was observed even in a fetus, which already had a high serum cholesterol level.[6]

Macrophages are known to produce more than fifty bioactive substances.[8] As it has become possible to culture vascular smooth muscle cells and endothelial cells in a tissue culture laboratory, many of the macrophage products have been shown to affect various functions of vascular cells *in vitro* (Fig. 1). For example, interleukin 1 (IL-1) induces expression endothelial-leukocyte adhesion molecule (ELAM-1) on endothelium which mediates adhesion of leuko-

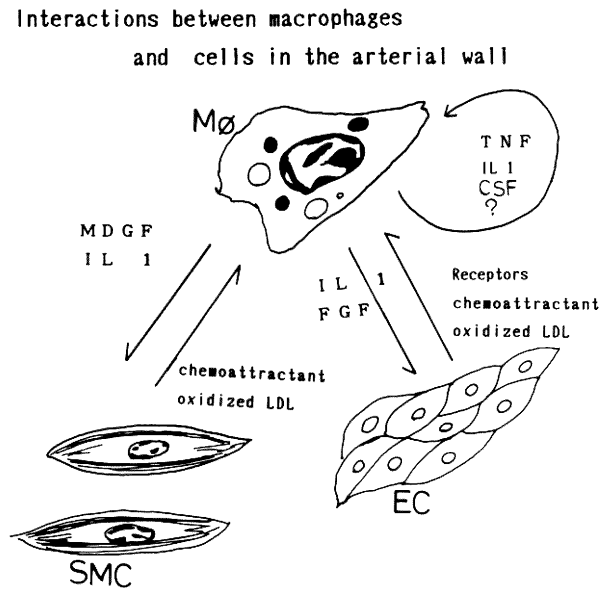


Fig. 1. Interactions of macrophages and other vascular cells.

*This work was supported in part by a grant from Ministry of Education.

cytes to endothelial cells. IL-1 increases endothelial production of tissue factor and prostacycline. On the other hand heparin-like activity and thrombomodulin of endothelial cells decrease after treatment with IL-1. IL-1 also induces endothelial production of granulocyte-monocyte colony-stimulating factor and IL-1 itself (see review by Shimokado 1987).[9] The modulation of cellular function of macrophage-products can play roles in progression and regression of atherosclerosis.

In this paper, we will discuss two categories of macrophage products which might be very important in atherogenesis.

GROWTH FACTORS

Macrophages produce growth factors for mesenchymal cells such as fibroblasts, vascular smooth muscle cells and glial cells. Those growth factors are supposed to play an important role in wound healing, fibrosis of various organs following chronic inflammation, and smooth muscle proliferation in atherosclerosis.[10-12]

The majority of tissue macrophages originate from mononuclear cells in the blood stream. Mononuclear cells do not show the growth promoting activity of mesenchyal cells until they differentiate into macrophages. Lipopolysaccharide (LPS), concanavalin A and some other stimulants for macrophages increases growth promoting activity of macrophages.[13]

Attempts to purify macrophage-derived growth factor (MDGF) lead to a couple of different substances. We tried to purify these substances from the conditioned medium of human alveolar and peritoneal macrophages (Fig. 2). Two activity peaks were obtained by gel filtration chromatography. Both activities were blocked by specific anti-platelet-derived growth factor (PDGF) polyclonal antibody. Furthermore, both of them competed binding of ^{125}I-PDGF to its receptor on cell surface. Those findings suggested that they consisted of PDGF-like molecules. Metabolic-labelling study using ^{35}S-cysteine showed that PDGF-like molecules were not contaminants from platelets and were actually synthesized by macrophages. Northern blot analysis using cDNA for B-chain of PDGF (v-*sis*) substantiated production of PDGF-like molecules by macrophages.[14]

Other groups found growth promoting activity different from PDGF in alveolar lavage and the macrophage-conditioned medium of patients with idiopathic pulmonary fibrosis (IPF). Macrophages from normal people or patients without IPF produced growth

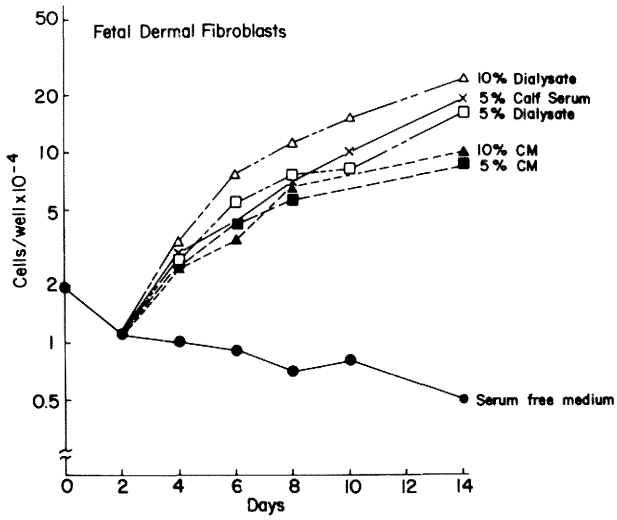


Fig. 2. Growth-promoting activity of macrophage-conditioned medium: Human macrophages are prepared from dialysate of CAPD. Macrophages are cultured in a Neuman-Tytell medium supplemented with 0.2% BSA. A conditioned medium or cell-free dialysate is concentrated ten-fold by ultrafiltration. Fetal dermal fibroblasts are plated in 24 well trays. After 24 hours, the medium is changed to test it. CM is conditioned mediune.

factors only after stimulation. The factors acted synagisitically with PDGF.[11]

Interleukin 1 was found to stimulate proliferation of fibroblasts and vascular smooth muscle cells.[15] Recently it was shown that growth-promoting activity of IL-1 was mediated by inducing the production of PDGF A-chain through smooth muscle cells. Namely, IL-1 induces production of A-chain of PDGF through smooth muscle cells and growth activity of IL-1 is blocked by anti-PDGF A-chain antibody.[16]

Basic fibroblast growth factor (FGF) is a growth factor produced by macrophages.[17] This factor is different from most of the other growth factors in cell specificity. It stimulates growth of not only smooth muscle cell or fibroblasts but also vascular endothelial cells. It also induces neoangiogenesis *in vivo*. Basic FGF is membrane-bound and is not secreted into culture medium. This factor might be released into the environment when the macrophages are destroyed in the lesion.

Transforming growth factor (TGF) alpha and beta were also found to be synthesized by macrophages.[18,19] Although TGF beta produces fibrosis and neoangiogenesis when injected subcutaneously, it suppresses growth of vascular smooth muscle cells *in vitro*. Because TGF beta stimulates PDGF production of endothelial cells, it is possible TGFs induce smooth muscle cell proliferation through PDGF produced by

endothelial cells in a paracrine fashion.

If any of those growth factors are secreted by macrophages in atherosclerotic lesions, what stimulates macrophages to produce growth factors? We conducted an experiment to elucidate the possibility that the uptake of denatured low density lipoprotein triggered the secretion of PDGF by macrophages. However, the uptake of acetylated LDL decreased mRNA of PDGF in macrophages (Fig. 3).

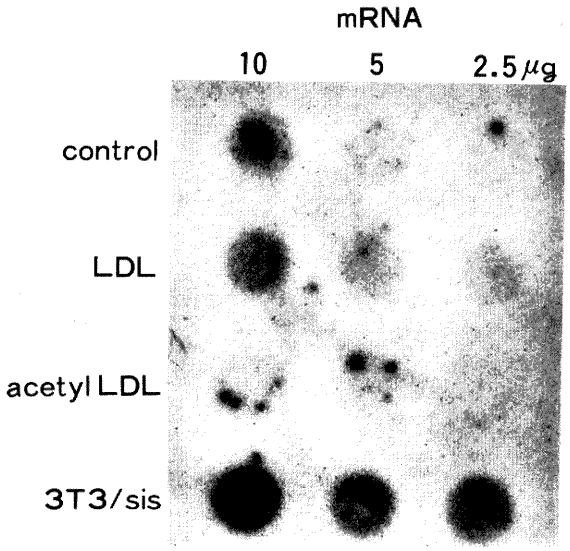


Fig. 3. Effect of acetylated LDL of expression of mRNA of PDGF B chain by mouse peritoneal macrophages (dot blot analysis): mRNA prepared from cultured mouse peritoneal macrophages are transferred to nitrocellulose paper and hybridized with cDNA of *v-sis* under lower stringency. Macrophages cultured with acetylated LDL express less mRNA of B chain as compared with those cultured with native LDL or without LDL. 3T3/sis-mRNA from Balb 3T3 cells transfected with *v-sis*.

UPTAKE OF DENATURED LDL

As mentioned earlier, macrophages in the subendothelial space become lipid-laden foam cells (Fig. 4). The major lipid component accumulated in the lipid particles in macrophages is cholesteryl oleate. Macrophages have receptors for denatured LDL but very few or no receptors for native LDL. Cellular uptake of native LDL is tightly regulated by regulating the number of cell surface LDL receptors. When LDL is taken up by cells, the number of LDL receptors is down-regulated and inflow of LDL decreases. In contrast, a receptor for denatured LDL is not down-regulated and macrophages continue to take up denatured LDL until they become full of lipid droplet.[20] This hypothesis was proposed based on experiments using chemically modified LDL such as acetylated LDL, which is not generated in the human body. Today the most likely candidate of denatured LDL generated in the human body is so-called "oxidized LDL"[21] Macrophages, as well as endothelium and smooth muscle cells, can produce active oxygen

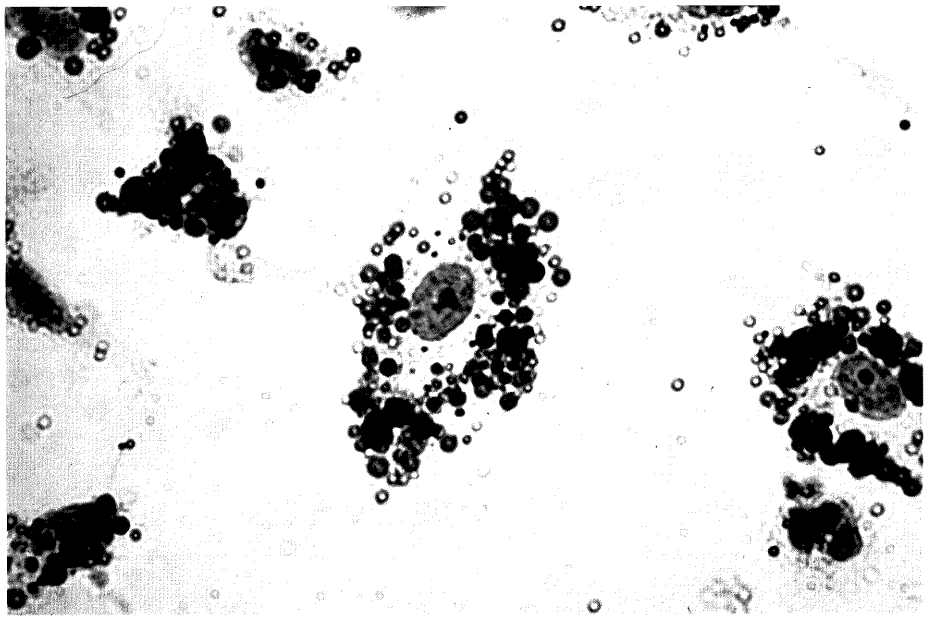
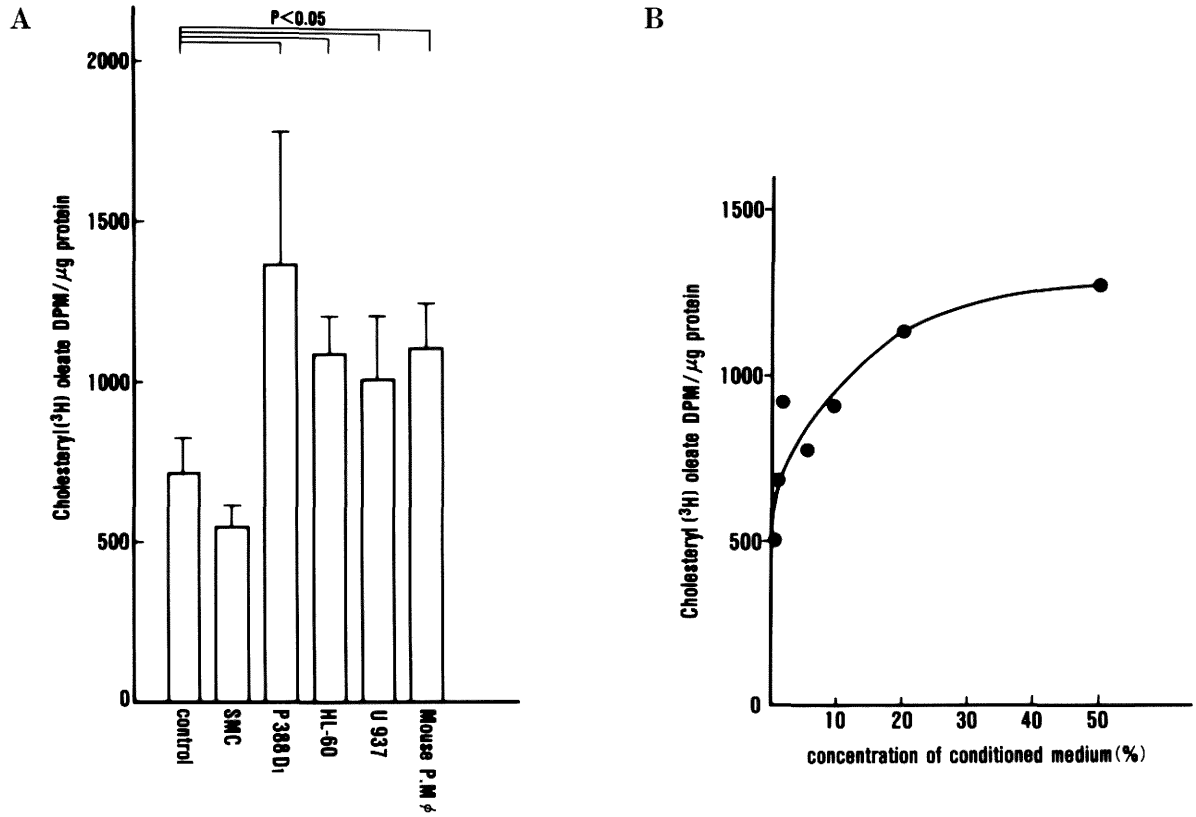


Fig. 4. Macrophage-derived foam cells: mouse peritoneal macrophages cultured with acetylated LDL (100 micro grams) for 72 hours.



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	Cholesteryl(³H) oleate DPM/mg protein
control	544.000±70.000
SMC	598.000±75.000
BAEC	404.000±20.000
Fibro.	610.000±87.000
P388D1	728.000±130.000*
mouse Mφ	749.000±110.000*

mean±SEM(n=4)
* P<0.05

Fig. 5. Effects of macrophage-conditioned medium on uptake of acetylated LDL by macrophages: Macrophage-conditioned medium is added to mouse peritoneal macrophages cultured with acetylated LDL. Uptake of acetylated LDL is measured by incorporation of 3H-oleate into cholesteryl oleate. Macrophages and related cell lines secrete substance which increase the uptake (A and B). Other types of cells do not stimulate uptake of acetylated LDL (C). SMC: smooth muscle cells, BAEC: bovine aortic endothelial cells, Fibro: fibroblasts, P338D: macrophage-related cell line, Mφ: macrophages.

and "oxidize" LDL nearby.[22] Lecithin in native LDL is degraded to lysolecithin and apo B is broken down to smaller polypeptide fragments. As a result, denatured LDL, which has increased density and more negative charge, becomes recognizable to the denatured LDL receptor.[23]

Although uptake of denatured LDL is not regulated by a negative feedback mechanism, the uptake is affected by products of macrophages. Human monocyte-derived macrophages secrete substances which stimulate uptake of denatured LDL by macrophages.[24] This factors effective only when it is added to differentiating macrophages and no longer has the effect on mature macrophages. Mouse peritoneal macrophages and various macrophage cell lines also produce mediators which increase uptake of acetylated LDL by mouse peritoneal macrophages[25] (Fig. 5). Endothelial cells, smooth muscle cells or fibroblasts do not secrete this mediator. Although TNF-alpha has a similar effect, this substance secreted by non-stimulated macrophages seems to be different from TNF-alpha.

STUDIES *IN VIVO*

Does any of the growth factors and monokines discussed above really play a role in atherogenesis? Some have tried to answer this question by using immunocytochemistry, Northern blot analysis or *in situ* hybridization technique. Data are still sparse and there are discrepancies between reports.

Increased expression of mRNA of B chain of PDGF was found in human atherosclerotic plaques excised during carotid endoarterectomy. The amount of mRNA of B chain correlated well with the amount of mRNA of *fms* (oncogene expressed in macrophage) but did not correlate with that of von Willbrand factor, a marker of endothelial cells. This suggests that the major source of B chain of PDGF is macrophages, but not endothelial cells which are another type of PDGF-producing cells.[26,27] However, *in situ* hybridization analysis using the same kind of specimen could not detect expression of B chain mRNA on macrophages in atherosclerotic plaque.[28] The latter does not necessarily rule out production of PDGF by arterial macrophages. It is possible that mRNA of B chain of PDGF in macrophages is more susceptible to degradation or that PDGF is expressed at an earlier stage of athorosclerosis.

CONCLUSION

There are many macrophages in atherosclerotic lesions. *In vitro* experiments suggest that many substances produced by these macrophages can modulate various functions of vascular cells. Although it has not been completely proved yet, data which suggest actual participation of monokines in atherogenesis are being accumulated.

REFERENCES

1) Geer JC, McGill HCJ, Strong JP: The fine structure of human atherosclerotic lesions. *Amer J Path* 38: 263-287, 1961.
2) Gown AM, Tsukada T, Ross R: Human atherosclerosis II. *Amer J Patth* 125: 191-207, 1986.
3) Tsukada T, Rosenfeld M, Ross R, Gown AM: Immunocytochemical analysis of cellular components in lesions of atherosclerosis in Watanabe and fat-fed rabbit using monoclonal antibodies. *Arteriosclerosis* 6: 601-604, 1986.
4) Faggiotto A, Ross R, Harker L: Studies of hypercholesterolemia in the nonhuman primate I & II. Arteriosclerosis, 4: 323-356, 1984.
5) Gerrity RG: The role of the monocyte in atherogenesis I & II. *Amer J Path* 103: 181-200, 1981
6) Rosenfeld ME, Tsukada T, Chait A, Bierman EL, Gown AM, Ross R: Fatty streak expression and maturation in Watanabe Heritable Hyperlipidemic and comparably hypercholesterolemic fat-fed rabbits, Arteriosclerosis 7: 24-34, 1987.
7) Steinberg RW, Rondolph RK, Jokinen MP, Clarkson TB, Barakat H: Relationship of plasma lipoproteins and the monocyte-macrophage system to atherosclerosis severity in cholesterol-fed pigeons. Arteriosclerosis 6: 614-626, 1986.
8) Nathan CF, Murray HW, Cohn ZA: The macrophage as an effector cell. *N Eng J Med* 303: 622-626, 1980.
9) Shimokado K, Numano F: Effects of Interleukin-1 on brain, muscle and connective tissue. *Nihon-Rinsho* 45: 995-1001, 1987.
10) Leibovich SJ, Ross R: The role of the macrophage in wound repair. *Amer J Path* 78: 71-90, 1975.
11) Bitterman PB, Rennard S, Hunninghake GW, Crystal RG: Human alveolar macrophage growth factor for fibroblasts. *J Clin Invest* 70: 806-822, 1982.
12) Kulonen E, Aalto M, Ronnemaa T, Sundstrom C, Vilpo J: Liberation of a fibrogenic factor for human blood monocytes, ascites cells, culture histiocytes and transformed mouse macrophages by treatment with Si02. *Scand J Clin Invest* 40: 311-318, 1980.
13) Glenn KC, Ross R: Human nonocyte-derived growth

factor (s) for mesencymal cells. *Cell* 25: 603-615, 1981.
14) Shimokado K, Rines EW, Madtes DK, Barrett TB, Benditt EP, Ross R: A Significant part of macrophage-derived growth factor consists of at least two forms of PDGF. *Cell* 43: 277-286, 1985.
15) Schmidt JA, Oliver CN, Lepe-Zuniga JL, Green I, Gery I: Silica-stimulated monocytes release fibroblast proliferation factors identical to interleukin 1. *J Clin Invest* 73: 1462-1468, 1984.
16) Raines WE, Dower SK, Ross R: Interleukin-1 mitogenic activity for fibroblasts and smooth muscle cells is due to PDGF-AA. *Science* 243: 393-396, 1988.
17) Baird A, Mormade P, Bohlen P: Immunoreactive fibroblast growth factors in cells of peritoneal exudate suggests its identity with macrophage-derived growth factor. *Biochem Biophy Res Com* 126: 358-364, 1985.
18) Assoian PK, Konoriya A, Meyers CA, Miller DM, Sporn MB: Transforming growth factor-beta in human platelets. *J Biol Chem* 258: 7155-7160, 1983.
19) Madtes DK, Raines EW, Sakariassen SK, Astoian RK, Sporn MB, Bell GL, Ross R: Induction of transforming growth factor alpha in activated human alveolar macrophages. *Cell* 53: 285-293, 1988.
20) Brown MS, Goldstein JK, Krieger M, Ho YK, Anderson RGW: Reversible accumulation of cholesteryl esters in macrophages incubated with acetylated lipoproteins. *J Cell Biol* 82: 597-613, 1979.
21) Steinberg D: Lipoproteins and atherosclerosis. *Arteriosclerosis* 3: 283-301, 1983.
22) Hiramatsu K, Rosen H, Heinecke JW, Wolfbauer G, Chait A: Superoxide initiates oxidation of low density lipoprotein by human monocytes, *Arteriosclerosis,* 7: 55-60, 1987.
23) Parthasarathy S, Printz DJ, Aoyd D, Joy L, Steinberg D: Macrophage oxidation of low density lipoprotein generates a modified form recognized by the scavenger receptor. *Arteriosclerosis* 6: 505-510, 1986.
24) Mazzone T, Jensen M, Chait A: Human arterial wall cells secrete factora that are chemotactic for monocytes. *Proc Natl Acad Sci USA* 80: 5094-5097, 1983.
25) Shimokado K: Autoregulation of lipid metabolism in macrophages. *J Japan Atherosclerosis Society* 16: 1111-1115, 1988.
26) Barrett TB, Gajdsek CM, Schwartz SM, McDougall JK, Benditt EP: Expression of the *sis* gene by endothelial cells in culture and in vivo. *Proc Natl Acad Sci USA* 81: 6772-6774, 1984.
27) Barrett TB, Benditt EP: *Sis* (PDGF-B) gene transcript levels are elevated in human atherosclerotic lesions compared to normal artery. *Proc Natl Acad Sci USA* 84: 1099-1103, 1987.
28) Wilcox JN, Smith KS, Williams LT, Schwartz SM, Gordon D: Platelet-derived growth factor mRNA detection in human atherosclerotic plaques by in situ hybridization. *J Clin Invest* 82: 1134-1143, 1988.