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ORIGINAL ARTICLE

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Ultrastructural localization of tissue factor on monocyte-derived macrophages and macrophage foam cells associated with atherosclerotic lesions

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Abstract The expression of tissue factor (TF) antigen by circulating monocytes, cultured macrophages, and macrophages associated with atherosclerotic lesions was ultrastructurally analysed using immunogold labeling. A subpopulation of macrophages associated with the intimal surface overlying lesions had a significant TF expression. Macrophages and macrophage foam cells that projected from the intima into the arterial lumen also expressed a high level of TF (14-fold increase over control). In contrast, circulating monocytes and macrophages in culture did not express TF above background control levels. This TF expression by macrophages in vivo but not by macrophages cultured from either normal or hypercholesterolemic animals suggests that monocyte activation and macrophage transition, as measured by TF expression, is lesion-dependent and not stimulated solely by intimal attachment, surface migration, or hypercholesterolemia. These results further suggest that macrophages and foam cells associated with early lesions of atherosclerosis can initiate fibrin formation, which could contribute to lesion complications and transition to a fibromuscular stage.

Key words Thromboplastin · Atherosclerosis
Electron microscopy · Cell culture

Introduction

Tissue factor (TF), or thromboplastin, is recognized as the primary physiological initiator of coagulation [2]. Tissue factor is an integral membrane polypeptide that binds factor VII, forming a TF-VIIa complex which activates factors IX and X. The end result of these initial proteolytic events is the polymerization of fibrinogen to fibrin. As an initiator of coagulation, TF distribution is localized to aid the body in the prevention of blood loss. Recent immunohistochemical studies suggest a "hemostatic barrier" of TF, as the protein is constitutively found within the adventitial layer of the vasculature, within the epithelium of the skin and within certain organ capsules [4, 7]. Normally, however, TF is not constitutively expressed by cells that are in direct contact with the blood. Monocytes and/or monocyte-derived macrophages do have the ability to express TF though, and this ability is expressed after stimulation by phorbol ester, endotoxin, or cytokines [5, 15, 22, 24]. Additionally, TF expression by monocyte-derived macrophages and vascular endothelium has been associated with pathological conditions such as cancer, infection, and inflammation [6, 17, 25]. This current report is directed at TF localization on cells associated with the intimal surface of atherosclerotic lesions. Specifically, we examined monocytes adherent to the endothelium, macrophages residing on the endothelium and those projecting into the intima, as well as macrophage foam cells that projected from the intima into the arterial lumen (Fig. 1). Our data document the expression of TF by all of the cell types described above with the exception of unstimulated monocytes adherent to the endothelium. Tissue factor expression by these cells is an important finding, as it indicates a possible mechanism by which thrombotic events may be initiated by cells associated with early foam cell lesions.

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Materials and methods

Ten random bred White Carneau pigeons were obtained from a closed colony at the Bowman Gray School of Medicine. Seven of the animals were fed an atherogenic diet consisting of cholesterol-free pigeon pellets supplemented with 0.3% cholesterol and 10% lard. The remaining three control animals were fed an unsupplemented diet. The diet regimen lasted 8–10 months, a period of time previously shown to exacerbate lesion formation in the thoracic aorta [12, 13].

Monocytes were isolated from the blood of normocholesterolemic animals or hypercholesterolemic animals by centrifugation over Isolymph (Gallare-Schlesdinger, Carle Place, N.Y.) for 45 min at 400 *g* [3]. The cells then were collected from the plas-

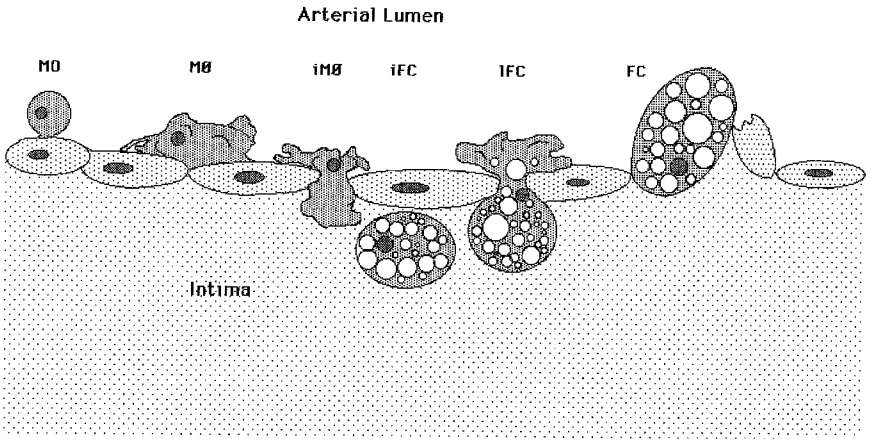

Fig. 1 Illustration of the various cell types observed during the study. From left to right, these cells are: undifferentiated monocytes on the endothelial surface (*MO*), monocyte-derived macrophages on the endothelium (*MØ*), macrophages on the endothelium with subendothelial contact (*iMØ*), intimal foam cells (*iFC*), foam cells projecting into the lumen (*lFC*), and foam cells projecting from areas of endothelial damage (*FC*)

ma/Isolymph interface. Some of the cells were fixed immediately for 10 min in dilute, 1% glutaraldehyde buffered in 0.1 M sodium cacodylate (pH 7.3). The remaining cells were plated on 35 mm culture dishes following previously published procedures [10, 11], and were allowed to spread and differentiate. Following culture for 5–7 days, the cells were washed in phosphate-buffered saline (PBS) and fixed as described above.

Segments of atherosclerotic lesions from the thoracic aorta were collected from animals fed a cholesterol-supplemented diet. The techniques used for anesthesia and perfusion have been previously published [14]. Briefly, the animals were anesthetized with an injection of sodium pentobarbital (0.4 ml). Five milliliters of blood for lipid analysis was collected into 3.8% sodium citrate by cardiac puncture and the plasma was analysed for total cholesterol concentration using the autoanalyser II method. Following blood collection, the vasculature was perfused under pressure (140 mm mercury) with PBS, pH 7.4, for 5 min to clear the remaining blood. A segment of the thoracic aorta extending from the left ventricle to the abdominal aorta was removed. This segment contained the test site, a well characterized region of lesion formation on the aorta immediately anterior to the bifurcation of the celiac artery [12, 13]. The arterial segment was immediately rinsed in fresh PBS, followed by a 5 min fixation in 1% buffered glutaraldehyde. The artery was then opened, and selected areas of the lesion were dissected out while immersed in fixative. These areas were fixed for an additional 5 min.

For TF immunogold labeling and electron microscopy, monocyte suspensions, macrophages in culture, and lesion segments were rinsed extensively (three times 5 min) in Gey's balanced salts following fixation. Free aldehydes were blocked with Gey's salts containing 0.01% glycine (three times 5 min), and this was followed by an additional rinse using Gey's without glycine (5 min). The material then was incubated with either the primary antibody (rabbit anti-human tissue factor, kindly provided by DR. L.V.M. Rao) or non-immune rabbit serum at a concentration of 20 μg/ml for 20 min [7, 19]. Following this treatment, the material was washed in Gey's (three times 5 min) and incubated with the secondary antibody mixture for 20 min. This mixture was a combination of two antibodies, a specific goat-anti-rabbit IgG (GAR) and a non-specific goat-anti-mouse IgG (GAM). The specific and non-specific antibodies were attached to gold colloids of different size in order to distinguish one from another (GAR-30 nm gold was used with GAM 15 nm gold, or GAR-15 was used with GAM-5).

After immunogold labeling, the material was extensively washed in Gey's salts and refixed in 2.5% cacodylate-buffered glutaraldehyde. Tissue for transmission electron microscopy then was post-fixed for 1 h in 1% buffered osmium tetroxide. Samples for scanning electron microscopy were not post-fixed. After further buffer washes, the samples were dehydrated in graded ethanols. Samples for transmission electron microscopy were infiltrated with propylene oxide and eventually Spurr's resin [26]. Semithick (0.45 μm) sections were cut from the samples and examined in a Philips CM-30 intermediate voltage electron microscope operating at 300 keV. Samples for scanning electron microscopy were dried from carbon dioxide by the critical point method and sputter-coated with gold-palladium prior to observation in a Philips 501 scanning electron microscope.

For morphometrics, TF expression in monocytes, cultured macrophages, and lesion-associated cells was quantified from random micrographs. The exposed surface of each cell was measured using a standard map wheel and the measurement was converted to distance (μm). For cells in suspension, this measurement would be the circumference of the cell section. For cultured cells, the apical surface not associated with the substrate was measured. For cells associated with the artery, only the surface of the cell exposed to the lumen was measured (Fig. 1). The number of gold colloids on each cell was determined, and this value was converted to number of gold particles/μm cell surface length. The overall tabulation of data encompassed 3729 specific (30 nm) gold particles and 3307 μm of surface from 108 different cells. For cells adherent to arteries 1338 specific (30 nm) gold and 49 non-specific (15 nm) particles were counted. The immunolabeling procedures involved samples from two to four animals for experimental condition. A total of 19 immunolabeling experiments were done. Group comparisons were performed using a single comparison Anova [30].

Controls designed to test the specificity of the immunolabeling included the use of non-immune serum, and the use of non-related antibodies in every immunolabeling procedure. Additionally, a variety of colloidal gold sizes was used to ensure that the probe size did not affect binding.

Results

Total plasma cholesterol

White Carneau pigeons receiving the 0.3% cholesterol-supplemented diet had significantly elevated plasma cholesterol levels (699±81 mg/dl) when compared to animals receiving the control, non-supplemented diet (130±8 mg/dl). Upon dissection, all animals receiving the atherogenic diet had developed fatty streaks and atherosclerotic plaque at the celiac bifurcation of the thoracic aorta, in agreement with earlier findings in our laboratory [12, 13].

TF expression by monocyte-derived macrophages and macrophage foam cells in situ.

Tissue factor procoagulant activity is not expressed in vitro by either freshly-isolated monocytes or monocyte-

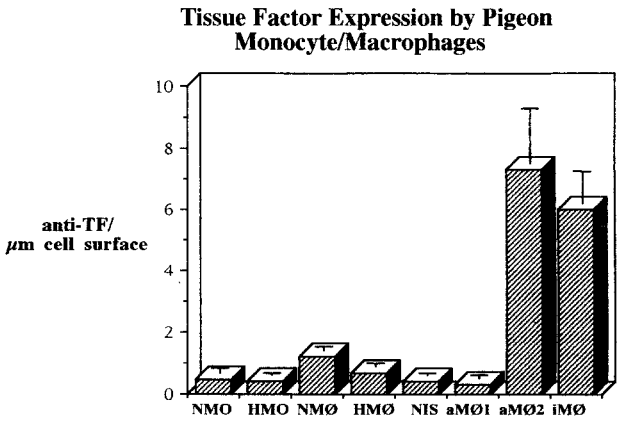

Fig. 2 Histogram comparison of tissue factor (TF) expression (number of immunogold beads/μm cell surface) by: monocytes from normal (*NMO*) and hypercholesterolemic (*HMO*) animals, macrophages cultured from normal (*NMØ*) and hypercholesterolemic (*HMØ*) animals, macrophages adherent to atherosclerotic lesions (*aMØ1* and *aMØ2*), and by macrophages and foam cells projecting into the arterial lumen from the subendothelial intima (*iMØ*). Note that the adherent macrophages are divided into two distinct populations, those expressing TF (*aMØ2*) and those not expressing TF (*aMØ1*). *NIS* indicates non-immune serum control labeling of monocytes from normocholesterolemic animals. Standard error of the mean is indicated for each category. Total number of cells used in the analysis = 110

derived macrophages maintained in culture. However, upon stimulation of either monocytes or macrophages with endotoxin or following lipid-loading of macrophages with oxidized low density lipoproteins significant procoagulant activity is associated with the cells [9]. The lack of procoagulant activity in non-stimulated cells is consistent with the low level of immunochemically-identifiable TF antigen in the plasma membrane. As illustrated in Figure 2, monocytes isolated from normocholesterolemic and hypercholesterolemic animals had approximately 1 immunogold particle for each 2 μm of plasma membrane. With maintenance in culture and concomitant macrophage transformation, the level of TF antigen in the plasma membranes increased approximately two-fold (1.2 particles/μm and 0.7 particles/μm respectively for normocholesterolemic and hypercholesterolemic macrophages); however, this degree of immunogold staining was not statistically different from the freshly isolated control cells. Interestingly, both monocytes isolated from hypercholesterolemic animals and macrophages cultured in hypercholesterolemic serum bound fewer immunogold particles than their normocholesterolemic counterparts. In marked contrast to these baseline levels of antigen expression, TF antigen was substantially increased on macrophages and macrophage-derived foam cells at the luminal surface of atherosclerotic lesions. As summarized in Figure 2 and illustrated in Figures 3–5, the number of gold particles observed on lesion-adherent macrophages (7.3 gold particles/μm cell surface) was seven-ten-fold greater than macrophages cultured in vitro. Most importantly, this degree of antigen expression was 24-fold greater than monocyte/macrophages newly adherent to the lesion surfaces. On these latter cells, the density of label was approximately 0.3 particles/μm of surface. The difference between the newly adherent cells and the fully activated macrophages or macrophage foam cells was statistically significant ($P<0.005$) and with respect to TF resulted in a bimodal distribution of cells on the vascular surface. The amount of immunogold on monocyte/macrophages newly adherent to lesions was similar to that found for the various in vitro studies and for all cells from experiments in which the anti-TF globulin was replaced by non-immune serum as a first-stage control antibody (Fig. 2).

The distribution of the anti-TF immunogold was generally uniform on luminally-adherent activated macrophages. Although these cells often had tortuous surfaces resulting from elaboration of membrane ruffles and microvilli, preferential localization of the antibody was not apparent (Fig. 3). Occasionally, the abluminal surfaces of the adherent cells had slightly less label than was found with the luminal aspect. This difference, however, may have reflected immunogold access rather than antigen distribution, for the labeling of intimal foam cells which projected into the lumen was restricted to the exposed surface of these cells (Fig. 4). As with adherent/activated macrophages, the distribution of label on the luminally-exposed foam cell surfaces was uniform. Notably, the immunogold labeling of endothelial cells either underlying the adherent macrophages (Fig. 3) or flanking intimal foam cells that protruded into the lumen (Fig. 4) was minimal. In all animals, the degree of endothelial cell label with the 30 nm specific anti-TF globulin was essentially equal to the density (less than 1 particle /μm of cell surface) of the non-specific, 15 nm, internal control. Typically, on the TF positive cells, the non-specific control was randomly scattered in relatively small amounts among the specific immunogold particles (Fig. 5). The ratios of specific to non-specific gold were not quantitated for all experimental samples, but a random sampling of 20 arterial micrographs involving 1360 immunogold particles resulted in an average ratio specific:non-specific of 35:1. The range of ratios was 8:1–109:1. Furthermore, many arterial areas and in vitro cells totally lacked the non-specific label.

Sampling error is always a major concern with transmission electron microscopy since preparative techniques dictate relatively small sample size. To circumvent this concern and provide a large sample basis, selected arterial segments were processed for immunogold microscopy in conjunction with scanning electron microscopy. The observations from these experiments supported the results obtained with sectioned material. As illustrated in Figure 6, TF expression by surface-adherent macrophages and intimal foam cells that projected into the lumen was uniform and dense over the entire surface of the involved cells. Consistent with the transmission electron microscopy observations, immunogold binding to the endothelium was minimal (Fig. 6).

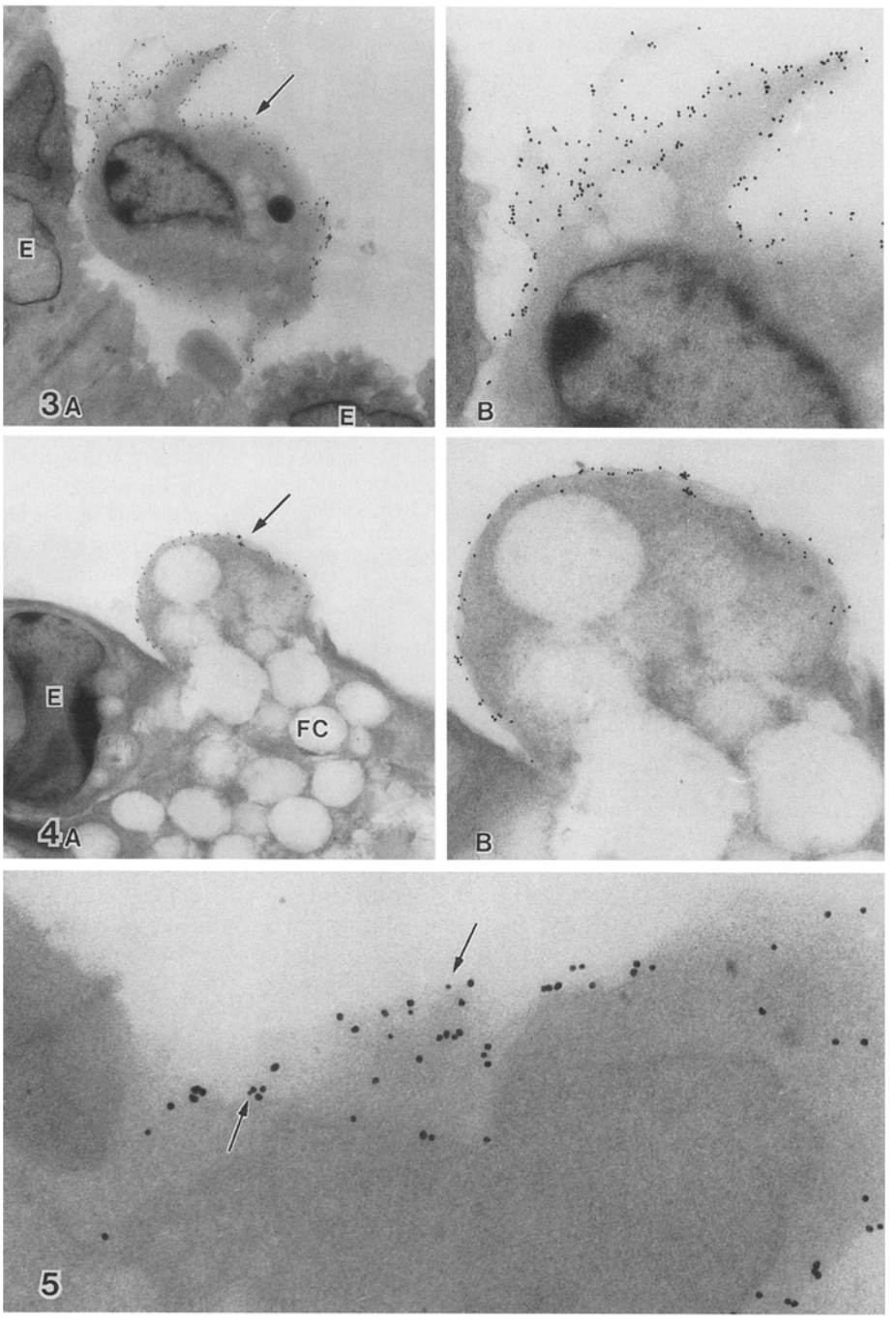

Fig. 3 Adherent monocyte-macrophage (*arrow*) on the endothelial (*E*) surface of an atherosclerotic lesion. **A** Low magnification of a labeled macrophage. X 6,900. **B** Higher magnification. The cell is heavily labeled with anti-TF antibodies [goat anti-rabbit IgG (GAR)-30 nm gold] whereas the non-specific control antibody (goat anti mouse 15 nm gold) is not present. X 16,300

Fig. 4 Macrophage foam cell (*FC*) protruding through the endothelium (*E*) into the vascular lumen expresses TF illustrating localization of the gold to the luminally exposed area *(arrow)*. **A** Low magnification. X 6,500. **B** Higher magnification of the same cell with anti-TF 30 nm gold. X 14,450

Fig. 5 Small segment of a foam cell protruding from a lesion in an area of endothelial damage is covered with TF. This high magnification micrograph illustrates the predominance of the specific 30 nm immunogold. A few nonspecific 15 nm gold particles *(arrows)* are scattered among the larger particles. X 37,000

Discussion

This study demonstrates TF expression by macrophages residing on the endothelium overlying foam cell lesions, and by macrophage foam cells exposed to the vascular lumen. Our findings are important because they suggest a mechanism for the initiation of thrombotic complications which may play an important role in lesion transition from the fatty streak stage to the more complex plaques that are characterized by the presence of fibrinogen and fibrin [1]. Previous studies involving TF localization in endarterectomy samples from human carotid lesions have localized TF to macrophage foam cells and to the extracellular matrix within the lesion necrotic core [28, 29]. In the study by Wilcox et al. [29], TF was found throughout the macrophages and not just on the plasma membrane of the cells, a distribution possibly resulting from either synthesis of TF or from macrophages ingesting one another following cell death. Our current study extends these observations by showing that: 1) TF is present on the plasmalemma of foam cells exposed to the arterial lumen as well as macrophages residing on the endothelial surface, and 2) TF is present at early stages of fatty streak formation.

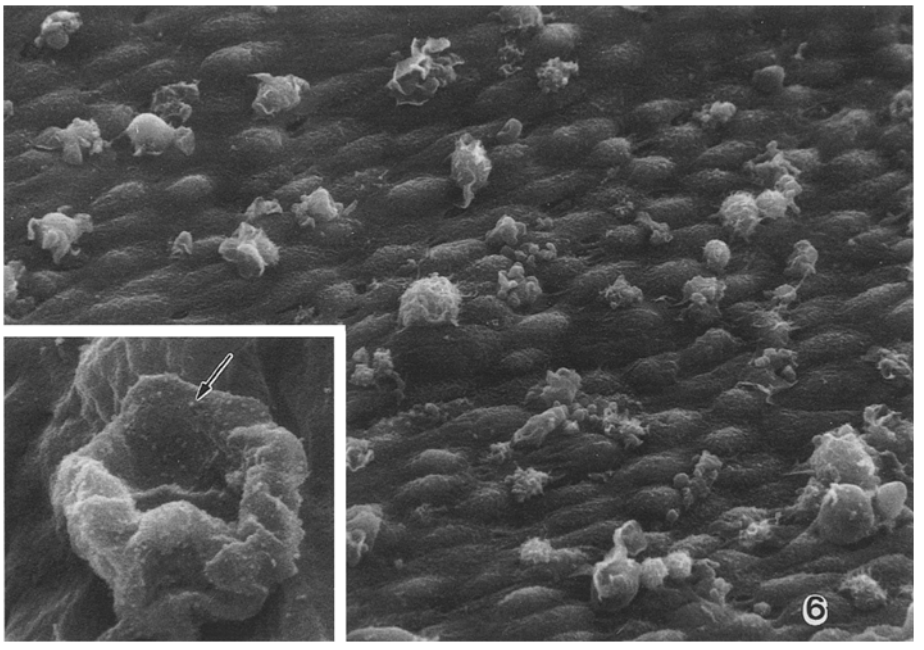

Fig. 6 Scanning electron micrographs of macrophages associated with a foam cell lesion. Low magnification demonstrates the abundance of monocyte-derived macrophages adherent to the endothelium over a lesion. X 815. *Inset:* higher magnification of a macrophage protruding from the intima into the vascular lumen. The cell surface is covered with anti-TF antibodies (GAR-30 nm gold, *arrow*). The surface of the endothelium is clearly not labeled with anti-TF. X 10,800

Most significantly, this study shows that TF on the surface of foam cells can gain access to the plasma compartment. These cells can thus mediate clot formation without the necessity for plaque rupture, a relatively late stage event in atherogenesis. Foam cells located partially within the intima and partially in the luminal compartment were reported by us [14] in a study of lipoprotein uptake by foam cell lesions. Such cells are reminiscent of the egressing foam cells reported by Gerrity [8] in atherosclerotic lesions in pigs. From earlier work in our laboratory, it is thought that foam cells in the White Carneau pigeon will egress from the intimal lesions into the vascular lumen if lesion regression is induced. Additionally, we have noted short term lesion regression is accompanied by a transient increase in clot formation on the lesion (Lewis, unpublished data). Possibly, TF expression by egressing foam cells and macrophages is responsible for the increased occurrence of clots in those earlier experiments.

If macrophages and macrophage foam cells express TF, then why are these cells not covered with fibrin polymers at all times? The most likely reason that TF expression does not always correlate with the presence of fibrin polymers is the presence of extrinsic pathway inhibitor (EPI, also known as tissue factor pathway inhibitor). This well studied plasma protein [2, 16, 20] reacts with factor Xa to bind to the TF-VIIa complex, halting the extrinsic pathway of coagulation. Further, when lipoproteins are isolated from the plasma through gel filtration, 50% of the plasma EPI is found in the low density lipoproteins (LDL) and very low density (VLDL) fractions and 44% is found in the high density lipoproteins fraction [16]. The circulation and delivery of EPI via lipoproteins would ensure that the atherosclerotic lesion will be supplied with pathway inhibitor. Our earlier study of lipoprotein uptake in pigeon lesions [14] demonstrated that luminally-exposed macrophages and foam cells bind and internalize modified LDL. Possibly, after modification in the artery wall LDL is a source of EPI for foam cells in the lesion. Another possible source of EPI is the endothelium, as EPI has been shown to bind to glycosaminoglycans on the luminal surface of the endothelium and may be a major site for EPI storage [20]. We would hypothesize then that in addition to the presence of TF on the macrophage and foam cell surfaces, a shift in the distribution of EPI would be required to initiate clot formation on the plaque surface.

The absence of TF on circulating monocytes and cultured monocyte-derived macrophages in this study indicated that macrophage activation, as measured by TF expression, is not stimulated solely by surface contact or hypercholesterolemia. If this were the case, TF expression in vitro by normal macrophages or macrophages from hypercholesterolemic animals would be expected without any further stimulation. Typically, mediators such as phorbol esters, endotoxin, immune complexes, or interferon gamma are required to stimulate monocytes or macrophages to express TF in vitro [15, 18, 22, 24], a finding that also has been demonstrated in pigeon macrophages [9]. Another stimulus that causes TF expression in monocytes and macrophages is modified LDL [23]. The results of this latter study suggest a possible mechanism for the activation of TF expression by developing foam cells in the lesion, because the ingestion of modified LDL is though to contribute significantly to foam cell formation [27]. Also, the study of Schuff-Werner [23] indicates the simultaneous expression of the scavenger receptor along with TF on activated macrophages, in agreement with this current study that demonstrates TF on cells previously shown to internalize modified LDL [14].

In conclusion, we have demonstrated that macrophages and macrophage foam cells associated with early fatty

streak lesions express TF. This expression was not observed in monocytes freshly isolated from normal or hypercholesterolemic animals, nor was it observed in cultured monocyte-derived macrophages from the same animals. These results reveal a possible mechanism by which macrophages and foam cells associated with early lesions of atherosclerosis can initiate fibrin formation, which can possibly contribute to lesion progression to the fibromuscular stage.

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References

1. Bini A, Fenoglio J Jr, Sobel J, Owen J, Fejgl M, Kaplan KL (1987) Immunochemical characterization of fibrinogen, fibrin I, and fibrin II in human thrombi and atherosclerotic lesions. Blood 69:1038–1045
2. Carson SD, Brozna JP (1993) The role of tissue factor in the production of thrombin. Blood Coagul Fibrin 4:281–292
3. Denholm EM, Lewis JC (1987) Monocyte chemoattractants in pigeon atherosclerosis. Am J Pathol 126:464–475
4. Drake TA, Morrissey JH, Edgington (1989) Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. Am J Pathol 134:1087–1097
5. Drake TA, Ruf W, Morrissey JH, Edgington TS (1989) Functional tissue factor is entirely cell surface expressed on lipopolysaccharide-stimulated human blood monocytes and a constitutively tissue factor-producing neoplastic cell line. J Cell Biol 109:389–395
6. Edwards RL, Rickles FR, Cronlund M (1981) Abnormalities of blood coagulation in patients with cancer. Mononuclear cell tissue factor generation. J Lab Clin Med 98:917–928
7. Fleck RA, Rao LVM, Rapaport SI, Vorki N (1990) Localization of human tissue factor antigen by immunostaining with monospecific, polyclonal anti-human tissue factor antibody. Thromb Res 57:765–781
8. Gerrity RG (1981) The role of the monocyte in atherogenesis. II. Migration of foam cells from atherosclerotic lesions. Am J Pathol 103:191–200
9. Gupta M, Doellgast GJ, Cheng T, Lewis JC (1993) Expression and localization of tissue factor-based procoagulant activity (PCA) in pigeon monocyte-derived macrophages. Thromb Haemost 70:963–969
10. Henson DA, St Clair RW, Lewis JC (1989) β-VLDL and acetylated-LDL binding to pigeon monocyte macrophages. Atherosclerosis 78:47–60
11. Henson DA, St Clair RW, Lewis JC (1989) Morphological characterization of β-VLDL and acetylated-LDL binding and internalization by cultured pigeon monocytes. Exp Mol Pathol 51:243–263
12. Jerome WG, Lewis JC (1984) Early atherogenesis in White Carneau pigeons. I. Leukocyte margination and endothelial alterations at the celiac bifurcation. Am J Pathol 116:56–68
13. Jerome WG, Lewis JC (1985) Early atherogenesis in White Carneau pigeons. II. Ultrastructural and cytochemical observations. Am J Pathol 119:210–222
14. Landers SC, Lewis JC (1993) acLDL binding and endocytosis by macrophages and macrophage foam cells in situ. Exp Mol Pathol 59:38–50
15. Lyberg T, Prydz H (1981) Phorbol esters induce synthesis of thromboplastin activity in human monocytes. Biochem J 194:699–706
16. Novotny WF, Girard TJ, Miletich JP, Broze GJ Jr (1989) Purification and characterization of the lipoprotein-associated coagulation inhibitor from human plasma. J Biol Chem 264:18832–18837
17. Østerud B, Flaegstad T (1983) Increased tissue thromboplastin activity in monocytes of patients with meningococcal infection: related to an unfavourable prognosis. Thromb Haemost 49:5–7
18. Prydz H, Lyberg T, Deteix P, Allison AC (1979) In vitro stimulation of tissue thromboplastin (factor III) activity in human monocytes by immune complexes and lectins. Thromb Res 15:465–474
19. Rao LVM (1988) Characterization of anti-tissue factor antibody and its use in immunoaffinity purification of human tissue factor. Thromb Res 51:373–384
20. Rapaport SI (1991) The extrinsic pathway inhibitor: a regulation of tissue factor-dependent blood coagulation. Thromb Haemost 66:6–15
21. Rush RL, Leon L, Turrell J (1970) Automated simultaneous cholesterol and triglyceride determination on the Autoanalyzer II instrument. In: Barton EC, De Cross MJ, Erdich MM (eds) Technicon Internation Congress Futura. Mt. Kisco, New York, pp 503–507. Thurman Associates. Miami, Fla.
22. Scheibenbogen C, Moser H, Krause S, Andreesen R (1992) Interferon-gamma-induced expression of tissue factor activity during human monocyte to macrophage maturation. Haemostasis 22:173–178
23. Schuff-Werner P, Claus G, Armstrong VW, Köstering H, Seidel D (1989) Enhanced procoagulatory activity (PCA) of human monocytes/macrophages after in vitro stimulation with chemically modified LDL. Atherosclerosis 78:109–112
24. Semararo N, Biondi A, Lorenzet R, Locati D, Mantovani A, Donati MB (1983) Direct induction of tissue factor sythesis by endotoxin in human macrophages from diverse anatomical sites. Immunology 50:529–535
25. Semararo N, Lattanzio A, Montemurro P, Papanice M, DeLucia O, DeBellis C, Giordano D (1985) Mechanisms of blood clotting activation in inflammation: the role of monoculear phagocytes. Int J Tissue React VII(4):313–320
26. Spurr AR (1969) A low viscosity epoxy resin embedding medium for electron microscopy. J Ultrastr Res 26:31–43
27. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL (1989) Beyond cholesterol. Modifications of low density lipoprotein that increase its atherogenicity. N Engl J Med 320:915–924
28. Tipping PG, Malliaros J, Holdsworth SR (1989) Procoagulant activity expression by macrophages from atheromatous vascular plaques. Atherosclerosis 79:237–243
29. Wilcox JN, Smith KM, Schwartz SM, Gordon D (1989) Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. Proc Natl Acad Sci USA 86:2839–2843
30. Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice Hall, New Jersey, p 718