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Short Communication

Angiogenic Macrophages Produce the Angiogenic Inhibitor Thrombospondin 1

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Previous investigations have shown that macrophages play a pivotal role in the induction of angiogenesis in both physiological and pathological settings. This investigation examines the relative production of the angiogenic modulator thrombospondin-1 (TSP1) by activated and nonactivated monocytes and macrophages. TSP1, a multifunctional extracellular matrix molecule, has been reported recently to inhibit angiogenesis both* in vitro *and* in vivo. *To examine the relationship between the level of TSP1 production by macrophages and expression of the angiogenic phenotype, murine monocytelike cells (WEHI-3) and human peripheral blood monocytes were each activated* in vitro *and examined for TSP1 production and angiogenic activity in rat corneal bioassay. Nonangiogenic monocytes produced low levels of TSP1 messenger RNA. Surprisingly, activated, potently angiogenic monocytes and macrophages exhibited as much as a sixfold increase in steady state TSP1 messenger RNA over unstimulated levels. Biosynthetic labeling studies demonstrated that TSP1 protein secretion increased in conjunction with increased TSP1 messenger RNA levels in angiogenic macrophages. The results demonstrate that activated monocytes and macrophages actively produce the angiogenic modulator TSP1 and suggest that TSP1 production may be a component of the angiogenic phenotype. In addition, the data suggest that the ability of macrophages to mediate angiogenesis results from a complex interplay of positive and negative regulators. (Am J Pathol 1993, 143:678–684)

The process of angiogenesis is an important component of a number of physiological and pathological processes.[1] Whereas many cell types play a role in modulating capillary growth, the tissue macrophage has been shown to be a key mediator of angiogenesis.[2,3] The capacity of macrophages to induce angiogenesis has been documented in physiological settings such as wound repair, as well as in pathological settings such as tumorigenesis and rheumatoid arthritis.[4–7] Macrophages are believed to regulate angiogenesis primarily through the production of soluble mediators. Macrophages may also influence the angiogenic process by modulating the composition of the extracellular matrix.[8,9] However, the precise molecular genetic events that comprise the angiogenic phenotype in macrophages are not well understood.

In 1985, Jaffe, et al.[10] reported that both resting and activated macrophages produce the glycoprotein thrombospondin-1 (TSP1). TSP1 is a large, multifunctional molecule that is a member of a family of extracellular matrix molecules. TSP1 exhibits a complex set of functional interactions with endothelial cells as well as with other proteins of the extracellular matrix.[11,12] Of particular interest is the recently documented ability of TSP1 to modulate angiogenesis, the process of new capillary growth. TSP1 has been reported to inhibit the angiogenic response of endothelial cells both *in vivo* and *in vitro*.[13–15] TSP1 inhibits endothelial cell proliferation and the migration of endothelial cells toward angiogenic mediators *in vitro*.[13,14,16,17] In corneal angiogenesis bioassays, TSP1 clearly inhibits the induction of angiogenesis by growth factors such as basic fibroblast growth factor.[13,14] As activated macrophages have been shown

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to be angiogenic, the production of TSP1, an inhibitor of angiogenesis, by these cells, is paradoxical. To determine if the production of TSP1 by macrophages is temporally linked to the angiogenic phenotype, an analysis of TSP1 synthesis in activated and nonactivated monocytes and macrophages was performed.

Materials and Methods

Cells and Cell Culture

The murine monocyte/macrophage line WEHI-3 was grown in complete Roswell Park Memorial Institute (RPMI) medium (RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin [100 U/ml] and streptomycin [100 μg/ml]) at 37 C with 5% CO_2. Human peripheral blood monocytes were prepared from donor buffy coats as previously described.[18] Cells were plated in complete RPMI, and cells that were adherent after 24 hours were used for experiments. Cells were activated by 2- to 24-hour treatment with lipopolysaccharide (LPS) (Sigma Chemical Co., St. Louis, MO, *Escherichia coli* 026:B6) at 0.1, 1, or 10 μg/ml. Activation by this method has been demonstrated previously to potently induce monocytes and macrophages to express angiogenic activity.[2,3,19]

Bioassay for Angiogenic Activity

Angiogenic activity was assayed in the avascular cornea of the rat eye as previously described.[6] Briefly, 24-hour conditioned media from cultures of quiescent and LPS-activated WEHI-3 cells and human peripheral blood monocytes were concentrated 20-fold by ultrafiltration through a UM10 Amicon membrane and incorporated into noninflammatory Hydron polymer (Interferon Sciences, New Brunswick, NJ), and 5-μl pellets were implanted into the corneal stroma, 1 to 1.5 mm from the limbus. Corneas were examined daily with a stereomicroscope to monitor capillary growth. Five to 7 days later, rats were perfused intraarterially with colloidal carbon to provide a permanent record of individual responses. Corneas were then excised, fixed in 2% glutaraldehyde 2.5% paraformaldehyde, flattened, and photographed.

RNA Analysis

Total cellular RNA was prepared by the method of Davis et al.[20] Northern analysis was performed by electrophoresis of RNA samples (10 μg/lane) through 0.8% agarose 2 mol/L formaldehyde gels in 20 mmol/L MOPS buffer, pH 7, containing 5 mmol/L sodium acetate and 1 mmol/L ethylenediaminetetraacetic acid. Gels were blotted onto Gene Screen Plus (DuPont-NEN, Wilmington, DE), and prehybridized for at least 15 minutes at 60 C in 1 mol/L NaCl, 1% sodium dodecyl sulfate (SDS), 10% dextran sulfate, and 100 μg/ml denatured and sheared salmon sperm DNA. Radiolabeled probe was added to a final concentration of 10^5 cpm/m, and blots were hybridized 16 to 24 hours at 60 C. Blots were washed for two 30-minute periods in 2× standard saline citrate (1× standard saline citrate = 0.15 mol/L NaCl, 0.015 mol/L Na Citrate), 1% SDS at 60 C. Autoradiography was performed with intensifying screens at −70 C for 3 to 48 hours. Densitometry was performed on an LKB Ultrascan XL.

The TSP1 probe was a mixture of two 1.5-kb *Pst*I fragments of the human TSP1 complementary DNA.[21] These fragments included the segments from base pairs 1,006 to 2,519 and from 2,853 to 4,300. This probe is highly similar to murine TSP1 and thus strongly cross-hybridizes with murine TSP1 messenger (m)RNA. As a control for RNA integrity, Northern blots were also hybridized to a 1.0-kb *Pst*I fragment of the human β-actin complementary DNA. Probes were labeled with ^{32}P by random priming[22] to a specific activity of at least 10^8 cpm/μg.

Immunoprecipitation

Immunoprecipitation was performed as previously described.[14] Cells were grown to approximately 70% confluence in 60-mm dishes and labeled for 4 hours in 1 ml cysteine- and methionine-free media supplemented with 100 μC [^{35}S]methionine-cysteine (Trans-label, ICN Radiochemicals, Costa Mesa, CA). In some experiments, cells were treated with LPS at 1 μg/ml during the labeling period (4-hour LPS treatment) or for 4 hours before and during the labeling period (8-hour LPS treatment). The culture medium was collected, and immunoprecipitation was performed with rabbit anti-TSP1 antiserum (a gift of Dr. Noel Bouck) or pre-immune rabbit serum. An equal number of trichloroacetic acid preciptible counts per sample were incubated for 1 hour at 4 C with a 1:30 dilution of anti-serum or pre-immune serum. Immune complexes were precipitated by incubation with 100 μl of Immunoprecipitation (GIBCO Bethesda Research Laboratories, Gaithersburg, MD) for 30 minutes at 4 C. The precipitates were washed extensively and then boiled

for 3 minutes in sample buffer (50 mmol/L Tris-Cl, pH 6.8, 100 mmol/L dithiothreitol, 2% SDS, 0.1% bromophenol blue, and 10% glycerol). Eluted proteins were resolved by Lammeli gel electrophoresis through an 8% SDS polyacrylamide gel under reducing conditions. Gels were stained with Coomasie Brilliant Blue, dried, and subjected to autoradiography at −70 C for 7 to 14 days.

Results

Angiogenic Phenotype of Activated Cells

Previous investigations have shown that stimulation of monocytes and macrophages with LPS evokes the angiogenic phenotype.[2,3,19] To assure that the cells investigated here were stimulated to produce angiogenic mediators, the conditioned media form LPS-treated WEHI-3 cells and human peripheral blood monocytes was assayed for angiogenic activity in the rat cornea. In each case, the LPS-activated WEHI-3 cells and human peripheral blood monocytes were potently angiogenic (Table 1, Figure 1).

TSP1 Levels Are Increased in Activated Monocytes and Macrophages

The steady state level of TSP1 mRNA in unstimulated (nonangiogenic) and LPS-stimulated (angiogenic) WEHI-3 cells and human monocytes was determined by RNA analysis. RNA was prepared from WEHI-3 cells that were exposed to LPS at 0, 0.1, 1, and 10 μg/ml for 24 hours, and human monocytes that had been exposed to 0 or 1 μg/ml LPS for 24 hours (Figure 2). RNA analysis with a TSP1-specific probe revealed that TSP1 mRNA levels were increased in LPS-activated WEHI-3 macrophages and human monocytes. Densitometric comparison of TSP1 mRNA in WEHI-3 cells revealed that the level of TSP1 mRNA increased 2.7-, 5.6-, and 6.1-fold over background when cells were treated for 24 hours with LPS at 0.1, 1, and 10 μg/ml respectively. The increase seemed to be relatively concentration-dependent, as greater levels of TSP1 mRNA were present in cells incubated with higher concentrations of LPS.

Table 1. *Angiogenic Phenotype of Activated and Nonactivated Macrophages*

Conditioned media	Proportion of positive responses (%)	
Cell-free control	0/6	(0)
Nonactivated WEHI-3	0/4	(0)
LPS-activated WEHI-3	5/5	(100)
Nonactivated human monocytes	0/4	(0)
LPS-activated human monocytes	5/5	(100)

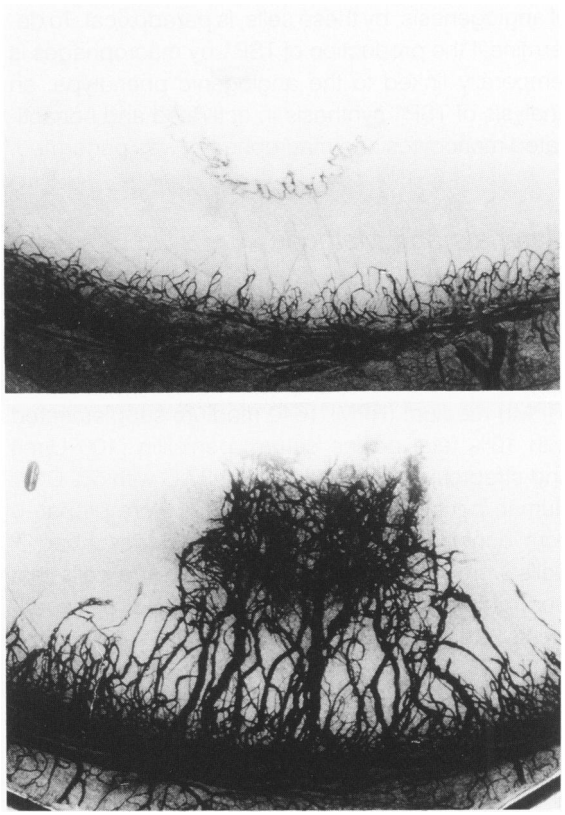

Figure 1. *Angiogenic activity of nonactivated and LPS-activated monocytes.* **A**: *Nonactivated human peripheral monocytes.* **B**: *LPS-activated human peripheral monocytes. Note the virtual absence of capillaries in* **A** *and the abundant capillary sprouts in* **B**. *Similar results were observed with nonactivated and LPS-activated WEHI-3 cells.*

The time course of induction of TSP1 mRNA by LPS in WEHI-3 cells was assessed by isolating RNA from cells exposed to 1 μg/ml LPS for 0, 2, 4, 6, 12, or 24 hours. RNA analysis with a TSP1-specific probe demonstrated that the level of TSP1-specific mRNA in these cells increased significantly after just 2 hours of LPS stimulation (Figure 2). TSP1 mRNA levels reached a maximum by 4 hours and then remained constant for the remainder of the 24-hour LPS exposure.

Production of TSP1 protein in LPS-stimulated WEHI-3 macrophages was assessed by biosynthetic labeling and subsequent immunoprecipitation of cell supernatants with polyclonal revealed that the level of TSP1 mRNA increased 2.7-, 5.6-, and 6.1-fold over background when cells were treated

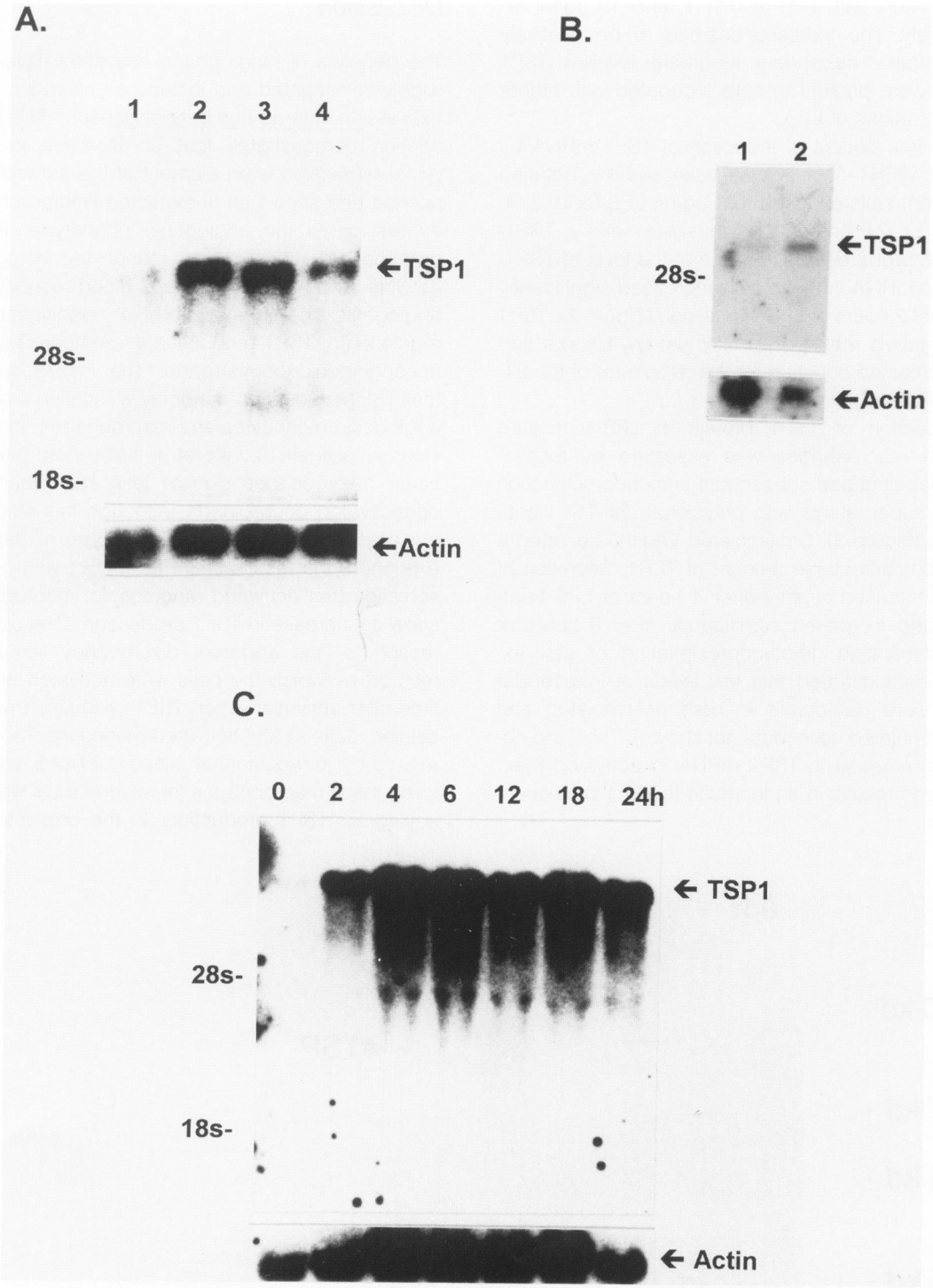

Figure 2. *Northern analysis of TSP1 mRNA levels in angiogenic and nonangiogenic monocytes and macrophages.* **A:** *RNA from WEHI-3, a murine monocyte-macrophage cell line. The blot was sequentially hybridized to probes for either TSP1 (top panel) or β-actin (lower panel). Each lane contains 10 μg total RNA isolated from WEHI-3 cells that had been incubated for 24 hours in 1) media, 2) media + 10 μg/ml LPS, 3) media + 1 μg/ml LPS, or 4) media + 0.1 μg/ml LPS. The locations of TSP1 mRNA, β-actin mRNA, and the 28s and 18s ribosomal bands are indicated.* **B:** *mRNA from human peripheral blood monocytes. The blot was sequentially hybridized to probes for either TSP1 (top panel) or β-actin (lower panel). Lanes contain 10 μg total RNA isolated from monocytes treated for 24 hours with 1) media alone or 2) media + 1 μg/ml LPS. The locations of TSP1 mRNA, β-actin mRNA, and the 28s ribosomal band are indicated.* **C:** *Time course analysis of TSP1 mRNA induction in activated WEHI-3 cells. The blot was sequentially hybridized to probes for either TSP1 (top panel) or β-actin (lower panel). Each lane contains 10 μg total RNA isolated from cells treated with 1 μg/ml LPS for 0, 2, 4, 6, 12, 18, or 24 hours. The locations of TSP1 mRNA, β-actin mRNA, and the 28s and 18s ribosomal bands are indicated.*

for 24 hours with LPS at 0.1, 1, and 10 μg/ml respectively. The increase seemed to be relatively concentration-dependent, as greater levels of TSP1 mRNA were present in cells incubated with higher concentrations of LPS.

The time course of induction of TSP1 mRNA by LPS in WEH1–3 cells was assessed by isolating RNA from cells exposed to 1 μg/ml LPS for 0, 2, 4, 6, 12, or 24 hours. RNA analysis with a TSP1-specific probe demonstrated that the level of TSP1-specific mRNA in these cells increased significantly after just 2 hours of LPS stimulation (Figure 2). TSP1 mRNA levels reached a maximum by 4 hours and then remained constant for the remainder of the 24-hour LPS exposure.

Production of TSP1 protein in LPS-stimulated WEHI-3 macrophages was assessed by biosynthetic labeling and subsequent immunoprecipitation of cell supernatants with polyclonal anti-TSP1 antibodies (Figure 3). Unstimulated WEHI-3 secreted a small but detectable amount of TSP1. Secretion of TSP1 increased slightly after 4 hours of LPS treatment and increased significantly after 8 hours of LPS stimulation. Immunoprecipitation of cell extracts demonstrated that low levels of intracellular TSP1 were detectable in both unstimulated and LPS-stimulated cells (data not shown). Thus, the observed increase in TSP1 mRNA in activated macrophages results in an increase in TSP1 protein secretion.

Discussion

The genetics of macrophage activation have been widely investigated and include an alteration in the expression of large numbers of genes.[23] This investigation demonstrates that an increase in TSP1 gene expression is an element of the activation response and shows an unexpected linkage of TSP1 expression to the angiogenic phenotype. In two systems—WEHI-3, a murine monocyte-macrophage cell line, and human peripheral blood monocytes—angiogenic cells exhibited a significant up-regulation of TSP1 production as compared to their nonangiogenic counterparts. The results suggest that TSP1 production may play a functional role for angiogenic monocytes and macrophages. In a previous investigation, Jaffe et al[10] observed that activated macrophages secrete less TSP1 than their nonactivated counterparts. Although this study did not assess the angiogenic phenotype of the cells examined, the results seem to conflict with our observation that activated, angiogenic, macrophages show an increase in TSP1 production. One possible reason for the apparent discrepancy lies in the method by which the cells were activated and the time after activation when TSP1 production was assessed. Jaffe et al[10] activated murine macrophages *in vivo* by intraperitoneal injection of LPS and cultured these macrophages for several days while assaying for TSP1 production. In the present study,

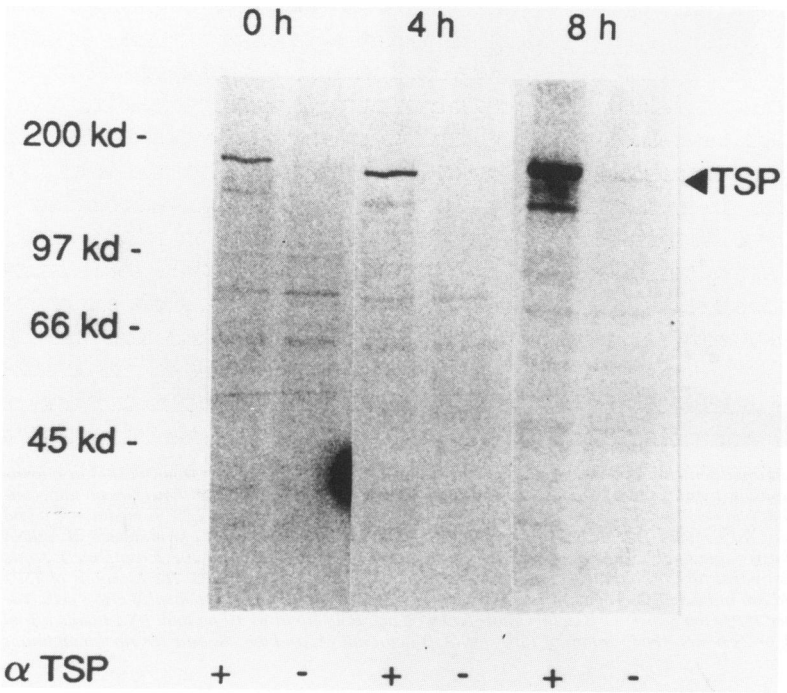

Figure 3. *SDS-polyacrylamide gel electrophoresis analysis of TSP1 immunoprecipitates of biosynthetically labeled WEHI-3 cells. Culture media from control or stimulated WEHI-3 cells was immunoprecipitated either anti-TSP1 (+) or pre-immune serum (−). Cells were exposed to LPS for either 0, 4, or 8 hours as indicated, all groups were biosynthetically labeled for 4 hours immediately before harvest.*

activation was performed *in vitro*, and TSP1 production was assessed immediately. Our findings are in accordance with a recent report by Varani et al,[24] who have shown that TSP1 production by the human monocytic cell line U937 increases during *in vitro* activation with phorbol myristate acetate. A recent investigation by Ostergaard and Flodgaard[25] also documents an increase in TSP1 production by monocytes that had been stimulated with human heparin binding protein, a molecule that is synthesized by neutrophils and is chemotactic for monocytes. We have also observed a similar increase in TSP1 production in WEHI-3 cells stimulated with phorbol myristate acetate or concanavalin A (L. DiPietro and P. Polverini, unpublished results). Taken together, the evidence strongly suggests that TSP1 production is a common feature of macrophage activation. The data reported here provide the first evidence that production of TSP1 occurs in tandem with the expression of the angiogenic phenotype.

The finding that activated, angiogenic, macrophages produce TSP1, a reported inhibitor of angiogenesis, would seem paradoxical. Macrophages have been shown to produce anti-angiogenic activity, and the angiogenic capability of macrophages *in vivo* may rely on a timely and/or balanced production of both positive and negative angiogenic mediators.[19,26,27] Alternatively, macrophage-derived TSP1 may not exert significant effects on endothelial cells, particularly if TSP1 is rapidly degraded or quickly sequestered into the extracellular matrix. In this case, the *in vivo* influence of activated macrophages upon endothelial cells may be dominated by diffusable angiogenic factors rather than extracellular matrix components such as TSP1. Several other functional roles for macrophage-derived TSP1 are possible. The adhesive capacity of TSP1 might facilitate migration of the activated macrophage. Macrophages have surface receptors for TSP1[28] and thus might lay down TSP1 upon the existing extracellular matrix as a scaffold upon which to migrate. This hypothesis is supported by a recent finding that anti-TSP antibodies inhibit macrophage migration through endothelial layers *in vitro*.[29] A second possibility is that macrophages produce TSP1 as a functional protease inhibitor. TSP1 has been recently described to act as a protease inhibitor *in vitro*, a function which may be extremely important to the activated macrophage as it produces other soluble mediators.[30] Finally, TSP1 has been shown to enhance neutrophil chemotaxis and to facilitate the phagocytosis of senescent neutrophils by macrophages.[31,32] The idea that angiogenic macrophages might produce TSP1 to effect neutrophil migration and clearance is an intriguing one, as it suggests a functional link between macrophages and neutrophils in the inflammatory process.

In this investigation, angiogenic macrophages are actively producing TSP1 at the time they are implanted in the corneal assay. However, we have not yet rigorously examined these cells to be certain if this same temporal relationship between TSP1 production and angiogenic activity exists *in vivo*. A recent investigation of Koch et al[33] provides evidence that angiogenic macrophages produce TSP1 *in vivo*. Using immunohistochemistry, Koch et al have shown that macrophages within the inflamed rheumatoid synovium of patients actively produce TSP1. In other investigations, rheumatoid synovial macrophages have also been shown to be angiogenic.[7] Taken together with this study, the data suggest that angiogenic macrophages *in vivo* may be a source of TSP1. Further *in vivo* investigations of TSP1 production by monocytes and macrophages at sites of active angiogenesis may determine whether the production of TSP1 is common characteristic of angiogenic responses that are associated with macrophages. Such investigations may lead to a better understanding of the functional significance of TSP1 production by angiogenic cells.

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References

1. Folkman J, Klagsbrun, M: Angiogenic factors. Science, 1987, 235:442–447
2. Polverini PJ, Cotran RS, Gimbrone MA, Unanue ER: Activated macrophages induce vascular proliferation. Nature 1977, 269:804–806
3. Koch AE, Polverini PJ, Leibovich SJ: Induction of neovascularization by activated human monocytes. J Leukocyte Biol 1986, 39:233–238
4. Thakral KK, Goodson WH, Hunt TK: Stimulation of wound blood vessel growth by wound macrophages. J Surg Res 1979, 26:430–436
5. Hunt TK, Knighton DR, Thakral KK, Goodson WH, Andrews, WS: Studies on inflammation and wound healing: angiogenesis and collagen synthesis stimulated *in vivo* by resident and activated wound macrophages. Surgery 1984, 96:48–54

6. Polverini PJ, Leibovich SJ: Induction of neovascularization *in vivo* and endothelial proliferation *in vitro* by tumor-associated macrophages. Lab Invest 1984, 51: 635–642
7. Koch AE, Polverini PJ, Leibovich SJ: Stimulation of neovascularization by human rheumatoid synovial tissue macrophages. Arthritis Rheum 1986, 29:471–479
8. Porras-Reyes, BH, Blair HC, Jeffrey JJ, Mustoe TA: Collagenase production at the border of granulation tissue in a healing wound: macrophage and mesenchymal collagenase production *in vivo*. Connect Tissue Res 1991, 27:63–71
9. Sunderkötter C, Goebeler M, Schulze-Osthoff K, Bhardwaj R, Sorg C: Macrophage derived angiogenesis factors. Pharmacol Ther 1991, 51:195–216
10. Jaffe EA, Ruggiero JT, Falcone DJ: Monocytes and macrophages synthesize and secrete thrombospondin. Blood 1985, 65:79–84
11. Frazier WA: Thrombospondin: a modular adhesive glycoprotein of platelets and nucleated cells. J Cell Biol 1987, 105:625–632
12. Lawler J: The structural and functional properties of thrombospondin. Blood 1986, 67:1197–1209
13. Rastinejad F, Polverini PJ, Bouck NP: Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppressor gene. Cell 1989, 56:345–355
14. Good DJ, Polverini PJ, Rastinejad F, LeBeau MM, Lemons RS, Frazier WA, Bouck NP: A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. Proc Natl Acad Sci USA 1990, 87:6624–6628
15. Iruela-Arispe ML, Bornstein P, Sage H: Thrombospondin exerts an antiangiogenic effect on cord formation by endothelial cells *in vitro*. Proc Natl Acad Sci USA 1991, 88:5026–5030
16. Bagavandoss P, Wilks JW: Specific inhibition of endothelial cell proliferation by thrombospondin. Biochem Biophys Res Comm 1990, 170:867–872
17. Taraboletti G, Roberts D, Liotta LA, Giavazzi R: Platelet thrombospondin modulates endothelial cell adhesion, motility, and growth: a potential angiogenesis regulatory factor. J Cell Biol 1990, 111:765–772
18. Vissers MCM, Jester SA, Fantone JC: Rapid purification of human peripheral blood monocytes by centrifugation through Ficoll-Hypaque and Sepracell-MN. J Immunol Methods 1988, 110:203–207
19. Polverini PJ: Macrophage-induced angiogenesis: a review. Cytokines. Edited by Sorg C, Basel S. Karger, 1989, pp 54–73
20. Davis LG, Dibner MD, Battey JF: Basic Methods in Molecular Biology. New York, Elsevier Science Publishing Co., Inc., 1986
21. Hennessey SW, Frazier BA, Kim DD, Deckwerth TL, Baumgartel DM, Rotwein P, Frazier WA: Complete thrombospondin mRNA sequence includes potential regulatory sites in the 3′ untranslated region. J Cell Biol 1989, 108:729–736
22. Feinberg AP, Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1983, 132:6–13
23. Adams DO, Koerner TJ: Gene regulation in macrophage development and activation. The Year in Immunology. Edited by Crose JM, Lewis RE Jr, Basel S. Karger, 1989, 4:159–180
24. Varani J, Stoolman L, Wang T, Schuger L, Flippen C, Dame M, Johnson KJ, Todd RF, Ryan US, Ward PA: Thrombospondin production and thrombospondin-mediated adhesion in U937 cells. Exp Cell Res 1991, 195:177–182
25. Ostergaard E, Flodgaard H: A neutrophil-derived proteolytic inactive elastase homologue (hHBP) mediates reversible contraction of fibroblasts and endothelial cell monolayers and stimulates monocyte survival and thrombospondin secretion. J Leukocyte Biol 1992, 51: 316–323
26. Besner GE, Klagsbrun M: Macrophages secrete a heparin-binding inhibitor of endothelial cell growth. Microvasc Res 1991, 42:187–197
27. Polverini PJ, DiPietro LA: Role of the macrophage in physiological and pathological angiogenesis. Angiogenesis in Health and Disease. Edited by Margoudakis ME, Gullino P. New York, Plenum Press, 1992, pp 43–53
28. Silverstein RL, Nachman RL: Thrombospondin binds to monocytes and macrophages and mediates platelet-monocyte adhesion. J Clin Invest 1987, 79: 867–874
29. Huber AR, Ellis S, Johnson KJ, Dixit VM, Varani J: Monocyte diapedesis through an *in vitro* vessel wall construct: inhibition with monoclonal antibodies to thrombospondin. J Leukocyte Biol 1992, 52:524–528
30. Hogg PJ, Stenflo J, Mosher DF: Thrombospondin is a slow tight-binding inhibitor of plasmin. Biochemistry 1992, 31:265–269
31. Mansfield PJ, Boxer LA, Suchard SJ: Thrombospondin stimulates motility of human neutrophils. J Cell Biol 1990, 111:3077–3086
32. Savill J, Hogg N, Ren Y, Haslett C: Thrombospondin cooperates with CD36 and the vitronection receptor in macrophage recognition of neutrophils undergoing apoptosis. J Clin Invest 1992, 90:1513–1522
33. Koch AE, Friedman J, Burrows JC, Haines GK, Bouck NP: Localization of the angiogenesis inhibitor thrombospondin in human synovial tissues. Pathobiology, in press.