Brief Review

Hypoxia/Hypoxemia-Induced Activation of the Procoagulant Pathways and the Pathogenesis of Ischemia-Associated Thrombosis

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Abstract—Although oxygen deprivation has long been associated with triggering of the procoagulant pathway and venous thrombosis, blood hypoxemia and stasis by themselves do not lead to fibrin formation. A pathway is outlined through which diminished levels of oxygen activate the transcription factor early growth response-1 (Egr-1) leading to de novo transcription/translation of tissue factor in mononuclear phagocytes and smooth muscle cells, which eventuates in vascular fibrin deposition. The procoagulant response is magnified by concomitant suppression of fibrinolysis by hypoxia-mediated upregulation of plasminogen activator inhibitor-1. These data add a new facet to the biology of thrombosis associated with hypoxemia/stasis and imply that interference with mechanisms causing Egr-1 activation in response to oxygen deprivation might prevent vascular fibrin deposition occurring in ischemia without directly interfering with other pro/anticoagulant pathways. (*Arterioscler Thromb Vasc Biol*. 1999;19:2029-2035.)

Key Words: tissue factor ■ hypoxia ■ ischemia ■ Egr-1 ■ PAI-1

In the 1850s, Virchow described the association between venous thrombosis and a triad of contributing factors, including hypercoagulability, vascular damage, and vascular stasis.[1] More recently, venous stasis has been linked to the rapid decline in intravascular oxygen tension and thrombus formation in veins of the lower extremities.[2–4] Definition of mechanisms through which low levels of oxygen cause blood to clot has been more elusive. The Wessler stasis model of venous thrombosis,[5,6] in which a rabbit vascular segment (typically the jugular vein) is occluded and a fibrin clot subsequently forms after addition of a procoagulant, demonstrated that acute lack of blood flow and/or hypoxia, although necessary, were not sufficient, at least acutely, to trigger clot formation. Without inclusion of a strong procoagulant stimulus, such as activated clotting factors (eg, Factors IXa, Xa, or thrombin), fibrin deposition did not occur. These observations have been extended to different types of vessels, and this has led to the same conclusion; acute occlusion of normal vessels with their normal blood content does not by itself promote fibrin formation. This situation must be differentiated from that of an atherosclerotic or other pathologic vessel, in which abundant neointimal tissue factor, a maximal prothrombotic stimulus, is immediately exposed to blood contents, triggering rapid coagulation. In fact, the intact, healthy vessel wall has very low levels of tissue factor

with an increasing gradient toward the adventitia. The cell type most uniformly accepted as being capable of expressing substantive amounts of tissue factor within the intravascular space in response to environmental stimuli is the mononuclear phagocyte, although polymorphonuclear leukocytes may also contribute, and endothelial cells have been shown to produce tissue factor in selected settings.[7,8] Thus it may not be surprising that a brief period of hypoxemia and/or stasis alone in a normal vessel are not sufficient to trigger fibrin formation.

Nonetheless, links between thrombosis and hypoxemia/stasis have remained strong even in the absence of mechanistic explanations. Studies in a canine model of limb immobilization showed stasis to be associated with a rapid fall in venous oxygen tension to virtually undetectable levels in the affected extremity.[2] Furthermore, hypoxemia was most severe in proximity to venous valve cusps, and nascent thrombi appeared to form on the apparently intact vessel wall at the parietal aspect of the valve cusp (this occurred during the period of hypoxemia). These accumulations of fibrin contained peripheral blood cells and erythrocytes and were speculated to result from hypoxemia-induced perturbation of the vessel wall. In fact, when untraumatized canine leg veins were exposed in vivo to intermittent periods of hypoxemia and reoxygenation, venous thrombi were shown to occur.[9]

Therefore, although in selected circumstances hypoxemia alone is sufficient to cause venous thrombosis, blood stasis results in ischemia that is associated with a myriad of changes in the vascular microenvironment, including diminished aerobic metabolism and accumulation of waste products. Thus the ischemic environment induces cell stress on many levels in addition to a contribution resulting from diminished blood oxygen. Another setting in which hypoxia has been considered to have a central role concerns vascular perturbation in response to high altitude, termed hypobaric hypoxia. Mountain climbing and other activities at high altitude are associated with vascular dysfunction, both prothrombotic events and increased vascular permeability (high altitude pulmonary and cerebral edema),[10-13] and these have been recently documented in graphic detail in the lay press.[14]

In the setting of ischemia, the most striking tissue injury occurs during reperfusion when leukocytes pour into the previously unperfused zone. We propose that mechanisms underlying pathologic events whose consequences are exaggerated during reperfusion are set in motion during the previous period of hypoxemia. An example of this principle is the critical period during organ preservation, when hypoxemia and stasis prime the graft vasculature for damage during reperfusion.[15] Such tissue injury occurring as soon as blood flow to the graft is reestablished is limited by strategies to diminish preservation-induced vascular dysfunction with more optimal preservation solutions during the period of organ storage. Our brief review will focus on mechanisms through which hypoxemia promotes fibrin formation in vivo and the results of recent studies to elucidate the bases for such activation of the procoagulant pathway.

A Model of Hypoxia-Induced Fibrin Deposition

We have developed a model system to examine the effects of hypoxia on intravascular thrombus formation, and this system has also recently been adapted by others in studies of mechanisms of hypoxia-induced thrombosis.[16,17] In this model, analysis of fibrin formation can employ 3 complementary techniques: morphological and immunoblotting studies with monospecific polyclonal antibody to a neo-epitope in fibrin gamma-gamma chain dimers (other investigators have used antibody to human fibrin beta chains with similar ability to detect murine fibrin),[16] electron microscopy, and deposition of radioiodinated fibrinogen. After exposure to hypoxia, vascular fibrin deposition occurs within approximately 6 hours.[18] Immunostaining shows close association of fibrin deposits with the vessel wall (Figure 1A), compared with lack of fibrin deposits in normoxic controls (Figure 1B). Electron microscopy shows that immunoreactive fibrin deposits from hypoxemic lung vasculature display ultrastructural properties of fibrin, especially the 22-nm periodicity (Figures 1C and 1D). Consistent with these observations, immunoblotting using fibrin-specific antibody and extracts from hypoxic lung treated with plasmin showed an immunoreactive band, whereas none was seen in normoxic controls or in hypoxic mice that had been pretreated with either hirudin
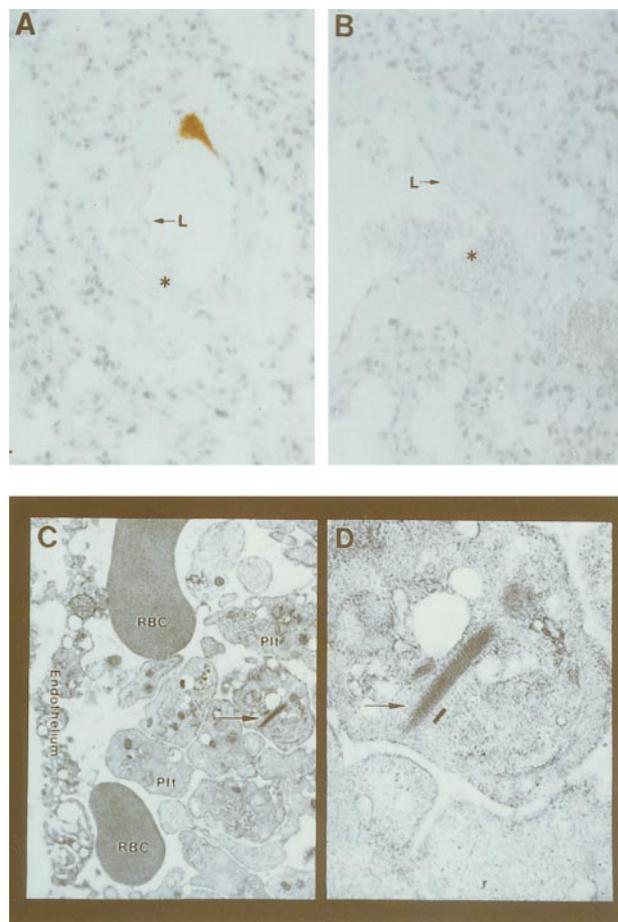

Figure 1. Fibrin deposition in pulmonary vasculature from mice exposed to hypoxia or normoxia. Mice were exposed to hypoxia (A), FiO$_2$ of $\approx$6%, or maintained in normoxia (B) for 8 hours, followed by administration of heparin before killing. Tissue was immunostained with antifibrin antibody. A and B, $\times$600; L indicates vascular lumen; arrows, vascular endothelium; and *, red blood cells. Electron micrograph of hypoxic (16 hours) murine pulmonary vasculature (C) showing endothelium, red blood cells (RBC), platelet clumping (Plt), and platelet-associated fibrin (arrow). Higher magnification (D) demonstrates 22.5 nm periodicity characteristic of fibrin; length of black bar is 112 nm. C, $\times$12 450; D, $\times$44 650. Adapted from reference 18.

or blocking antibody to tissue factor.[18] These data indicate that hypoxia/hypoxemia triggers a pathway leading to fibrin deposition in the lung.

Hypoxia-Associated Expression of Tissue Factor

Because blocking antibody to tissue factor suppressed fibrin accumulation in hypoxic murine lung, it was likely that oxygen deprivation caused increased expression of tissue factor in a compartment exposed to circulating blood. One possibility was that polymorphonuclear leukocytes (PMN) became adherent to the hypoxemic vessel wall due to translocation of P-selectin to the cell surface[15] or cytokine-mediated upregulation of intercellular adhesion molecule-1 expression.[19] Subsequent leukocyte activation could generate reactive oxygen intermediates, damaging the vessel wall and causing exposure of tissue factor in subendothelial layers of the vessel wall. However, antibody-induced depletion of PMNs had no effect on fibrin accumulation.[18] Another mechanism could be in-
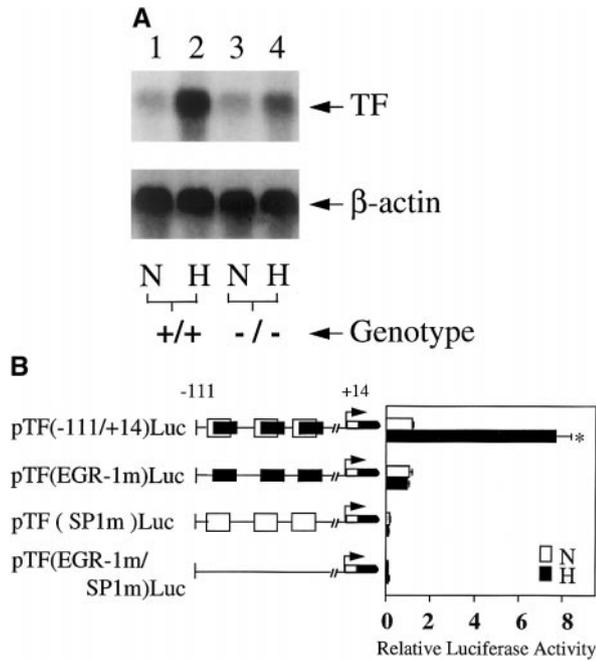
Figure 2. Hypoxia-mediated induction of tissue factor expression in murine lung and the role of egr-1. A, Egr-1 null mice show reduced tissue factor induction. Mice (wild-type, +/+; homozygous null mice, −/−) were subjected to normoxia (N) or hypoxia (H; 6% oxygen), lungs were rapidly harvested, total RNA was prepared, and Northern analysis (30 μg/lane of total RNA) was performed with ^{32}P-labeled cDNA for mouse tissue factor (upper) or β-actin (lower). Adapted from reference 20. B, Hypoxia-inducible tissue factor expression is due to transcriptional activation at Egr-1 sites. Transient cotransfection of HeLa cells was performed using either pTF($-$111/+14)Luc, pTF(EGR-1 m)Luc, pTF(SP1 m)Luc, or pTF(EGR-1 m/SP1 m)Luc, and pCMV-β–galactosidase. Cultures were transfected with each of the indicated constructs using the lipofectamine procedure, and then cells were exposed to normoxia (N) or hypoxia (H) for 5 hours. Luciferase and β-galactosidase activity were then determined, and relative luciferase activity normalized for β-galactosidase activity. Adapted from reference 20.

creased vascular permeability due to the direct effect of hypoxia on the endothelium or indirectly via hemodynamic changes (for example, pulmonary hypertension). However, increased vascular leakage in response to hypoxia occurs at later times and is not substantially different within 8 hours of being subject to the oxygen-deficient environment, at which time fibrin deposition was already evident.[18] In contrast, antibody-induced depletion of monocytes did suppress fibrin deposition in hypoxic lung,[18] suggesting that this cell type was likely to have an integral role in activation of the procoagulant pathway in this setting. This evidence led us to predict that monocytes were triggering the procoagulant pathway, probably by upregulating tissue factor, and platelets were subsequently amplifying procoagulant events.

To test this hypothesis, tissue factor expression in hypoxic murine lung was analyzed.[20] Northern analysis showed ≈20-fold increased tissue factor transcripts in hypoxic lung from wild-type animals compared with normoxic controls (Figure 2A). Immunohistochemical studies demonstrated increased tissue factor in hypoxic lung, colocalized with mononuclear phagocytes[20] from

wild-type mice, compared with normoxic controls. In vitro studies confirmed that mononuclear phagocytes placed in an hypoxic environment demonstrate transcriptional upregulation of tissue factor mRNA.[20] These data implicating tissue factor expression in hypoxia-driven thrombosis are especially provocative, given recent data showing the important role tissue factor plays in maintaining normal hemostasis. When tissue factor is expressed at very low levels in genetically altered mice, they exhibit a hemorrhagic tendency.[21]

In depth exploration of the mechanism by which hypoxia increases tissue factor transcription led to the identification of Egr-1 as the primary driving motif underlying hypoxia-induced tissue factor transcription. These data are in concordance with recent studies identifying an association between Egr-1 and vascular injury.[22,23] Using double-stranded DNA probes from the tissue factor promoter, electrophoretic mobility shift assays with extracts from mononuclear phagocytes showed an apparent increase in Egr DNA binding activity with no change in the DNA binding activity for Sp1 sites. The most striking data were obtained using consensus probes for Sp1 or Egr-1[20]; nuclear extracts showed no increase in the gel shift band with Sp1 in response to hypoxia. In contrast, similar experiments with the radiolabeled consensus Egr probe showed a gel shift band (supershifted with anti–Egr-1 antibody) in nuclear extracts from hypoxic cultures, although not in normoxic counterparts. Finally, transient transfection experiments were performed with constructs promoter-reporter gene constructs derived from the serum response region of the tissue factor promoter (Figure 2B). Increased expression of the luciferase reporter was seen with the wild-type construct, in which Sp1 and Egr-1 sites were intact, whereas mutational inactivation of Egr-1 sites blocked hypoxia-enhanced gene expression. Inactivation of Sp1 sites prevented both basal and hypoxia-induced expression of luciferase. Transfection studies with various promoter-luciferase reporter constructs showed that only constructs with an intact Egr site displayed increased expression in hypoxia.[20] These data emphasized the role of Egr-1 in hypoxia-modulated expression of tissue factor.

In vivo studies confirmed the biological importance of Egr-1–driven tissue factor transcription by hypoxia; homozygous Egr-1 null animals placed in a hypoxic environment showed a much smaller increment in tissue factor mRNA, with virtually no change in tissue factor antigen (Figure 2A) and virtually no fibrin deposition compared with their counterparts left in a normoxic environment.[20] Studies are in progress to trace the sequence of events increasing Egr-1 activation in hypoxia, and preliminary results indicate that oxygen deprivation enhances de novo Egr-1 synthesis and that this is driven by binding of ternary complex factor to ets/SRE sites in the Egr-1 promoter.[24]

Hypoxia-Associated Suppression of Fibrinolysis

The unusual presence of fibrin within the intravascular space in hypoxic mice, even with an apparently intact endothelial cell lining, suggested that in addition to enhanced procoagulant activity, oxygen deprivation might
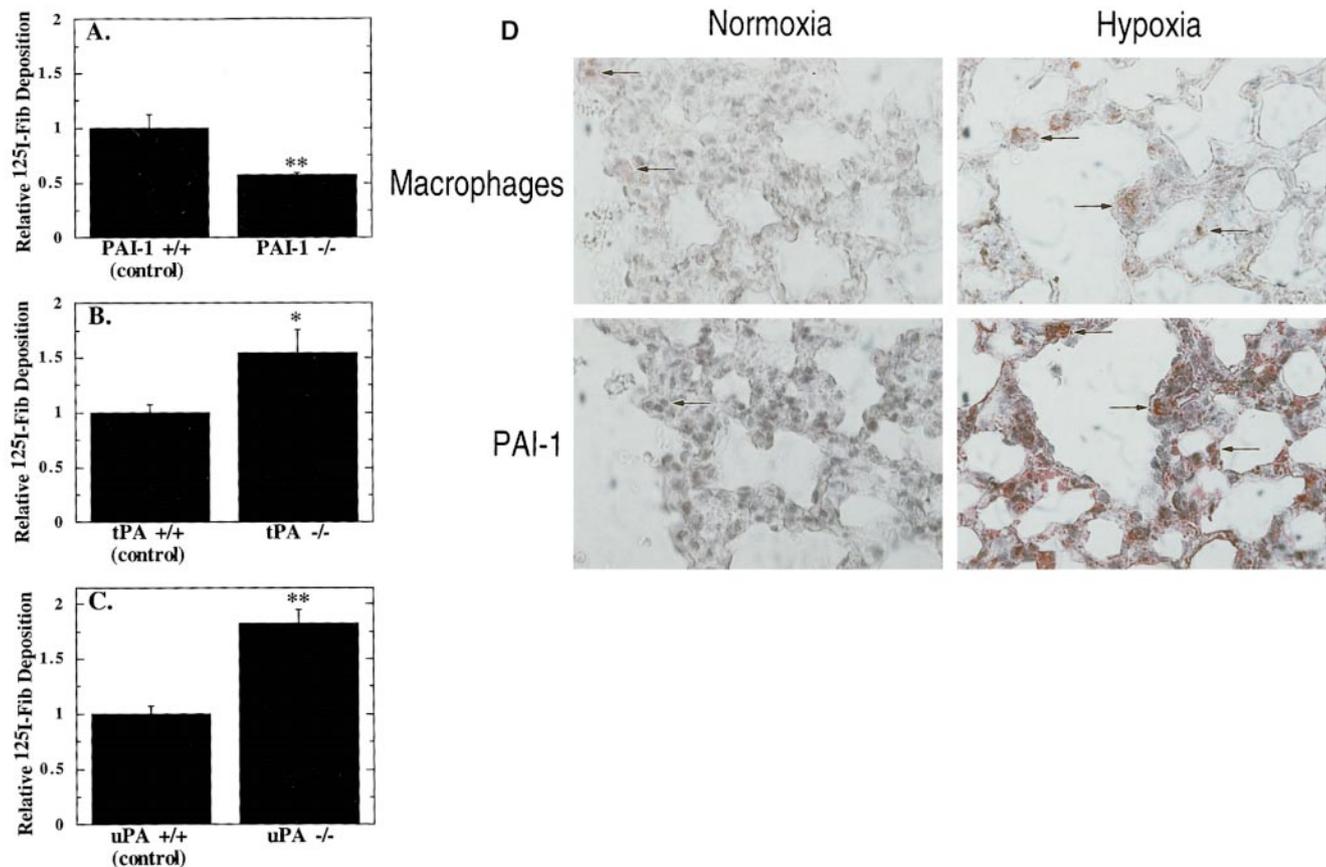
Figure 3. A to D, Role of fibrinolytic genes in hypoxia-induced fibrin formation. A to C, Effect of deletion of either the PAI-1 gene, the tPA gene, or the uPA gene on the hypoxia-induced accumulation of ^{125}I-fibrinogen/fibrin. At the onset of the 16-hour hypoxic period, ^{125}I-labeled murine fibrinogen was injected into control mice or mice that were homozygous null for either the PAI-1gene (A); the tPA gene (B); or the uPA gene (C). Relative deposition of ^{125}I fibrinogen/fibrin was calculated as the ratio of cpm/g tissue between hypoxic experimental and control animals, with a relative value of 1 representing approximately a 3.5-fold increase in the hypoxic/normoxic accumulation of ^{125}I-FIB; the modulating effect of a given gene on the hypoxia-induced accumulation of ^{125}I-FIB deposition is shown. Means±SEM are shown. *$P<0.05$ and **$P<0.01$ versus control. D, Immunohistochemical colocalization of PAI-1 antigen and mononuclear phagocytes in lung tissue from normoxic and hypoxic (PAI-1 +/+) mice. Tissue was obtained from mice under normoxic conditions (left panels) or after 16 hours exposure to normobaric hypoxia (FiO2 5% to 6%, right panels). Adjacent sections of paraffin-embedded tissue was immunostained for either PAI-1 (lower panels) or the mononuclear phagocyte marker F4/80[41] (upper panels). In hypoxic lung tissue, cells that stain as macrophages (arrows) stain strongly for the PAI-1 antigen (deep russet color). Magnification, ×400. Adapted from reference 26.

also be associated with diminished fibrin removal. Our first studies evaluating possible hypoxia-associated suppression of anticoagulant mechanisms focused on thrombomodulin.[25] However, hypoxia-mediate suppression of thrombomodulin expression was not observed until at least 24 hours subjecting cultures to oxygen deprivation,[25] and fibrin deposition had already occurred in the pulmonary vasculature by this time. In contrast, analysis of the fibrinolytic system has shown coordinated enhanced expression of plasminogen activator inhibitor-1 (PAI-1) and suppression of plasminogen activators.[26] Use of mice deficient in PAI-1, urokinase-type plasminogen activator (uPA), or tissue-type plasminogen activator (tPA) provided definitive evidence for the relevance of suppressed fibrinolysis to hypoxia-induced fibrin deposition. Whereas PAI-1 null mice showed no increase in fibrin formation on exposure to hypoxia, uPA or tPA deficient mice showed a strong potentiation of fibrin deposition (Figures 3A to 3C). Thus PAI-1 overexpression is likely to be an important factor preventing normally active fibrinolytic mechanisms

from removing fibrin deposits formed in hypoxemic vasculature. In concordance with these data, mice placed under conditions of normobaric hypoxia showed increased levels of PAI-1 transcripts in the lung and, at the protein level, increased PAI-1 antigen and activity levels compared with normoxic controls.[26] Immunocytochemical analysis of cells overexpressing PAI-1 in hypoxic lung pointed to an important contribution of mononuclear phagocytes[18] (Figure 3D). Further studies on hypoxic lung showed a time-dependent decrease in transcripts for tissue-type urokinase-type–plasminogen activators, lending support to the concept that net fibrinolytic activity of the hypoxic lung was decreased, thereby facilitating fibrin accumulation. The results of in vitro studies using transformed murine macrophages (RAW cells) as a model system for mononuclear phagocytes pointed to a strong effect of hypoxia on mononuclear phagocytes. RAW cells showed a 6-fold increase in PAI-1 transcription (by densitometric analysis of nuclear run blots) after placement in hypoxia. In addition to an increased rate of transcription of
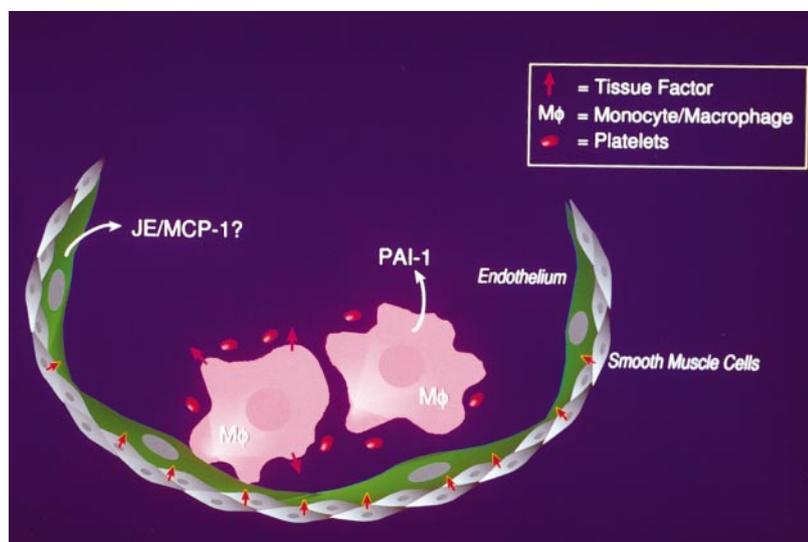
Figure 4. Schematic representation of events leading to fibrin accumulation within hypoxemic blood vessels.

the PAI-1 message, there was also an apparent increase in the stability of the PAI-1 message under conditions of hypoxia. These 2 observations, the increased rate of PAI-1 mRNA transcription and the increased stability of the PAI-1 mRNA after transcription, contributed to the marked increase in PAI-1 mRNA in hypoxic cells, which was observed by Northern analysis.[26]

Conclusion/Hypothesis

The adaptive response to hypoxia occurs on many levels. Transcriptional activation has been shown to involve hypoxia-inducible factor 1 (HIF-1), AP-1, NF-kB, and in the current work, Egr-1. The best studied of these is HIF-1, first identified during studies to elucidate the basis for erythropoietin expression stimulated in response to oxygen deprivation. Subsequently, HIF-1 has been shown to mediate other critical facets of the host response to hypoxia, including upregulation of glycolytic enzymes,[27–29] the non–insulin-dependent glucose transporter, the key angiogenic mediator vascular endothelial growth factor, and enzymes contributing to vasomotor control, such as nitric oxide and heme oxygenase type I. In addition, HIF-1 has an essential role in vasculogenesis, as illustrated by the phenotype of HIF-1 null mice; complete absence of HIF-1 alpha results in developmental arrest, neural tube and cardiovascular defects, and embryonic lethality by day 11.[30] Activation of AP-1 in response to reductive stress has been shown in vitro and results in expression of c-fos,[31–34] although assessment of the in vivo significance of these events has not yet been provided. Nuclear translocation of NF-kB has been observed by some groups,[35] although this may be cell-type specific, as others have not observed it.[31] Again, the physiological/pathophysiological implications of NF-kB activation for adaptive mechanisms to hypoxia have not yet been clarified in vivo.

Previous in vitro studies suggested a central role for Egr-1 in multiple cellular homeostatic events. Initially, Egr-1 was believed to play a role in macrophage differentiation,[36] although this has been called into question by a more recent report.[37] These possibilities were markedly drawn into focus after production of Egr-1 null mice, which appear to develop normally, and have only shown a phenotype after environmental stress. For example, female Egr-1 null mice are sterile

due to luteinizing hormone-β deficiency, an effect due to decreased transcription of the gene. Our studies have shown a central role for Egr-1 in monocyte expression of tissue factor after induction of hypoxia. In view of the multiple stimuli that have been shown to activate Egr-1, an immediate-early gene, it is unlikely that oxygen deprivation directly induces activation of Egr-1. Rather, hypoxic stress is likely to generate conditions leading to Egr-1 activation and triggering of downstream mechanisms, including tissue factor transcription-translation. In contrast to the apparently positive impact of HIF-1–mediated events on adaptation to hypoxia, the beneficial effects of tissue factor expression in monocytes and smooth muscle cells are less clear. Although local promotion of clotting would serve to isolate an ischemic area, the negative impact of vascular fibrin deposition eventuating in occlusive thrombosis can have obvious deleterious consequences. Egr-1 expression in hypoxia might be predicted to impact on cell-cell interactions through changes in the expression of cell adherence molecules and inflammatory mechanisms through effects on proinflammatory cytokines,[38] although definite proof of Egr-1 involvement in these events remains to be tested in Egr-1 null mice. Thus Egr-1-initiated mechanisms may subserve a quite different facet of the adaptive response to oxygen deprivation compared with that associated with HIF-1. However, recognition of a role for Egr-1 in potentially pathologic changes associated with hypoxia could point to novel approaches to limiting these events, ie, preventing activation of coagulation in hypoxia by suppressing Egr-1 activation rather than by interfering directly with the procoagulant mechanism.

The data presented in our brief review provide the outline of a pathway through which hypoxia triggers de novo expression of tissue factor in mononuclear phagocytes and smooth muscle cells (Figure 4). Lungs, which possess tissue-based (alveolar) macrophages in great abundance, are likely to recruit even more macrophages to intravascular locations through expression of the monocyte chemoattractant JE/monocyte chemoattractant protein-1 (MCP-1). Hypoxia-associated activation of Egr-1 causes transcription and subsequent cell surface expression of tissue factor in both vascular smooth muscle cells and macrophages, which can initiate the local procoagulant

response. Further amplification of the amount of fibrin deposited could be due to alterations in the balance of fibrinolytic mediators, such as may occur by increased expression of plasminogen activator inhibitor-1, thereby attenuating removal of vascular fibrin. It might be expected that any prothrombotic tendency, such as that in mice that have a targeted point mutation in the thrombomodulin gene,[16] would display an enhanced procoagulant response in this model. Thus patients with the Factor V Leiden mutation, those deficient in protein S, or those with other defects manifest as diminished endogenous antithrombotic activity would be vulnerable to enhanced fibrin deposition at hypoxemic sites in the vasculature.

This initial picture of hypoxia-associated triggering of the procoagulant pathway raises many questions. For example, by what mechanisms are monocytes attracted and retained in hypoxemic vasculature? In a previous study, we observed that hypoxia increased transcripts for the macrophage chemoattractant JE/MCP-1 in cultured endothelial cells[35] and that JE/MCP-1 antigen was increased in hypoxic lung (Lawson et al, unpublished observation, 1995). Thus it is possible that endothelial production of JE/MCP-1 draws in macrophages and serves as a cofactor in their activation and retention. JE/MCP-1 produced by hypoxic endothelium could also have an integral role in increased expression of tissue factor levels observed in smooth muscle cells[39,40] in hypoxemic vasculature. Aside from these questions of the basic biology of the procoagulant response, recognition of the relative vulnerability of the lung to hypoxemia-associated fibrin deposition in the mouse may also provide important insights. Although reasons for this are unclear, the combination of the lung's rich vasculature and the intensity of the vasoconstrictor response to hypoxia, in contrast to vasodilation in the systemic circulation, may serve to magnify activation of coagulation. Taken together, the series of events causing expression of tissue factor in the hypoxemic vasculature, especially in mononuclear phagocytes and smooth muscle cells, provides a new biologic context to consider mechanisms underlying and possible interventions to prevent pathologic thrombosis in settings of oxygen scarcity.

Acknowledgments

References

1. Virchow R. *Gessamelte Abhandlungen zur Wissenschaftlichen Medicin.* Frankfurt: A M Von Meidinger Sohn; 1856:525–520.
2. Hamer JD, Malone PC, Silver IA. The pO_2 in venous valve pockets: Its possible bearing on thrombogenesis. *Br J Surg.* 1981;68:166–170.
3. Malone P. A hypothesis concerning the aetiology of venous thrombosis. *Med Hypothesis.* 1991;5:189–201.
4. Malone PC, Morris CJ. The sequestration and margination of platelets and leukocytes in veins during conditions of hypokinetic and anaemic hypoxia: Potential significance in clinical postoperative venous thrombosis. *J Pathol.* 1978;125:119–129.
5. Millet J, Vaillot M, Theveniaux J, Brown NL. Experimental venous thrombosis induced by homologous serum in the rat. *Thromb Res.* 1996; 81:497–502.
6. Bara L, Bloch MF, Samama MM. A comparative study of recombinant hirudin and standard heparin in the Wessler model. *Thromb Res.* 1992; 68:167–174.
7. Folkman J. Seminars in Medicine of the Beth Israel Hospital, Boston. Clinical applications of research on angiogenesis. *N Engl J Med.* 1995; 333:1757–1763.
8. Zhang Y, Deng Y, Wendt T, Liliensiek B, Bierhaus A, Greten J, He W, Hach-Wunderle V, Waldherr R, Ziegler R, Mannel D, Stern DM, Nawroth PP. Intravenous somatic gene transfer with antisense tissue factor restores blood flow by reducing tumor necrosis factor-induced tissue factor expression and fibrin deposition in mouse meth-A sarcoma. *J Clin Invest.* 1996;97:2213–2224.
9. Hamer JD, Malone PC. Experimental deep venous thrombogenesis by a non-invasive method. *Ann R Coll Surg Engl.* 1984;66:416–419.
10. Lewis DM, Bradwell AR, Shore AC, Beaman M, Tooke JE. Capillary filtration coefficient and urinary albumin leak at altitude. *Eur J Clin Invest.* 1997;27:64–68.
11. Hultgren HN. High-altitude pulmonary edema: Current concepts. *Annu Rev Med.* 1996;47:267–284.
12. Severinghaus JW. Hypothetical roles of angiogenesis, osmotic swelling, and ischemia in high-altitude cerebral edema. *J Appl Physiol.* 1995;79: 375–379.
13. Johnson TS, Rock PB. Current concepts. Acute mountain sickness. *N Engl J Med.* 1988;319:841–845.
14. Krakauer J. *Into Thin Air: A Personal Account of the Mount Everest Disaster.* New York: Anchor Books; 1997.
15. Pinsky DJ, Naka Y, Liao H, Oz MC, Wagner DD, Mayadas TN, Johnson RC, Hynes RO, Heath M, Lawson CA, Stern DM. Hypoxia-induced exocytosis of endothelial cell Weibel-Palade bodies. A mechanism for rapid neutrophil recruitment after cardiac preservation. *J Clin Invest.* 1996;97:493–500.
16. Weiler-Guettler H, Christie PD, Beeler DL, Healy AM, Hancock WW, Rayburn H, Edelberg JM, Rosenberg RD. A targeted point mutation in thrombomodulin generates viable mice with a prethrombotic state. *J Clin Invest.* 1998;101:1983–1991.
17. Healy AM, Hancock WW, Christie PD, Rayburn HB, Rosenberg RD. Intravascular coagulation activation in a murine model of thrombomodulin deficiency: Effects of lesion size, age, and hypoxia on fibrin deposition. *Blood.* 1998;92:4188–4197.
18. Lawson CA, Yan S-D, Yan S-F, Liao H, Chen G, Sobel J, Kisiel W, Stern DM, Pinsky DJ. Monocytes and tissue factor promote thrombosis in a murine model of oxygen deprivation. *J Clin Invest.* 1997;99:1729–1738.
19. Shreeniwas R, Koga S, Karakurum M, Pinsky D, Kaiser E, Brett J, Wolitzky BA, Norton C, Plocinski J, Benjamin W, Burns DK, Goldstein A, Stern D. Hypoxia-mediated induction of endothelial cell interleukin-1a. An autocrine mechanism promoting expression of leukocyte adhesion molecules on the vessel surface. *J Clin Invest.* 1992;90: 2333–2339.
20. Yan S-F, Zou YS, Gao Y, Zhai C, Mackman N, Lee SL, Milbrandt J, Pinsky D, Kisiel W, Stern D. Tissue factor transcription driven by Egr-1 is a critical mechanism of murine pulmonary fibrin deposition in hypoxia. *Proc Natl Acad Sci U S A.* 1998;95:8298–8303.
21. Parry GC, Erlich JH, Carmeliet P, Luther T, Mackman N. Low levels of tissue factor are compatible with development and hemostasis in mice. *J Clin Invest.* 1998;101:560–569.
22. Khachigian LM, Lindner V, Williams AJ, Collins T. Egr-1-induced endothelial gene expression: A common theme in vascular injury. *Science.* 1996;271:1427–1431.
23. Khachigian LM, Williams AJ, Collins T. Interplay of Sp1 and Egr-1 in the proximal platelet-derived growth factor A-chain promoter in cultured vascular endothelial cells. *J Biol Chem.* 1995;270:27679–27686.
24. Yan S-F, Gao Y, Zhai C, Stern DM. Hypoxia-triggered signal transduction mechanisms underlying induction of tissue factor. *Circulation.* 1998;98:I-40.
25. Ogawa S, Shreeniwas R, Brett J, Clauss M, Furie M, Stern DM. The effect of hypoxia on capillary endothelial cell function: modulation of barrier and coagulant function. *Br J Haematol.* 1990;75:517–524.
26. Pinsky DJ, Liao H, Lawson CA, Yan S-F, Chen J, Carmeliet P, Loskutoff DJ, Stern DM. Coordinated induction of plasminogen activator inhibitor-1 (PAI-1) and inhibition of plasminogen activator gene expression by hypoxia promotes pulmonary vascular fibrin deposition. *J Clin Invest.* 1998;102:919–928.
27. Firth JD, Ebert BL, Ratcliffe PJ. Hypoxic regulation of lactate dehydrogenase A. Interaction between hypoxia-inducible factor 1 and cAMP response elements. *J Biol Chem.* 1995;270:21021–21027.

28. Semenza GL, Roth PH, Fang HM, Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J Biol Chem*. 1994;269:23757–23763.

29. Firth JD, Ebert BL, Pugh CW, Ratcliffe PJ. Oxygen-regulated control elements in the phosphoglycerate kinase 1 and lactate dehydrogenase A genes: Similarities with the erythropoietin 3′ enhancer. *Proc Natl Acad Sci U S A*. 1994;91:6496–6500.

30. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gearhart JD, Lawler AM, Yu AY, Semenza GL. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev*. 1998;12:149–162.

31. Rupec RA, Baeuerle PA. The genomic response of tumor cells to hypoxia and reoxygenation. Differential activation of transcription factors AP-1 and NF-kappa B. *Eur J Biochem*. 1995;234:632–640.

32. Kerppola TK, Curran T. Maf and Nrl can bind to AP-1 sites and form heterodimers with Fos and Jun. *Oncogene*. 1994;9:675–684.

33. Okuno H, Akahori A, Sato H, Xanthoudakis S, Curran T, Iba H. Escape from redox regulation enhances the transforming activity of Fos. *Oncogene*. 1993;8:695–701.

34. Abate C, Curran T. Encounters with Fos and Jun on the road to AP-1. *Semin Cancer Biol*. 1990;1:19–26.

35. Karakurum M, Shreeniwas R, Chen J, Pinsky D, Yan SD, Anderson M, Sunouchi K, Major J, Hamilton T, Kuwabara K, et al. Hypoxic induction of interleukin-8 gene expression in human endothelial cells. *J Clin Invest*. 1994;93:1564–1570.

36. Krishnaraju K, Nguyen HQ, Liebermann DA, Hoffman B. The zinc finger transcription factor Egr-1 potentiates macrophage differentiation of hematopoietic cells. *Mol Cell Biol*. 1995;15:5499–5507.

37. Lee SL, Wang Y, Milbrandt J. Unimpaired macrophage differentiation and activation in mice lacking the zinc finger transplantation factor NGFI-A (EGR1). *Mol Cell Biol*. 1996;16:4566–4572.

38. Khachigian LM, Collins T. Inducible expression of Egr-1-dependent genes. A paradigm of transcriptional activation in vascular endothelium. *Circ Res*. 1997;81:457–461.

39. Schecter AD, Giesen PL, Taby O, Rosenfield CL, Rossikhina M, Fyfe BSK, Fallon JT, Nemerson Y, Taubman MB. Tissue factor expression in human arterial smooth muscle cells. TF is present in three cellular pools after growth factor stimulation. *J Clin Invest*. 1997;100:2276–2285.

40. Schecter AD, Rollins BJ, Zhang YJ, Charo IF, Fallon JT, Rossikhina MG, Nemerson Y, Taubman MB. Tissue factor is induced by monocyte chemoattractant protein-1 in human aortic smooth muscle and THP-1 cells. *J Biol Chem*. 1997;272:28568–28573.

41. Lee S-H, Starky PM, Gordon S. Quantitative analysis of total macrophage content in adult mouse tissues. *J Exp Med*. 1985;161:475–489.

Arteriosclerosis, Thrombosis, and Vascular Biology

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