Blood Vessel Wall and Thrombosis

Volume I

Editor
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PREFACE

Thrombosis is one of the most serious medical problems of our time. The mechanisms by which thrombosis develops have been studied extensively for many decades. Attention has always been centered on the role of the blood vessel wall and its interaction with the platelets, the coagulation factors, and their inhibitors. More details are known about the intricate interactions among the multiple factors operative in thrombus formation and lysis. We have, as yet, been unsuccessful in finding the crucial determinant for effective prevention and treatment of this number one killer in the developed world.

The purpose of these multiauthor volumes is to present the latest, authoritative information on the regulatory role of the blood vessel wall on the process of hemostasis in health and disease. From this point of view, blood vessel wall and circulating blood can be considered as an indivisible system. Such an interpretation is also supported by the fact that pathological changes in hemostasis are not only involved in intravascular thrombus formation and bleeding from the injured vessel wall, but also contribute to various pathological injuries of the vessel wall e.g., in atherosclerosis, hypertension, diabetic angiopathy, immunopathology alterations, and tumor metastasis.

The regulation of hemostatic interactions involves a delicate balance of pro- and anticoagulant forces. Our knowledge of the structure and function of the blood vessel wall as well as of cellular and humoral factors of blood involved in hemostasis has logarithmically increased during the past few years. For specialists and nonspecialists these two volumes provide an easier survey of the vast range of the accumulated knowledge in this fascinating field of biomedicine.

Susan R. Hollán, M.D.
May 1986

THE EDITOR

Raymund Machovich received his M.D. degree from Semmelweis University Medical School (Budapest) in 1961 and subsequently specialized in internal medicine. He obtained the Ph.D. degree in 1972 for his work in the laboratory of Professor B. F. Straub on the biosynthesis of pancreatic enzymes. His research on thrombosis and hemostasis began in 1973, and he was awarded the D.Sc. degree in 1978 for his thesis, "Regulation of Thrombin Inactivation".

He spent 1 year (1970) in the laboratory of Professor E. Knox (Harvard Medical School) working with Dr. O. Greengard on the activation of thymidine kinase in tumor cells. Eight years later, he received the Canadian Heart Foundation Award and attended McMaster University for 1 year studying the role of heparin in hemostasis. In 1982, he spent 6 months at the Centre National de Transfusion Sanguine in Paris.

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Dr. Machovich has published about 70 papers and has edited a two-volume book *The Thrombin*, (CRC Press, 1984). He was an editor of *Thrombosis Research* from 1981 to 1985 and is currently a member of the editorial board of *Excerpta Medica*.

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Chapter 1

HEMOSTASIS

Raymund Machovich

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I. INTRODUCTION

The term hemostasis refers, on the one hand, to the ability of blood to arrest bleeding from an injured part of the blood vessel wall, and on the other hand, to prevent thrombus formation inside the blood circulation. The balance between these two extreme states is

rigorously controlled, i.e., various functions of endothelial cells, smooth muscle cells, liver cells, and platelets, as well as several molecular components of the blood coagulation-fibrinolytic system are involved in the regulation of hemostasis. Any disorganization in the processes of hemostasis may lead to hemorrhagic or thromboembolic diseases. Thrombus formation, however, is one of the most serious medical problems of our time.

The crucial questions in thrombosis are the following: (1) What is/are the trigger(s) causing initiation of plug formation in a nonappropriate part of the blood vessel wall and (2) how does a failure of stop-signal(s) for blood coagulation develop? Although several factors, both cellular and molecular, may participate in thrombus formation, one of the most essential components is certainly the blood vessel wall, especially the endothelial cells.

The purpose of this chapter is to give an overall view on hemostasis, to show the inherent features among the cellular and molecular components of it, and to discuss the role of blood vessel wall in hemostasis. Details about the cellular and molecular factors and their morphological and functional changes under pathological conditions are described in the appropriate chapters.

II. STRUCTURE AND FUNCTION OF BLOOD VESSEL WALL

Almost all types of human cells communicate with the external environment indirectly. Without blood vessels they are not able to exist and to accomplish their biological roles. Endothelial cells supply and, at the same time, limit, hormones, nutrients, oxygen, etc. for the metabolism of other cells. Even the regulation of the various cell functions occurs through the endothelium. Its most important roles are to maintain blood pressure and to direct blood-tissue exchange via both permeability and barrier functions. These functions, however, depend on the thromboresistant nature of the endothelial cells.

A. Morphology of Artery, Vein, and Capillary

The size of blood vessels varies according to their functions. When the inner diameter of a vessel is over or under 100 μm, it is called macro- or microvasculature, respectively.[1] The microvasculature includes the capillary, which is less than 7 μm in the diameter of lumen.

The structure of vessel walls, the macrovasculature in general terms, is described as intima, media, and adventitia.[1,2] The innermost stratum of the vessel wall consists of the intima, which is built up from endothelial monolayer on the subendothelial zone. The subendothelial zone is composed of basement membrane, elastin, and microfibrils. The intima is divided from media by the internal elastic lamina. The media is organized mainly of smooth muscle cells. The outermost layer of the blood vessel walls, the adventitia, is separated from the media by the external elastic lamina. The adventitia contains fibroblasts, extracellular connective tissues, small blood vessels, lymph vessels, and nerves. Artery, vein, and capillary differ not only in size, but also in the components and structure of blood vessel wall (Figure 1 and Figure 2).

Arteries can be found from the heart to the capillaries with a steadily diminished caliber. Since the total cross-sectional area of the arterial system expands to the capillaries, the rate of blood flow decreases also continuously.[3]

Blood is carried from the capillaries by the veins, with increasing caliber, toward the heart, and the blood flow is slow in veins compared to arterial flow. As for the histology of veins, in general, the media is poorly developed (or even absent in smaller veins), whereas the adventitia is several times as thick as the media in veins.

Capillaries, the greatest surface of the vasculature, are the principal vessels of the entire circulatory system. Practically, they consist of endothelial cell monolayer and basal lamina (basement membrane) with a lumen diameter of 7 μm or less. The extracellular connective

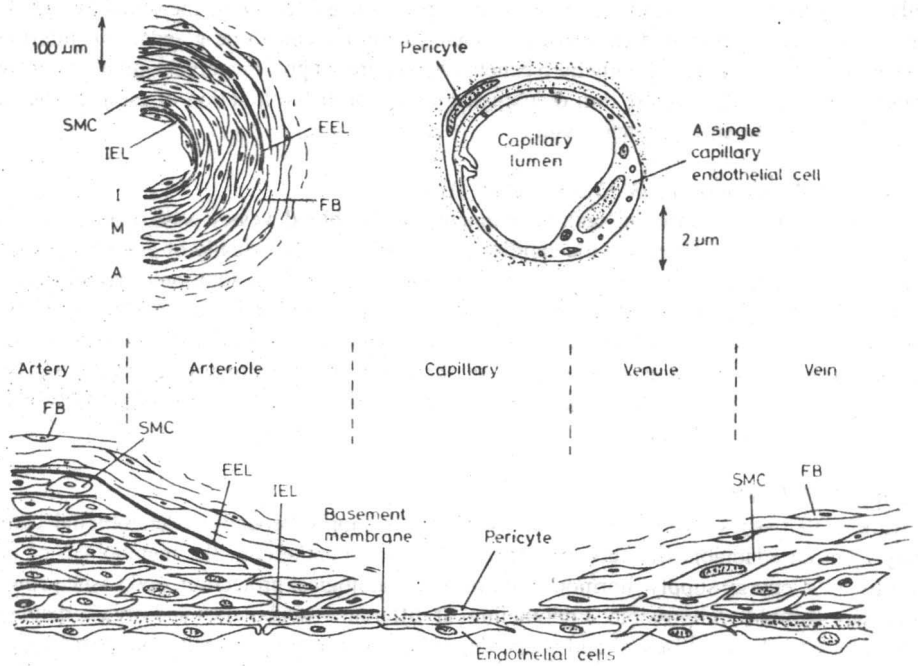

FIGURE 1. Schematic diagram of blood vessel walls. The main structure components of arteries and veins and their relative distributions are symbolized. Cross-segment of an arteriole and capillary are shown separately with indication of approximate sizes. Abbreviations: I, intima; M, media; A, adventitia; IEL, internal elastic lamina; EEL, external elastic lamina; SMC, smooth muscle cells; FB, fibroblast.

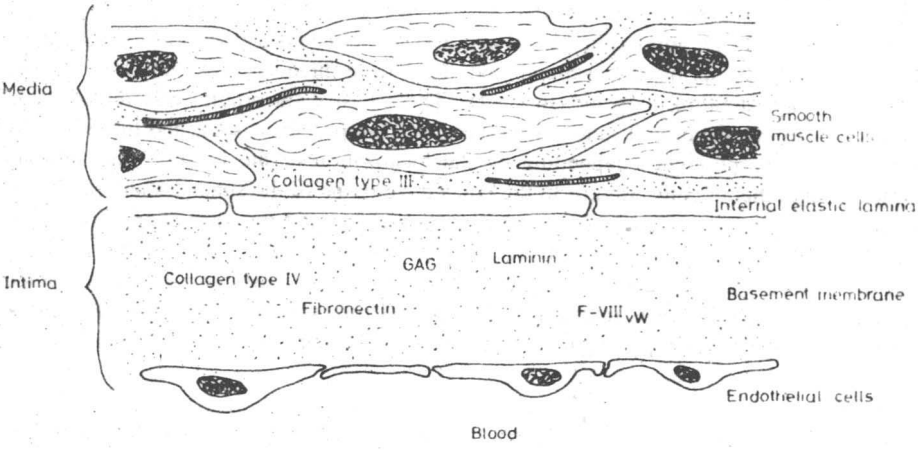

FIGURE 2. Structure of intima. Abbreviations used: GAG, glycosaminoglycans of vessel wall, mainly heparan sulfate, dermatan sulfate, chondroitin-4 sulfate, and chondroitin-6 sulfate; F-VIII$_{vW}$, F-VIII$_{vW}$.

tissue, produced primarily by endothelial cells, refers to collagen type IV, glycosaminoglycans, Factor VIII$_{von\ Willebrand}$ (F-VIII$_{vW}$); and glycoproteins such as fibronectin, laminin, and entactin[4] (Figure 2).

Two major types of capillaries can be distinguished: continuous and fenestrated.[5] The continuous capillaries of skeletal muscle, connective tissue, and CNS contain endothelial

cells with a thick area around the nucleus and thin cytoplasm covering the remainder of the luminal surface. Fenestrated capillaries of renal glomeruli, endocrine glands, and intestine have extremely attenuated endothelium penetrated by pores approximately 90 nm in diameter. These fenestrae are closed by thin diaphragms with a probable function of rapid exchange of molecules between blood and tissue.

1. Cellular Components of the Blood Vessel Wall

The occurrence of different cellular elements of the blood vessel wall depends on its function in blood circulation. In the capillary, the wall consists virtually of endothelial cells with a few pericytes. Large vessels, like arteries and veins, contain additional types of cells. In the subendothelial space of these vessel walls, mast cells can be found. The media is mainly a build-up of smooth muscle cells, whereas adventitia consists of fibroblasts, adipocytes, and rarely of some mast cells. The structural and functional integrity of a given vessel wall depends upon the integrity of its cellular components and of the extracellular matrix synthesized by these cells.[6]

a. Endothelial Cells

Almost all tissues live on blood supply, and this supply depends on endothelial cells. They form an adaptable life-support system;[7] without endothelium, the metabolism of the various cells, energy supply, hormonal control, immunological protection, and tissue growth and repair would be impossible. Endothelium at different vascular sites and organs displays individual peculiarities[8] in size and shape depending on the various predominant biological functions. Indeed, endothelial cells are continuously adapting to the composition of blood, to the pulsatile hydrostatic pressure, to the biologically active materials, etc. They also change according to their main functions, i.e., oxygen transport, gas and water exchange, metabolic equilibrium, and protein production for or removal from blood circulation. No matter how their roles are specialized, a common task for all endothelial cells is the thromboresistance under normal conditions. Since these are the stipulations of blood circulation, endothelium is discussed in this chapter, first from this viewpoint.

Endothelial cells are approximately 40 μm in diameter and 3 μm thick, forming a monolayer over every vessel basement membrane. They have three surfaces: cohesive, adhesive (abluminal), and nonthrombogenic (luminal).[5] The cohesive site adjoins endothelial cells to one another by means of cell junctions serving for transport processes, and these junctions show various morphologies depending on the localization of the vessel.[9] The abluminal surface of endothelial cells adheres to the connective tissue of the subendothelial zone. The surface that faces the lumen is the nonthrombogenic one and this thromboresistant nature of the membrane is further supported by factors synthesized and secreted by these cells.

From a morphological aspect, endothelial cells, like other types of cells, have nucleus, mitochondria, microtubules, microfilaments, pinocytotic vesicles, etc.[10] In addition, endothelial cells contain unique organelles, vesicles, and components characteristic for these cells:

1. Weibel-Palade body,[11] a rod-shaped tubular organelle with ca. 3-μm length and 0.1-μm diameter, derived from the Golgi apparatus,[12] probably serving as a storage vesicle for F-VIII$_{vw}$
2. Small, approximately 80-nm vesicles[10] for transport by endocytosis at the luminal surface and by exocytosis at the external surface or vice versa
3. F-VIII$_{vw}$ antigen
4. Type IV procollagen
5. Angiotensin converting enzyme
6. A characteristic glycocalyx

These can be considered as characteristic components of the endothelial cells.

b. Smooth Muscle Cells

The media of the blood vessel wall consists of elongated smooth muscle cells having a single nucleus and containing thick as well as thin filaments aligned with the long axis of the cells.[13]

The main functions of these cells are (1) contraction to control blood flow, (2) synthesis of various connective tissue elements, and (3) lipid uptake, synthesis, and turnover.[13,14]

1. Although the mechanism of contraction of smooth muscle cells of blood vessel is fundamentally the same as in skeletal muscle,* it differs functionally in two aspects: (a) the level of ATP-ase activity is lower in smooth muscle than in skeletal muscle cells and is subjected to a direct calcium regulation and (b) the myosin of smooth muscle cells is able to interact with actin molecule when its light chain is phosphorylated.[16] The cascade of reactions, by which the contraction of smooth muscle cells is activated, is triggered by the increase of Ca^{2+} level inside the cell. The effect of Ca^{2+} is mediated by calmodulin. The calmodulin-Ca^{2+} complex formed activates myosin light-chain kinase and as a consequence myosin is phosphorylated and becomes active.[17] The enzyme reaction that follows Ca^{2+} binding to calmodulin is relatively slow to activate smooth muscle cell contraction. As a result, smooth muscle cells rarely undergo quick contraction, but enable the vessel wall to maintain a constant tension with great efficiency.
2. Smooth muscle cells are able to synthesize and secrete collagen and elastin as well as glycosaminoglycans.
3. Lipid synthesis is stimulated in smooth muscle cells of blood vessels when the cell medium contains lipid-free serum, whereas it is inhibited when exogeneous lipid is present.[18] Human arterial smooth muscle cells preferentially bind and take up low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL). This type of cells also incorporates oleic and linoleic acids and participates in the synthesis and removal of different cholesterol esters.[19]

c. Other Cells

While intima contains endothelial cells and media contains smooth muscle cells, the main cellular components of adventitia are fibroblasts and mast cells.[5,20] Fibroblasts are useful for the synthesis of collagen type I and III, fibronectin, glycosaminoglycans, lipids, elastin, and molecular components with a procoagulant nature. Furthermore, fibroblasts, together with smooth muscle cells, are able to accumulate LDL and cholesterol, forming "foam cells" in the arterial wall, thereby playing a role in atherogenesis.[21] Mast cells, on the other hand, can produce and store heparin, histamine, serotonin, and hyaluronic acid.[22] The secretion of heparin may contribute to the nonthrombogenic character of the blood vessel wall.

2. Extracellular Components of the Blood Vessel Wall

Cells of the blood vessel wall synthesize and secrete several kinds of macromolecules (Table 1**) forming extracellular matrix, which in turn determines the mechanical properties of the blood vessel wall, contributes to the microenvironment of the cells, and influences

* Muscle contraction is a result of sliding of actin against myosin filaments. The head part of myosin undergoes an ATP dependent cycle by attaching to actin and detaching from that. The conformational change pulls one filament against the next. These functions are regulated by calmodulin and Ca^{2+}. When the Ca^{2+} level increases in response to neurogen stimulus, the myosin head is allowed to interact with actin, resulting in contraction.[15]

** A brief summary of the main components of hemostasis can be found in Table 1.

Table 1
SOME CELLULAR AND MOLECULAR COMPONENTS OF BLOOD AND BLOOD VESSEL WALL PARTICIPATING IN HEMOSTASIS

Origin	Component	Structure	Functions
Vessel wall (endothelial cells, smooth muscle cells, fibroblasts)	Collagen (several types)	Three-chain glycoprotein, with M_r approximately 300,000 daltons	Vascular structure, procoagulant
	Elastin	Insoluble, nonpolar amino acid rich, cross-linked protein	A network giving flexibility to vessel walls
	Glycosaminoglycans (GAGs)	Linear chain polysaccharides with various negative charges, with heterogeneous M_r	Structural part of vessels, transport processes, anticoagulant
	Lipoproteins LDL, HDL, VLDL	Complexes of proteins and lipids with various density and with heterogenous M_r, M_r from 300,000 up to 10^7 daltons	Transport for triglyceride and cholersterol, complex formation with GAG in vessel wall
	Fibronectin	A 2-chain glycoprotein with M_r 440,000 daltons	Cell-cell interactions
	Laminin	A 2-chain glycoprotein with M_r 850,000 daltons	Basement membrane structure for cellular-molecular interactions
	F-$VIII_{vw}$	Multimeric structure of a glycoprotein with M_r 220,000 daltons	Cell-cell interaction mainly for platelets and endothelial cells
	Tissue-factor	1-chain glycoprotein, M_r 52,000 daltons	Procoagulant in a complex of phospholipids
	Thrombomodulin	Insoluble membrane protein of endothelial cell, M_r 74,000 daltons	Cofactor for protein C activation, thrombin receptor
	Angiotensin converting enzyme	Endothelial cell membrane enzyme	Conversion of angiotensin I to II, inactivation of bradykinin
	Ecto-ADP-ase, adenosine	Endothelial cell membrane enzyme system	Adenosine formation from ADP (ATP, AMP) inhibition of platelet aggregation
	Prostacyclin (PGI_2)	Intermediate of arachidonic acid metabolism	Inhibitor of platelet aggregation
	Endothelium-derived relaxant factor	Unknown	Vascular relaxation