Review Article

Changing concepts of clotting mechanism of blood: From cascade model to cell-based model

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Abstract

The knowledge of the process of clotting of blood dates back to the period of Hippocrates and Aristotle. After many centuries of accumulation of knowledge, the theory of Macfarlane and Davies, which laid more importance on the protein coagulation factors in controlling the process of coagulation with cells only serving as substrates has been held as the most acceptable doctrine. The cascade model proposed by them consists of two different pathways ending in a common pathway, with less interaction between the two. The new model, called the cell-based model of coagulation, proposes highly interconnected reactions occurring over three overlapping phases called initiation phase, amplification phase, and propagation phase, with cells playing central and controlling role over the protein coagulation factors.

Key words

Cell-based model, Initial phase, Amplification phase, Propagation phase.

Introduction

Hemostasis is the physiological process that evolved to prevent blood loss due to injury, thus protecting the organism from potential death. It constitutes a small part of the bigger process that sustains life, the homeostasis, which helps to maintain the constancy of the internal environment despite continuous changes inflicted upon it. Hemostasis, which starts immediately after injury, is not simply concerned with the prevention of blood loss, it has other roles like, prevention of redundant clotting in normal tissues and lysis of clot to restore blood flow to the occluded tissue, end up as an overlapping

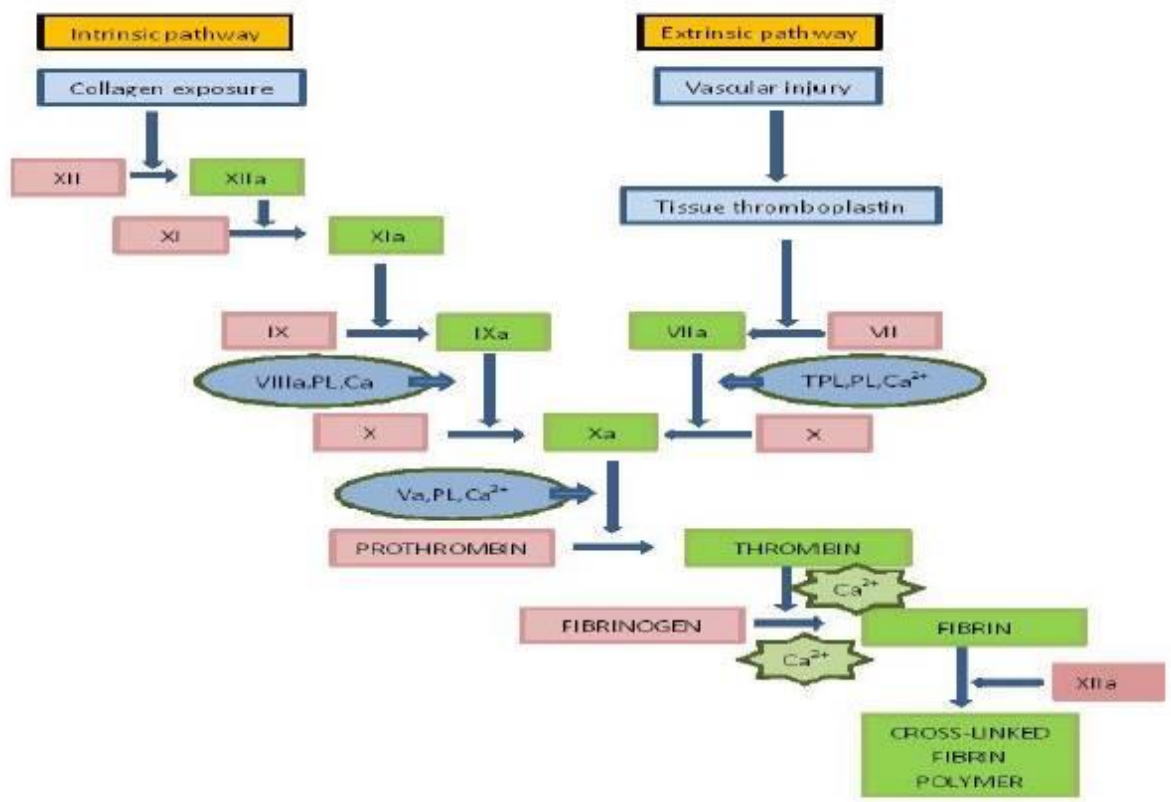
phase with the repair of the injured tissue [1]. So the hemostatic process occurs spanning over days to weeks to accomplish its goal. Three sequential processes are involved in this tightly regulated and balanced process. They are primary hemostasis, secondary hemostasis, and tertiary hemostasis. The primary hemostasis starts with exposure of the subendothelial collagen to the von Willebrand factor (vWF) and platelets to form loose, unstable platelet plug over the injured site [2]. In secondary hemostasis, enzymatic activation of a cascade of reaction causes the formation of active clotting factors from inactive clotting factors happens over the activated platelets and injured endothelium, forming fibrin meshwork that entraps RBCs and WBCs, strengthening the loose, unstable platelet plug formed during the primary hemostasis [3]. The platelet plug is converted into a definitive clot during secondary hemostasis. Tertiary hemostasis activates the fibrinolytic system to lyse the clot [4] so that the normal architecture of the endothelium is restored and occluded vessel lumen is re-canalized to allow normal circulation to resume through the affected tissues. In this review article, the authors discuss the timeline of the development of theories of coagulation, the widely accepted cascade model of coagulation, some of its lacunas, and the emerging concept of cell-based coagulation.

Historical perspective

The notion that blood becomes solidified when it flows out of blood vessels was observed way back during the periods of Hippocrates, Aristotle, Celsius, and Galen. Though they made detailed observations of various internal and external bleeding tendencies, they could only conjecture that contact of blood with atmospheric air is the reason for bleeding to stop [5]. This theory was in vogue until two thousand years later, in the early 1720s, when a French surgeon, Jean-Louis Petit, made the critical observation that bleeding from the amputated limb was arrested due to small clots in the damaged blood vessels, associating for the first time in the history of medicine the relation between blood coagulation and hemostasis [6]. Momentum was gained for the concept of the importance of blood coagulation for hemostasis when Swiss physician Friedrich Hopff, in the 1820s, made the seminal observation that hypercoagulability is associated with a bleeding disorder, when he studied males with a familial bleeding disorder, now recognized as hemophilia, an X-chromosome linked bleeding disorder [5]. In 1882, Giulio Bizzozero, an Italian professor of general pathology, identified platelets as new particles apart from RBCs and WBCs in the blood that played a similar role in hemostasis as well as thrombosis [7]. During the nineteenth century, the inter-relationship between plasma clotting factors, platelets, and endothelium was brought out by German pathologist, Rudolph Virchow in 1856, when he proved that clots in the veins of the legs travelled to the lungs and caused pulmonary embolism and clots form whenever there is disturbance exist in the form of hypercoagulability, stasis, and injury to the endothelium, commonly referred to as Virchow's triad [8]. In 1905, Paul Oskar Morawitz, a German Internist and Physiologist, described four coagulation factors viz fibrinogen (I), prothrombin (II), thrombokinase (III) and calcium (IV) involved in the process of clotting of blood. He proposed that prothrombin is converted into thrombin by thrombokinase in the presence of calcium and the formed thrombin converted fibrinogen into fibrin enabling the formation of fibrin clot [9]. The understanding biochemical process of blood coagulation started when Paul Owren, a Norwegian physician, studied a woman whose bleeding diathesis could not be explained by the four-factor concept of blood coagulation, in 1947 [10]. Following his discovery, many coagulation factors were discovered between the period of 1940s and 1950s in various parts of the world and an international committee was formed in 1954 to establish a common nomenclature for the new clotting factors to avoid misunderstanding of results of a blood test of bleeding disorders. The international committee which met in Rome, in 1958, agreed to use Roman numerals for

identifying various clotting factors and the numerals given to the factors were according to the order of their discovery and not related to their level of importance in the clotting cascade. The detailed steps of coagulation were described as a series of reaction in which activation of one clotting factor leads to the activation of next one in the series and so on until thrombin is formed, by two independent groups of biochemists in 1960s and one group headed by Macfarlane proposed the cascade model of blood coagulation in the journal Nature in the year 1964, and another group lead by Davie and Ratnoff advanced the waterfall model of blood coagulation in the journal Science in the same year [11, 12].

Figure - 1: Cascade model of blood coagulation depicted with an intrinsic pathway, extrinsic pathway and common pathways [30] - diagram redrawn from the source



*TPL – Tissue phospholipid, PL – Platelet phospholipid

The traditional coagulation cascade model

This model suggested that blood coagulation is initiated either intrinsically when blood is exposed to activated contact factor in the blood, or extrinsically when tissue factor released from the injured tissue initiates the process [13, 14]. Both pathways culminate with the production of a prothrombin activator, which starts the common pathway and finally ends with the production of fibrin meshwork [15]. The intrinsic pathway is initiated when factor XII comes in contact with a negatively charged surface like glass or membrane of the activated platelets, which converts factor XII into XIIa [16, 17] ('a' behind the Roman numeral indicates activated form of the clotting factor). A protein called High molecular weight kininogen (HMWK) acts as a cofactor and helps factor XII to get anchored to the charged surface of platelets [18-20]. The activation of factor XII in this way is slow in reaction. But another cofactor called kallikrein, formed from prekallikrein by the action of XIIa, once formed activates the further conversion of factor XII into XIIa through positive feedback mechanism [21, 22, 23-26], resulting in an explosive release of enormous quantities of XIIa

into the blood. The next in series in the cascade, factor XI is converted into factor XIa which in turn converts factor IX into IXa. This activated factor (IXa), with the help of another two downstream factors, thrombin, and Xa, activate factor VIII to form VIIa. A trimolecular complex called Tenase is formed when factor IXa combines with VIIa in the presence of calcium and phospholipid. This enzyme complex converts factor X to Xa. The extrinsic pathway involves the interaction of between tissue factor (also called as factor III or tissue thromboplastin) and factor VII [27, 28]. The tissue factor is constitutively expressed as an integral membrane protein in the nonvascular tissues, functions as a receptor to the plasma protein factor VII [29, 30]. Any tissue injury exposes factor VII to tissue factor, which converts it to factor VIIa. Once activated, the factor VIIa forms a complex with tissue factor (similar to Tenase complex of the intrinsic pathway) that activates factor X to factor Xa (**Figure – 1**).

Once factor Xa is formed, either through an intrinsic or extrinsic pathway or both, common pathway proceeds to form Prothrombinase complex which is an enzyme complex formed when factor Xa combines with factor Va in the presence of tissue phospholipid and calcium [31, 32]. This complex converts prothrombin to thrombin which in turn converts a soluble plasma protein, fibrinogen to fibrin monomer which is still soluble. The monomers polymerize to form long chains of fibrin thread. These fibrin threads are interlinked by covalent cross-linking by factor XIIIa, which is formed from factor XIII by the action of thrombin. The cross-linked polymers of fibrin thread form a meshwork called stable fibrin which is insoluble in the plasma [32]. For diagnosing bleeding disorders due to intrinsic pathway abnormalities, activated partial thromboplastin time (aPTT) is used [33]. For diagnosing bleeding disorders due to extrinsic pathway abnormalities, prothrombin time (PT) is used [33]. For diagnosing bleeding disorders due to common pathway abnormalities, both activated partial thromboplastin time and prothrombin time can be used [34].

Constraints in traditional coagulation cascade model

The traditional model is depicted as two separate independent pathways that can function independently of each other with very little interaction between them which is not true in vivo. The scope for the interaction of coagulation factors with cells like endothelial cells and platelets is highly sabotaged. Segregating the pathways involved in coagulation helps us in identifying the deficient clotting factors when laboratory tests are done to assess the risk of bleeding or thrombosis, as aPTT has done for diagnosing intrinsic pathway disorders and PT done for extrinsic pathway disorders. Still, the environment in which blood clotting occurs inside the body is not replicated in the laboratory domain. For example, the clotting mechanism is a balanced activity between procoagulants and anticoagulants inside the body. When it comes to testing in the laboratory, though the platelet-poor plasma still contains both procoagulants and anticoagulants, the role of thrombomodulin in activating the anticoagulants is missed as endothelial cells (which is the source of thrombomodulin) are not part of the laboratory environment [35]. Hence the true risk of bleeding or thrombosis is not correctly reflected in these laboratory tests if there are changes in the endogenous anticoagulant system. Deficiency of clotting factors such as factor XII, high molecular weight kininogen, and prekallikrein that produce a prolonged aPTT result is not associated with any bleeding tendency in the concerned persons [36]. The normal extrinsic and common pathways in people with isolated deficiency of factor VIII (hemophilia A) or factor IX (hemophilia B) do not compensate for the deficient clotting factors and achieve hemostasis, but manifests as severe bleeding disorders [37]. Deficiency of factor XI is not manifested uniformly as bleeding tendency as some people remain normal despite abnormal aPTT results [38]. Even though factor XII is not normally present in some mammalian species like whales and dolphins, they do not suffer from any bleeding disorder [39]. Thus the above-

mentioned discrepancies lead the researchers to conclude that the blood clotting mechanism is less likely to work as separate pathways in vivo [40-44].

Cell-based model of coagulation

The cell-based model of coagulation was initially proposed by Hoffman, Monroe [9], and later expanded by K.G. Mann, S. Buenas [31]. The modern view of coagulation lays more stress on the process of coagulation over the surface of cells involved in the process [45]. The process of coagulation is portrayed as three overlapping phases such as the Initiation phase, the Amplification phase, and the Propagation phase [46] in the cell-based model in contrast to two separate and less interacting intrinsic and extrinsic pathways in the traditional model. The tissue factor (TF) bearing cells and platelets are the two pivotal cell-related components whereas thrombin and fibrinogen are the clotting proteins that interact with the cellular components to accomplish hemostasis [47]. Recent studies have shown the importance of the third cellular elements in the hemostasis, the RBCs, which form an impermeable complex structure with platelets and fibrin in the clot [48, 49] so maintaining a critical level of hematocrit gives a potential survival advantage in the face of life-threatening hemorrhage [50].

The initiation phase

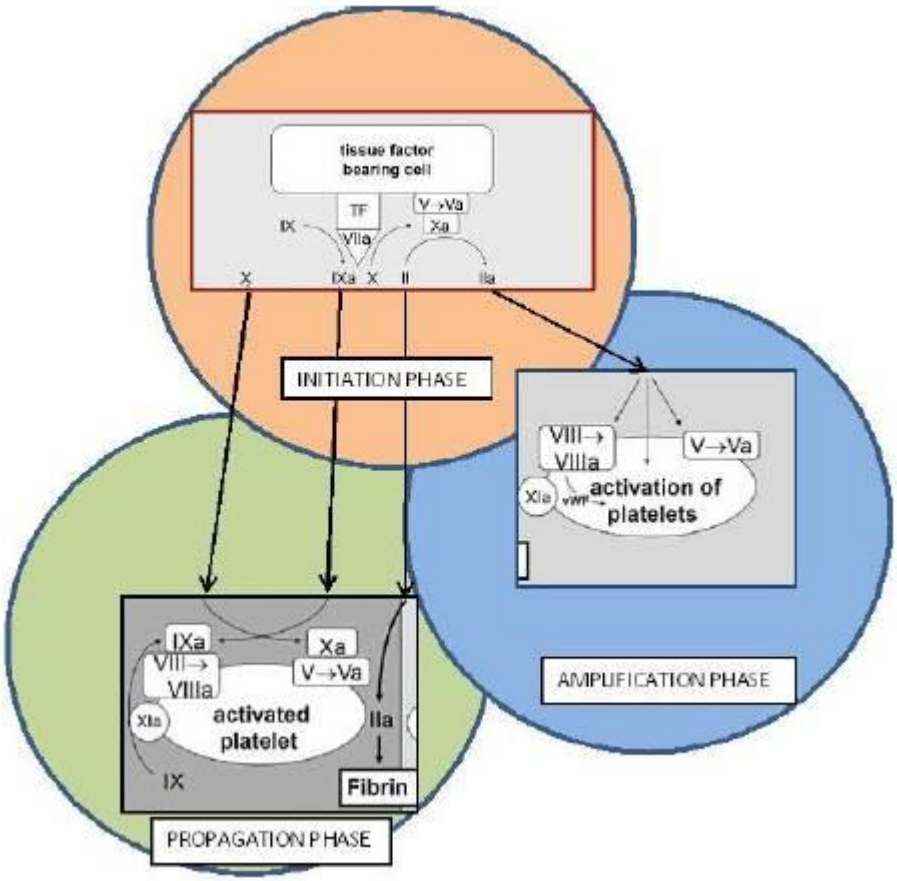
When blood is exposed to cells that bear tissue factor (TF) on their membrane, the process of hemostasis is set in motion at the site of injury. The TF is commonly present in the smooth muscle cells and fibroblasts of the subendothelial layer and seen in endothelial cells, macrophages, and circulating platelets only during inflammatory conditions [51]. The TF acts as a receptor as well as a cofactor for factor VII. The exact mechanism of activation of factor VII upon binding with TF is not yet clear. The complex TF/VIIa, called extrinsic Tenase complex on the cells activate factor X and IX (**Figure - 2**), both of which have an independent and specific role in the initiation of blood clotting [52]. The factor Xa forms a complex with Va on the TF-bearing cells, called Prothrombinase complex which converts prothrombin to thrombin over the TF-bearing cells whereas the factor IXa, diffuse to nearby activated platelets and bind with the surface receptor [53] and interact with the cofactor VIIa present on the activated platelets. This complex can directly activate factor X to Xa that breakdown prothrombin to thrombin over the activated platelets. The amount of thrombin formed during the initiation phase, called thrombin spark [54], is very less as the activity of factor Xa is inhibited by the endothelium-derived components like the Tissue factor pathway inhibitor (TFPI) and Antithrombin III (ATIII) [9].

The amplification phase

Intrinsic Tenase complex is formed when factor IXa combines with factor VIIa on the membrane of the activated platelets (sometimes on the endothelial cells and microparticles also) in the presence of calcium [28]. This intrinsic Tenase complex is very potent in producing the required amount of factor Xa needed to sustain hemostasis during this amplification phase. The amount of factor Xa produced by the intrinsic Tenase complex is 50 to 100 times greater than that is produced by the extrinsic Tenase complex produced during the initiation phase [55]. Not only the intrinsic Tenase complex potency is enhanced, but the potency of Prothrombinase complex is also augmented due to their co-localization on the phospholipid membrane of the activated cells in the presence of calcium [56]. The Prothrombinase complex alone by itself has 300,000 times higher capacity to generate thrombin when compared to factor Xa [57]. The ability of the platelet to bind both the Tenase complex and Prothrombinase complex enhances its ability to generate more thrombin [58-60]. Thrombin is formed rapidly within a short period to form a stable clot due to the positive feedback effect of these activated factors. Thrombin increases the expression of the factor Va on the platelet membrane when it binds and activates through its receptor GpIb on the

platelets which also results in degranulation of α-granules [41]. Platelet aggregation is potentiated by the thrombin when it acts through GpIb/IIIa receptors. Thrombin increases the levels of factor VIIa and factor XIa, by breaking VIIa:vWF complex and liberating the factor VIIa free and converting inactive factor XI to its active form [44]. The Tenase and Prothrombinase complexes acting cooperatively on the membrane of the platelets during this phase produce an enormous amount of thrombin, called "burst of thrombin".

Figure - 2: The Initiation phase on the TF bearing cell (fibroblast), Amplification phase on the platelets, and the Propagation phase on the activated platelets of Cell-based model of coagulation. (Adapted from Maureen Hoffman, Dougald M. Monroe [9].



The propagation phase

The "burst of thrombin" that started in the amplification phase should be judiciously channelized to effectively form the needed quantity of fibrin in the propagation phase to produce hemostasis. Platelets play a major role in localizing the burst of thrombin formation at the site of injury as they adhere to the exposed TF. Also, plasma factors like vWF and thrombin, platelet factor like its receptor, and vessel wall factor-like collagen assist platelets to get localized to the site of injury [23]. The stage is set utterly when intrinsic Tenase complex and Prothrombinase complex are available at the same site in the presence of cofactors like calcium and phospholipid membrane over the activated platelets. The burst of thrombin formed convert large quantities of fibrinogen to fibrin monomers that polymerize to form a gel of fibrin threads. Factor XIIIa formed by thrombin, links the fibrin polymer threads covalently to form stable fibrin clot [31]. The freshly formed fibrin clot is protected from the plasmin mediated fibrinolysis by the action of thrombin. Thrombin activates thrombin-activatable fibrinolysis inhibitor (TAFI) that removes lysine residues

from the fibrin clot so that the binding site of the plasmin is removed, thwarting clot lysis [54, 55].

Conclusion

The current understanding of the process of hemostasis has shifted our focus from the factor components of hemostasis to cellular components. The queries that prevailed in the cascade mode were answered by this cell-based model. This comprehensive understanding of the intricate and interdependent processes of blood coagulation will help us in strengthening our insights into the diagnosis of bleeding disorders with more accurate diagnostic tools and targeted therapeutic interventions.

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