Wound Healing

Wound Healing:

Molecular Pathways, Therapies, Shortcomings, and the Potential of Natural Products

By

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and Shalini Ramalingam

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The authors dedicate this book to
His Holiness Jagadguru Sri Shivarathri Deshikendra Mahaswamigalavaru,
a beacon of wisdom, compassion and selfless service.

Drug design and development based on plant based natural products (bioactive compounds) as leads, will yield potent drugs for wound healing. Even the *Sumatran orangutan* of Indonesia seems to know this; It treats wounds on its face using the plant *Fibraurea tinctoria*!

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PREFACE

I grew up in a village, raised by parents in a needy and impoverished family of seven children. I had a very difficult time completing my college and university education. From my school days, I used to dream of becoming a good academic/scientist. Today, I have a certain sense of fulfilment and self esteem, looking back on my academic/scientific accomplishments.

Some years back, one of my brothers, Krishnan, passed away. He was a diabetic and also suffered from foot ulcer. It was the foot ulcer that took his life. I asked myself why foot ulcers could not be cured. I therefore, decided to look into the problems of wound healing. After thoroughly surveying the literature, and a deep study, I decided to write a book on wound healing. When I discussed this with Dr. Shalini Ramalingam, a good teacher and a brilliant researcher who has carried out some advanced research in wound healing, she readily agreed to my proposal.

Research on wound healing is a neglected area as it falls outside the priority disease areas of the World Health Organization. Multiple factors can cause impaired wound healing by affecting one or more phases of the healing process. The influence of these factors are not mutually exclusive. Several investigations and in particular investigations based on growth factors, recombinant growth factors, gene theraphy, stem cell therapy, bioengineered skin substitutes, hyperbaric oxygen therapy, antimicrobial peptides, nanomaterials and plant based natural products have been carried out to develop efficacious therapies for wound healing.

We conducted a thorough survey of the literature on these investigations. Based on this, as well as our own research findings, we have emerged with this book. We hope and trust that the medical community, and in particular those working in the field of wound healing research will find this book interesting and useful.

Ootacamund,
May, 2025

Moola Joghee Nanjan
Shalini Ramalingam

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CHAPTER 1

INTRODUCTION

1.1. Skin

The skin is the largest organ in the body. It acts as the primary protective barrier against various environmental factors such as chemical, physical, thermal, microbial and immunological action (Nagar 2016, 1–8). In addition to several other functions, the skin also initiates the important biochemical process of Vitamin D production, essential for calcium absorption and bone metabolism. The skin consists of three layers, namely the epidermis, dermis and hypodermis [**Fig.1**–1]. These layers vary in their anatomy and function.

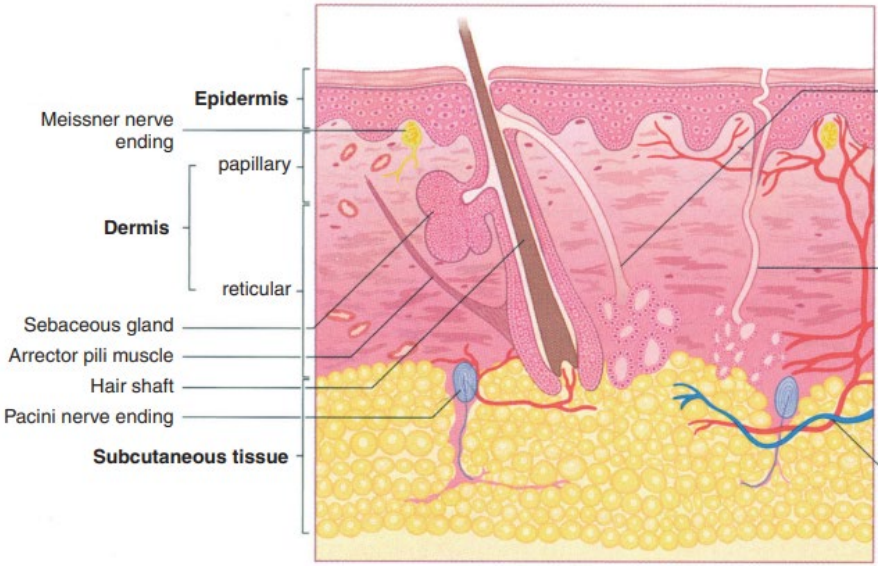

Fig. 1–1 Skin layers
(Courtesy of: Kolarsick, Paul A. J., et al. "Anatomy and Physiology of the Skin" *Journal of the Dermatology Nurses' Association* 3, no. 4 (July 1, 2011): 203–13)

1.1.1. The Epidermis

The epidermis layer is the top and outermost layer of the skin made up of the stratified and squamous epithelium layer. It consists of four sublayers based on the morphology and the position of the keratinocytes, namely the stratum germinativum (basal cell layer), stratum spinosum (squamous cell layer), stratum granulosum (granular layer), stratum lucidum and stratum corneum (cornified or horny cell layer) (Murphy 1997, 5–45). These layers are continuously renewed and give rise to sebaceous apparatuses, nails and sweat glands. The layers also contain various kinds of cells, namely, keratinocytes, dendritic cells, melanocytes, langerhans cells and merkel cells. Among these, keratinocytes are the predominant cells, which are differentiated and migrate to the upper layer, a process known as keratinization. Melanocytes are responsible for synthesising melanin pigment that is transferred to keratinocytes (Chu 2008, 57–73).

1.1.1.1. Basal cell layer

The basal layer,which is the deepest, consists of column-shaped keratinocytes attached to the basal membrane zone. This zone is a single layer zone attached to the basement membrane through desmosomes. Basal cells are mitotically active and appear dark due to the presence of pigment in the melanocytes. During the wound healing process, basal cells migrate from the basal layer to the cornified layer to occupy the wound bed. The basal cells require 14 days to reach the cornified layer, and another 14 days to reach the epidermis (Kolarsick 2011, 203–13).

1.1.1.2. Squamous cell layer

Above the basal layer, a 5–10 cell thick layer of squamous cells is arranged, making up the squamous cell layer. The squamous cell layer contains a variety of cells. The suprabasal spinous cells consist of polyhedral cells with a rounded nucleus. The upper spinous layer consists of larger, flatter cells with lamellar granules that act as lysosomes by producing various kinds of hydrolytic enzymes such as lipases, proteases, acid phosphatases and glycosidases. It also contains some membrane bound organelles with glycoproteins, glycolipids, phospholipids and free sterols. The intracellular spaces between the squamous cells are occupied by desmosomes (Murphy 1997, 5–45 & Chu, DH 2008, 57–73).

1.1.1.3. Granular layer

Above the squamous cell layer, the 1–3 cell layer thickness of flattened cells having keratohyaline granules is known as stratum granulosum. These cells are responsible for keratinization. Keratohyaline granules are irregular in shape and size, and basophilic in nature. They are first converted to interfibrillary matrix and finally to the soft keratin by enzymatic activity. These granules hold the keratin filaments together, undergo a differentiation process and form the cornified layer (Murphy 1997, 5–45 & Chu 2008, 57–73).

1.1.1.4. Stratum lucidum

The granular layer under the palms of the hands and the soles of the feet is known as stratum lucidum. This layer is 10 times thicker than the granular layer, and provides the site for the transformation of keratohyalin to eleidin (Chu 2008, 57–73).

1.1.1.5. Cornified or horny cell layer

The cornified layer is the outermost layer of the epidermis and acts as a barrier against foreign substances in order to prevent dehydration. The cornified layer consists of large, flat and polyhedral shaped horny cells called corneocytes. This layer is rich in protein and lipids. It provides mechanical support to the epidermis layer (Murphy 1997, 5–45 & Chu 2008, 57–73 & Kolarsick 2011, 203–13).

1.1.2. Dermis

The dermis layer is a combined system of blood vessels, lymph vessels, connective tissues, fibroblasts, macrophages and mast cells. The dermis is responsible for the elasticity and tensile strength of the skin, and its predominant component is collagen. Collagen is found in tendons, ligaments of the dermis. Collagen constitutes 70% of the skin dry weight. Collagen fibres are constantly degraded by proteolytic enzymes and replaced by new fibres. Collagen fibres are highly enriched with glysine, hydroxyproline and hydroxylysine (James 2019, 13th ed).

1.1.3. Hypodermis

The hypodermis, otherwise known as the subcutaneous fat layer, is the deepest layer of the skin. This layer contains adipose lobes and lipocytes.

Subcutaneous layers are responsible for buoyancy and act as a storehouse of energy. Liptin, a product of lipocytes, regulates body weight (Kolarsick 2011, 203–13).

1.2. Wounds

A wound is a disruption of cellular and anatomic continuity of living tissues. Any discontinuity or loss of integrity of the skin leads to the disruption of the structure and function of the underlying tissues, causing a wound. In everyday pathology, wounds remain a challenging clinical problem due to the continued production of inflammatory markers and insufficient production of granulation tissues (Berlanga–Acosta 2010, 255–261).

Based on the cause, wounds are divided into two types; open wounds and closed wounds. In open wounds, blood escapes from the tissues and can be clearly observed in incised wounds, puncture wounds, penetration wounds and tear wounds. In closed wounds, blood escapes from the circulatory system but remains under the skin as in hematomas (blood tumors), contusions and crush injury (Nagori 2011, 392–405).

Based on their physiology, wounds are divided into acute wounds and chronic wounds. In acute wounds, the progression of the reparative process takes place in a well–orchestrated manner within the stipulated time, resulting in an epithelial integrity. It can result in the destruction of both the layers, namely the epidermis layer and superficial dermis, or subcutaneous layer.

In chronic wounds, the progression of the healing process does not take place in a well–orchestrated manner within the stipulated time. It can result in the destruction of all the layers, namely epidermis, superficial dermis and subcutaneous layers (Berlanga-Acosta 2010, 255–261 & Menke 2007, 19–25).

Based on the extention of affected area, wounds are divided into superficial, partial and full thickness wounds. In superficial wounds, loss of the epidermis and upperdermis takes place. In partial thickness wounds, only the loss of the top two layers takes place and does not go beyond the dermis layer. In full thickness wounds, a complete loss of tissue takes place, resulting in the loss of the epidermis, dermis and subcutaneous layer (Gaspar-Pintiliescu 2019, 854–65).

Based on their appearance, wounds are divided into necrotic, sloughy, granulating and infected wounds. Dead tissues accumulate in necrotic wounds. Wet and gristly rehydrated necrotic tissues are present in sloughy wounds. Large granulation tissues with excessive exudate accumulate in granulation wounds. Microbial colonization with inflammation and pus formation are seen in infected wounds (Gaspar-Pintiliescu 2019, 854–65).

1.3. Wound healing

Wound healing is an intricate, dynamic and multi–phased process of regeneration or repair of broken tissues (Nagar 2016, 1–8). Wound healing involves a complex series of events which aim to restore the structural and functional integrity of the damaged tissues. It consists of four highly programmed overlapping phases, namely hemostasis, inflammation, proliferation and remodelling, which must take place in their proper sequence at a specific time and in a regulated manner (Hu 2019, 305–317). The progression of wound healing depends upon the timed release of diverse signalling molecules that orchestrate the behavior of inflammatory cells, endothelial cells, keratinocytes and fibroblasts. A failure in these signals increases dermal thickness and cellularity, and leads to impaired wound healing.

Wound healing remains a challenging clinical problem in modern biomedical sciences mainly due to its unknown mechanism of healing. The predominant reason for the neglect of wound research is that it falls outside the WHO's priority disease areas. According to the WHO, wounds are a "world wide pandemic" and their management is a public health issue best managed by an inter–professional team because a large number of cell types including neutrophils, macrophages, lymphocytes, keratinocytes, fibroblasts and endothelial cells are involved. Multiple factors can cause impaired wound healing by affecting one or more phases of the healing process. These factors may be local or systemic. The influence of these factors are not mutually exclusive. Single or multiple factors may play a role in any individual phase, contributing to the overall outcome of the healing process. Another major problem is the unavailability of suitable drugs capable of stimulating the process of wound repair as a number of parameters like inflammation, angiogenesis and epithelialisation, in addition to the large number of cell types are involved in successful healing. There is a lack of in–depth knowledge on the pathogenesis of wounds and in particular, the phase at which wound healing is halted, leading to complications. An effective wound healing

treatment, therefore, depends on the identification of the molecular defects in the pathophysiology of the wound, and exactly where the drug interacts with the target and induces mitogenesis.

Present day therapy generally involves treatment through the application of antibiotics systemically and antiseptics topically. These agents only help the wound area to be sterile in order to prevent further infection. They are not, however, involved in the physiological and molecular repair processes. Determining the molecular mechanisms of an investigatory treatment of the healing process is challenging. A few targeted approaches which have been developed like gene therapy, cell therapy, growth factors and recombinant growth factors, etc., are not effective either, due to the complex nature of the wound. Wound healing has thus become a serious health problem across the world today due to its inefficacious therapy. There is a need, therefore, for further intensive research for designing and developing alternative therapies/newer drugs for wound healing.

In this context, since ancient times medicinal plant extracts have been used for treating cuts, burns and wounds due to their affordability and suitability for chronic type of wounds. Several of these plant-based natural products (isolated bioactive compounds) have been screened scientifically for their wound healing activity, and some of these have also been successfully developed as wound healing drugs.

Further, natural products, elaborated within living systems, are known to have the ability to mask and fine tune the reactivity of their labile functional groups so that a small molecule can retain its kinetic stability which is needed for it to reach and specifically inhibit biological targets, either by a covalent mechanism or by employing the exquisite structural complementarity between the small molecule and its biological target (Clardy 2004, 829–37). Subjecting plant based natural products as leads for structure–activity relationships, target–based drug discovery followed by animal studies and clinical trails, therefore, has a tremendous potential for developing drugs. It is hoped that the research and development community engaged in wound healing research sees the potential of natural products for the design and development of newer potent drugs for wound healing.

CHAPTER 2

THE MOLECULAR PATHWAY

Wound healing is an intricate process which consists of four overlapping phases, namely hemostasis, inflammation, proliferation and remodeling. These four phases constitute complex and fragile processes. They are not discrete events; they occur both sequencially and simultaneously. Each of the four phases are equally important and the failure of any one of the phases can disrupt the healing process, leading to the development of chronic wounds. A comprehensive understanding of the biological molecular mechanism of these four phases, however, has not yet been clearly established. Several variations on the molecular pathway of wound healing exist in the literature. In what follows, the best possible summary of the molecular processes that take place during wound healing is presented. Wound healing, which is a worldwide pandemic, however, remains a challenging clinical problem today.

2.1. Hemostasis

In the first phase of wound healing, namely hemostasis covalently cross-linked fibrin clots are formed to plug the wound and stop bleeding. Fibrin clot formation involves the interaction of platelets with the damaged endothelial cells. This is initiated by platelets and leukocytes with a series of steps. Platelets contain many receptors, namely integrins, immunoglobulin superfamily receptors, leucine–rich repeat receptors, lipid receptors, miscellaneous platelet receptors, prostaglandin receptors, selectins, tetraspanins, transmembrane receptors and tyrosine kinase receptors for their activation and to help with their adhesion to other platelets and leukocytes (Saboor 2013, 891-6).

2.1.1. Primary hemostasis

The primary hemostasis phase starts in response to the injury. The main role of primary hemostasis is to form a platelet plug at the site of the injury. It has four orchestration and overlapping phases, namely

vasoconstriction, platelet adhesion, platelet activation and platelet aggregation (Shoshana 2013, 5–13).

2.1.1.1. Vasoconstriction

During vasoconstriction, immediate blood vessel constriction takes place by a neurological response, to initiate interactions of platelets with the exposed collagen in the extracellular matrix (ECM). Further constriction and activation of platelets are mediated by serotonin and the lipid mediator, TxA_2, secreted by platelet α–granules (Shoshana 2013, 5–13).

2.1.1.2. Platelet adhesion

During platelet adhesion, the platelets marginate and migrate along the vessel wall and attach to the wall with the help of adhesion molecules. Initially platelets bind with the exposed collagen. More than 300 proteins are released by human platelets for the activation of thrombin, the most potent platelet-aggregating agent [**Fig. 2–1**] (Judith 2003, 2096-2104). Adhesion is usually mediated by integrin α2β1 (GPIa–IIa), GPVI, GPIb–IX–V receptors and VWF. At the lower shear, platelets directly bind to collagen, fibronectin and laminin, while at the greater shear, the subendothelial matrix produces vWF and undergoes conformational change to facilitate the binding of platelet receptor, GP–Ib–IX–V, to collagen. In addition to the GP–Ib–IX–V receptor, GPVI and integrin receptors α2β1, α5β1, α6β1, αLβ2, αIIbβ3 and αVβ3, are needed for the firm adherence of the platelets to collagen. The integrin receptors, α2β1, α5β1, α6β1, αLβ2, αIIbβ3 and αVβ3, bind to collagen, fibronectin, laminin, leukocyte, fibrinogen and vitronectin, respectively. The GP–Ib–IX–V receptor initiates hemostasis as well as thrombosis.

2.1.1.3. Platelet activation

GPVI, which belongs to the immunoglobulin superfamily, plays a central role in platelet activation. It contains two domains; D1 and D2. The D1 domain has a specific amino acid sequence, Gly–Pro–Hyp, which helps in collagen binding. In the absence of vWF and fibronectin, collagen binding is mediated by integrin receptors which facilitate the binding of most of the ECM proteins. Integrin receptors also stop platelet translocation and allow collagen interaction with GPVI (Nuyttens 2011, S26-29 & Nieswandt 2001, 2120-2129). During activation, platelets which adhere to the subendothelium are transformed into irregular spheres with multiple filipodia so as to increase their area and make additional contacts.

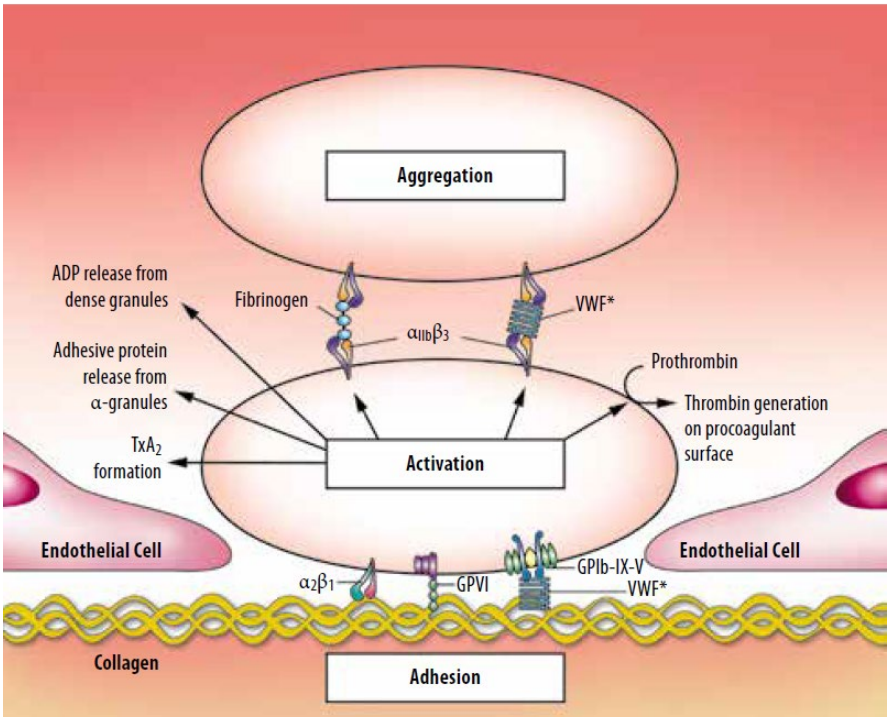

Fig. 2–1 Binding of platelet receptors with collagen
(Courtesy of: Blanchette, Victor S., Veronica R. Breakey, and Shoshana Revel–Vilk, eds. 2013. "*SickKids"*)

During this change in shape, granules coalesce in the centre of the platelets to interact with the surface-connected canalicular system and release their contents to secrete the aggregating agent, ADP, and a second messenger agent, TxA_2, from the platelet granules. The platelets then undergo remodelling that exposes the phosphatidylserine on the cell surface. The negatively charged aminophospholipid provides a procoagulant surface for the assembly of the coagulation factors and generate the potent aggregating agent, thrombin (Shoshana 2013, 5–13 & Davì 2007, 2482-94).

2.1.1.4. Platelet aggregation

After initial adhesion, the endothelial Weibel-Palade bodies release adhesion molecules, P–selectin, V–selectin, fibrinogen, epinephrine, thrombin, ADP

and a large amount of vWF (Shoshana 2013, 5). ADP binds to $P2Y_{12}$, TxA_2 binds to thromboxane and thrombin binds to PAR1 and 4 on the platelets to extend the platelet plug. Additionally, the $P2Y_{12}$ receptor coupled with the neutrophil receptor, $G\alpha_{i2}$, cause phosphoinositide hydrolysis and raise the cytosolic Ca^{2+} concentration, leading to the re-arrangement of the actin cytoskeleton and changing the shape of the platelet for easy movement (Brass 2003, 18S–25S & Dorsam 2004, 340–45). After the aggregation of platelets, P–selectin triggers monocytes and macrophages leading to the production of chemoattractants and growth factors. They also activate platelets for the production of protein CD40 in the wound site, which helps in the production of IL–1β, ROS and matrix degrading enzymes, matrix metalloproteases MMP–2, MMP9, and NADPH oxidases. NADPH oxidases are primarily responsible for the production of ADP and O_2^-, which act as scavengers of nitric oxide (Davì 2007, 2482-94). Vasodilation takes place to increase the vascular permeability which is mediated by prostaglandin A, prostaglandin D, prostaglandin E and prostacyclin. The vascular endothelial gaps increase and trigger the flow of plasma proteins and fluid into the interstitial space and increase the inflammatory cells into the wound site. At this stage all the white blood cells and macrophages create a barrier against microbial infection and decrease the activity of the injured cells (Wild T. 2010, 862–66).

2.1.2. Secondary hemostasis

Secondary hemostasis is a cascade of enzymatic reactions in which inactive zymogens are converted to the active serine proteases. At the end of the secondary hemostasis the soluble fibrinogen is converted to insoluble fibrin by coagulation and thrombosis. It is an intricate pathway that involves many zymogens of serine proteases: FII, FVII, FIX, FX, FXI and FXII, cofactors TF, FVIII and FV and the zymogen of transglutaminase, FXIII and fibrinogen. Fibrin formation, consisting of a series of enzymatic reactions, is divided into three pathways; the extrinsic pathway, intrinsic pathway and common pathway. The protease FVII, and tissue factor (TF) are involved in the extrinsic pathway. Factors VIII, IX, and XI are involved in the intrinsic pathway, and Factors II, V, X are involved in the common pathway. The major aim of these pathways is to form three enzyme complexes: the TF complex, TF/FVIIa; the tenase complex, FVIIIa/FIXa; and the prothrombinase complex, FVa/FXa. These three complexes help in the conversion of IX to IXa, FX to FXa and prothrombin to thrombin, respectively. The end product, fibrin, helps in

the formation of clots and in preventing bleeding from the injured vessels (Cugno 2014, 40–48, Gailani 2018, 2034–50 & Stavrou 2010, 210–15).

2.1.2.1. Extrinsic pathway

The tissue factor (TF), a 263/261 amino acid transmembrane protein, contains 3 domains, (1) an extracellular domain representing the NH_2^- terminal part of the molecule (residues 1 to 219) composed of 2 fibronectin type III domains, (2) a transmembrane domain which anchors TF to the membrane (residues 220 to 242) and (3) a cytoplasmic –COOH terminal domain (residues 243 to 263). The cellular domain of TF, exposed to the blood, complexes with FVIIa to form TF/FVIIa, an active form of FVII, which in concert with phospholipids (PL) converts FIX to FIXa, and FX to Xa. This sequence is called the 'extrinsic pathway' because under physiological conditions TF is not present in the blood [**Fig. 2–2**].

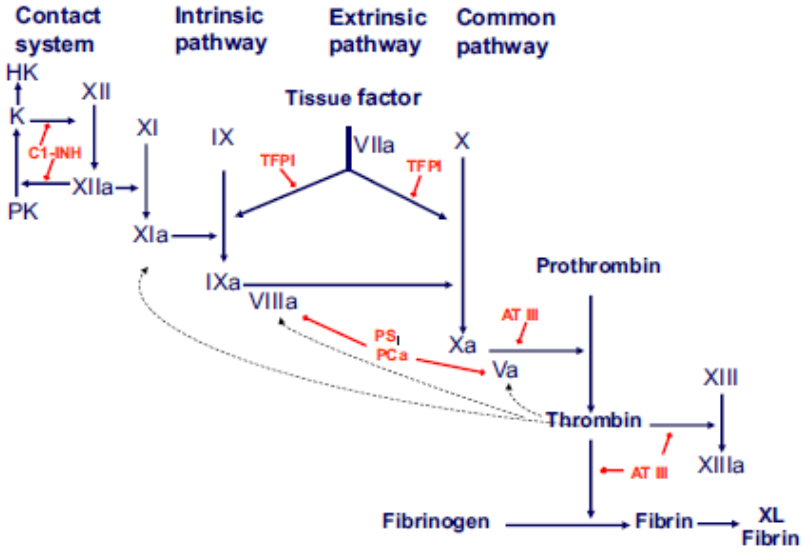

Fig. 2–2 Coagulation cascade
(Courtesy of: Cugno, Massimo, Roberta Gualtierotti, Adriana Tedeschi, and Pier Luigi Meroni. 2014. "Autoantibodies to Coagulation Factors: From Pathophysiology to Diagnosis and Therapy". *Autoimmunity Reviews* 13 (1): 40–48.

The transmembrane domain enhances the attachment of TF to the membrane and the cytoplasmic domain helps in the transduction pathway. The active FXa then enters the blood stream as a zymogen consisting of heavy and light chains held together by disulphide bonds (Butenas 2009, 1989–96).

2.1.2.2. Contact Activation System

The Contact Activation System is a connecting point of coagulation and inflammation. It either starts the coagulation process by activating FXII and FXI on the surface of platelets, or the inflammatory process by activating FXII, prekallikrein (PK) and the high molecular weight kininogen (HK) on the surface of endothelial cells. FXII, otherwise known as Hageman factor, is secreted by the liver (Pathak 2018, 66). It is an 80 kDa glycosylated protein and contains heavy and light chains held together by disulphide bonds. Zymogen protein, FXII, comprises of many domains, such as the fibronectin type II domain, EGF like domain, fibronectin type I domain, Kringle domain and the catalytic domain. FXII is autoactivated and converted into FXIIa by the cleavage of the specific amino acid sequence R_{353} and V_{354}. FXIIa converts the thromboplastin, FXI, to the active Factor XIa (Stavrou 2010, 210–15).

2.1.2.3. Intrinsic pathway

The intrinsic pathway includes factors that circulate in the blood. It starts with the antiactivation/activation of the zymogen precursor, thromboplastin, XI, to the active one, XIa. XI is a 160kDa disulphide linked dimer and contains 607 amino acid subunits of four apple domains (A1, A2, A3, A4) at the N–terminal end, and a C–terminal trypsin–like catalytic domain. In the circulating blood, FXI complexes with the high molecular weight kininogen (HK). When this complex cleaves at the Arg369–Ile370 bond, it produces high molecular weight kininogen, bradykinin and active FXIa (Emsley 2010, 2569–77). Active FXIa helps in converting FIX to FIXa. TF/VIIa complex activates plasma thromboplastin antecedent FIX, a 57k Da glycoprotein that contains the Gla domain, 2 EGF domain, activation domain and catalytic domain. The Gla domain contains γ–carboxylated glutamate, important for recruiting Ca^{2+}, thereby facilitating the binding of factor IX to the phospholipid membrane. The two EGF domains help in protein–protein interaction. The activation domain gets cleaved at the Arg145–Arg180 bond and releases 35 amino acid residue, the active FIXa (Smith 2008, 87–98). FIXa binds to 330–kDa glycoprotein substrate, FVIIIa, and forms tenase complex, FXa/ FVIIIa. The tenase complex

converts the inactive zymogen, FX, to active FXa (Butenas 2009, 1989–96). FVIII, an antihemophilic factor, contains 2332 amino acid residues with three different domains; A, B and C, with the subdomains A1–a1–A2–a2–B–a3–A3–C1–C2 (Cugno 2015, 40–48). Factor VIII binds to the substrate binding site and cleaves at the Arg15–Ile16 peptide bond present on FX which gets converted to FXa. FXa, a 52 amino acid protein, mainly depends on Vitamin K for its activity and acts as a substrate for both extrinsic and intrinsic coagulation pathways (Chandler 2019, 907–908). FXa combines with the 236 amino acid glycoprotein, Leiden, FV, to form prothrombinase complex, FXa/ FV (Cugno 2015, 40–48) that enters the common pathway.

2.1.2.4. Common pathway

The common pathway, which is the final pathway, predominantly deals with the conversion of prothrombin to thrombin and fibrinogen to fibrin. The prothrombinase complex, FXa/FV, formed in the intrinsic pathway cleaves at Arg271–Thr272, and Arg320–Ile321 bonds in the 71.6–kDa glycoprotein prothrombin, and gets converted to the active thrombin (Kalogeropoulos 2019, 170). Thrombin then cleaves 340–kDa homo dimeric glycoprotein, fibrinogen. Fibrinogen is made up of a 2Aα chain, 2Bβ chain and 2γ chain held together by 29 disulphide linkages synthesised in hepatocytes. Fibrinogen assembly plays an essential role in clot formation and fibrosis. The assembly of fibrinogen takes place in a sequential manner in which Aα binds to γ, and Bβ binds to γ to form an end complex, $(A\alpha/B\beta/\gamma)_2$. Under normal conditions the concentration of fibrinogen is 2 to 5mg/ml but in the case of injury, the concentration of fibrinogen increases to more than 7mg/ml (Kattula 2017, e13–e21). Fibrinogen then allows the resultant fibrin monomers to polymerise. The polymer formed is stabilized by FXIIIa and crosslinked to form a fibrin network.

2.2. Inflammation

The achievement of hemostasis leads to the immediate onset of inflammation. Inflammation plays a predominant role in wound healing; it takes place immediately after the injury and lasts for up to 2 weeks of wound healing. Inflammation, caused by microbial infection or tissue injury, acts as a protective response against the various environmental cues. Inflammation occurs in a series of processes, namely neutrophil recruitment, infiltration of neutrophils, removal of microbes, monocyte infiltration, transformation of monocytes into macrophages, removal of

cellular debris, and macrophage polarization. In response to tissue injury, the danger-associated molecular proteins, namely DNA, histones, high mobility group protein B1 (HMGB1), N–formyl peptides, Adenosine triphosphate (ATP), and interleukin–1α (IL–1α), act as chemoattractants which are sensed by neutrophils through the G–protein coupled receptors (GPCRs). In addition, the neighbouring tissues for the production of a lipid mediator are activated: leukotriene B4 (LTB4) and chemokine (CXCL8) that are strong chemoattractants of neutrophils. CXCL8 chemokines interact with glycosaminoglycans in the ECM and form chemokine gradients which help in the migration of neutrophils to the damaged tissue (Wang 2018, 2785–7). Several other factors also bind to different pattern recognition receptors. Among the different pattern recognition receptors, Toll like receptors (TLRs) play a predominant role in injury and infection. In non-infection inflammation also known as sterile inflammation, some endogenous molecules, namely ECMs, breakdown products, heat shock proteins, the S100 family of proteins and high mobility group box–1 (HMGB1) bind to TLR, activate macrophage, pro-inflammatory cytokines and chemokines and provide a link to generate adaptive immune responses (Peiseler 2018, 335–49). The non-infection inflammation (or sterile inflammation) is activated by TLRs. There are two types of TLRs, namely TLR2 and TLR4. TLR4 plays a critical role in the tissue regeneration process (Suga 2014, 117–24). ATP and uridine–5′–triphosphate bind to the family of P2 receptors as well as to human neutrophil receptors, P2X7, P2Y4, P2Y6 and P2Y11 receptor subtypes, especially P2X7, and activate the inflammasome, a multiprotein complex. The inflammasome activates a NOD like receptor and converts pro caspase–1 to active caspase–1 which then induces the secretion of IL–1β and pro–IL18 (Gendaszewska–Darmach 2011, 193–206 & Mirza RE 2014, 1103–14). This predominant signaling pathway [**Fig. 2–3**] by TLRs leads to the activation of NF–κB, AP–1, C/EBPβ, PU–1 and IRF (Peiseler 2018, 335–49). In cytosol, NF–κB is always associated with the inhibitor protein molecule IKBS. This inactive complex, NF–κB–IKBS, gets degraded by proteasome to an active form of NF–κB, translocates into the nucleus and starts the transcription of pro–inflammatory cytokines, IL–1β, IL–12, IL18 and TNFα, chemokines, cell–adhesion molecules and nitric oxide synthase, neutrophil elastase and cathepsin G which are involved in microbicidal activity (Fujiwara 2005, 281–86).