

Historical review

HISTORY OF TISSUE FACTOR

'It is natural that a striking feature such as coagulation of blood always inspired researchers and that it is not surprising that since the beginnings of scientific research there were numerous hypotheses and theories which tried to explain this phenomena' wrote Morawitz in his famous overview (Morawitz, 1905). 'Clotting is the most striking and best known property of blood. It is indeed a remarkable phenomenon. One can realize that nature had a difficult problem to solve in creating a circulating medium for the animal body that would remain entirely liquid while in the blood-vessels, but would promptly set into a gel as soon as the vessels were wounded and the blood was in danger of being lost' wrote Howell 30 years later (Howell, 1935). How the clotting process starts was, and still remains, one of the main questions asked by researchers. The evolution of tissue factor or tissue thrombokinase from the late nineteenth century to the end of the twentieth century reflects very well the evolution of the clotting theory and the development of biochemistry and molecular biology in this period. This article describes the history of the term and concept of tissue thrombokinase in 1905 and its evolution to the concept and fact of tissue factor as a glycoprotein and the main physiological initiator of clotting *in vivo* in 1999.

Viewed as the only activator of prothrombin at the end of the nineteenth century and beginning of the twentieth century, the discovery of other clotting factors and characterization of their deficiency states in humans diminished the impact of the concept of tissue factor (TF) as the main initiator of coagulation. Its status was lowered further by the proposal of the 'clotting cascade' and the 'waterfall theory', which favoured activation of a cascade of reactions through factor XII, the so-called intrinsic pathway (Davie & Ratnoff, 1964; Macfarlane, 1964). This together with the absence of purified tissue factor protein made some researchers relegate tissue factor as the putative principal initiator of clotting *in vivo* to the background.

The nomenclature and terminology in haemostatic research up to 1962 was a confusing issue. In 1952, Milestone wrote 'The concept of "tissue factor" has entered into the definition of clotting factors, even though the meaning of 'tissue factor' is vague and the 'tissue factor' problem has not been solved. Consequently, there have been ambiguities in the usage of the old terms, 'thrombokinase' and 'thromboplastin'. It has become necessary, either to sharpen the definitions of the old terms, or else to replace them with new ones' (Milestone, 1952). In his overview, he showed clearly how much confusion existed concerning the

term and concept of thrombokinase, tissue thromboplastin or tissue factor in his time. Later, in his review, he wrote 'without some detailed specifications, the meaning of tissue factor is vague. It is not at present a clear and simple expression which can be used to define a particular clotting factor'. Between 1954 and 1962 I. S. Wright made an effort to chair a committee which introduced a numerical nomenclature to the clotting system. This committee renamed fibrinogen as factor I, prothrombin as factor II, tissue factor as factor III and ionized calcium as factor IV (Wright, 1962).

The new history of tissue factor began in the early 1980s with the purification of the protein, continued in the late 1980s with the cloning of the gene and concluded in the 1990s with the crystallization of the protein and firm establishment of tissue factor as the principal initiator of clotting *in vivo*.

Early history

To introduce the concept and term of tissue factor, one has to look back to the late nineteenth century and early twentieth century and review the understanding of clotting and the terms in use.

Fibrin, the first term used with respect to coagulation, was introduced by Plato (1892) and is still in use today. Fibrinogen was established as the precursor of fibrin by Virchow (1856). The first attempts to purify fibrinogen were undertaken by Hammersten in the 1870s (Morawitz, 1905; Hammersten, 1911). The observation that serum induced coagulation of hydrocele fluid led to the conclusion that there must be another factor which converts fibrinogen to fibrin (Buchanan, 1845). This substance was described in the 1890s as fibrin ferment, and subsequently as thrombin (Schmidt, 1892). Schmidt realized that thrombin could not be present in circulating blood, as it would otherwise clot. He postulated that thrombin was a product of the clotting process and its precursor was named prothrombin by Pekelharing (Quick, 1957).

Previously, De Blainville (1834) had reported the astonishing observation that intravenous injection of a suspension of brain tissue was immediately lethal, occluding the animal's blood vessels with clots. Buchanan confirmed the clot-promoting effect of tissue extracts (Buchanan, 1845; Morawitz, 1905). The injection of a variety of tissue extracts into different animals was reproduced many times at the turn of the nineteenth century and was one of the models used to advance the understanding of clotting at that time. In 1862, Alexander Schmidt suggested that tissues provide a zymoplastic substance which converts prothrombin to thrombin and subsequently fibrinogen to

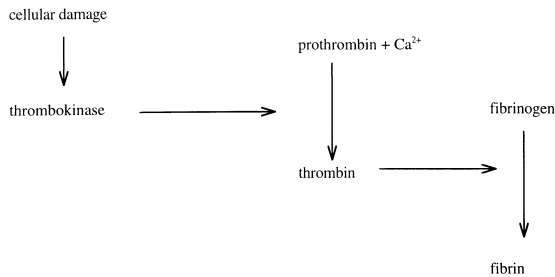


Fig 1. Morawitz's clotting theory (Morawitz, 1905).

fibrin (Morawitz, 1905). At the same time, Rauschenbach realized an additional unknown factor was required to convert prothrombin to thrombin. He found it in tissue extracts and called it protozym (Morawitz, 1905). He wrongly assumed this factor was predominantly found in tissues and cells with a high content of nucleoproteins, such as leucocytes and spermatocytes.

Another observation was 'if (blood) trickle from a wound or flow over an extensive surface, concretion almost immediately ensues, but if...it issue in a full stream and be received into a proper vessel, several minutes elapse before this process commences' (Thackrah, 1834). Thus, it was known that when blood came in contact with tissue clotting was much faster *in vitro* and *in vivo* (Morawitz, 1905). It was also known that the active clot-promoting substance of tissue was not stable, but it could be vacuum dried and stored for a long time. Other investigators confirmed tissue extracts do not clot fibrinogen directly, rather they change prothrombin to thrombin (Fuld & Spiro, 1904; Morawitz, 1905). Another component widely studied at that time was salts, specifically calcium salts. They were described as having 'specially favourable' effects on the clotting process by Hammersten in 1875 (Morawitz, 1905). Morawitz stated in his review that the reaction between thrombokinase and prothrombin, or thrombogen as he named it, is calcium dependent and inhibited by adding plasma treated with oxalate, in which calcium is precipitated and unavailable (Morawitz, 1905).

Morawitz was the first to use the term thrombokinase to describe the clot-promoting substance found in tissues and introduced it into his clotting theory. He proposed a clotting theory which influenced research in this field over the next 50 years (Fig 1). The four components were thrombokinase, calcium, prothrombin and fibrinogen. The theory purported that thrombokinase in the presence of calcium converts prothrombin to thrombin, which in turn converts fibrinogen to fibrin (Morawitz, 1905).

Morawitz believed thrombokinase was an enzyme. 'We will therefore in future designate the active substance of tissue juices as thrombokinase, since we are thinking of the analogy with the action of enterokinase and trypsinogen'. In his theory, he postulated two sources of thrombokinase; first, in platelets and leucocytes released upon injury of a vessel or after prolonged stagnation of the blood, and second from tissue exposed after tissue injury. The earlier observation of De Blaineville, Buchanan and others that the

injection of tissue extract, particularly brain extracts, into animals led to disseminated intravascular coagulation supported his theory (De Blaineville, 1834).

Morawitz was aware of experiments in which cell-free plasma collected in paraffin-lined tubes did not clot, but that transfer into glass tubes induced immediate clotting. At the end of the last century these observations led to the firm belief that clotting was an intrinsic property of the blood itself and prevented *in vivo* by the lining of the vessel walls; only upon contact with a foreign or 'wetable' surface did coagulation occur. This could be seen as a contradiction to Morawitz's theory because he believed thromboplastin was a *sine qua non* for coagulation. To explain this observation, he postulated platelets continuously liberated small amounts of 'plasma thrombokinase', which upon contact with 'wetable' or foreign surfaces, such as glass, induced clotting.

So Morawitz renamed and defined the zymoplastic substance of Alexander Schmidt and the protozym of Rauschenbach as 'thrombokinase'. Later, in 1908, Nolf used the term tissue thromboplastin, and even later Howell introduced the term tissue factor (Howell, 1935; Nolf, 1938).

Some of the ideas at the beginning of this century were quite unorthodox. Howell, an influential professor from Harvard Medical School, suggested blood contained an inhibitor which binds to prothrombin. When tissue thromboplastin was added to plasma, the phospholipid portion of the complex detached the inhibitor from prothrombin, thus freeing thrombin for clotting. Jules Bordet held similar ideas. He believed thrombin was a result of the formation of a complex of tissue thromboplastin (which he called cytozyme) and prothrombin (serozyme) (Nolf, 1938). At the beginning of the twentieth century, there were as many clotting theories as researchers in the field. Only Morawitz's idea survived for another 50 years.

The 'middle ages' of tissue factor history

The classical concept of the clotting theory of Morawitz enabled Quick to develop his quantitative prothrombin test (PT) (Quick, 1935). This test is based on the assumption that clotting time of blood plasma in the presence of optimal calcium concentration and excess tissue thrombokinase (tissue factor) is directly dependent upon the prothrombin concentration. The principle of this test was a direct logical conclusion based on the classical coagulation doctrine of Morawitz. His source of thrombokinase (tissue factor) was an acetone extract of rabbit brain. He concluded in his paper that the bleeding tendency observed in patients with obstructive jaundice was due to a prothrombin deficiency because their PT was prolonged. The normal PT in plasma samples from haemophiliacs '...suggests that in haemophilia prothrombin is normal in quantity and quality, but thromboplastin is deficient' (Quick, 1943). The PT was widely used in clinical practice to monitor oral anticoagulation, introduced in clinical practice at that time. In the 1940s, anticoagulation was thought to influence only prothrombin levels. However, there were test results which could not be explained by the classical four-factor theory of

Morawitz. The other vitamin K-dependent proteins that have an influence on the PT, factors VII and X, were not yet discovered. Quick (1943) observed that normal plasma stored frozen for a long period of time yielded a prolonged PT. Addition of plasma from anticoagulated patients or fresh plasma corrected this prolongation. These results could only be explained by a new factor, which he termed 'labile factor' or 'prothrombin B', later renamed factor V (Quick, 1943). Independently, Owren (1947) published a case history of a woman with a lifelong bleeding tendency. Her PT was prolonged and could be corrected by adding 'prothrombin-free' plasma pretreated with aluminium hydroxide, fresh plasma or plasma from patients anticoagulated with dicoumarol. He concluded that his patient was lacking a new factor, which he called factor V. Only a few years later, Alexander described a patient with a prolonged PT which could be normalized by adding serum lacking factor V and prothrombin, or by adding normal plasma (Alexander *et al.*, 1951). Koller *et al.* (1951) experimented with a protein they extracted from serum and called it factor VII. It was the same protein which was missing in Alexander's patient. They realized that factor VII was present in serum and plasma and that its concentration decreased very rapidly upon treatment with dicoumarol. The numerical term, factor VII, was adopted by Wright and his co-workers in 1953 and was thought to interact with tissue thromboplastin. In the 1950s, Hougie presented the case of a lay preacher named Stuart and Telfer the case of a young woman named Miss Prower (Telfer *et al.*, 1956; Hougie *et al.*, 1957). Their PT was prolonged and could be corrected by factor VII-deficient plasma. Again, a new factor (Stuart-Power factor, later called factor X) was added to the ever-expanding list.

Until 1962, 12 distinct clotting factors were described, however there was no theory to unite them into one system. In the 1950s, these new evolving insights led to the proposal of an extrinsic (tissue factor activated) system and an intrinsic (contact activated or glass activated) system. It was suggested there were two thromboplastins, plasma thromboplastin and tissue thromboplastin. Plasma thromboplastin was active when plasma came into contact with foreign surfaces and was thought to interact with factors VIII, V, IX, XI, X and XII and calcium, platelets or phospholipids. Tissue thromboplastin was thought to be a lipoprotein which interacted with factors VII, X and V, prothrombin and calcium (Macfarlane, 1972).

In 1964, Davie and Macfarlane independently formulated the 'waterfall' or 'cascade theory' of *in vivo* clotting. Both theories were essentially the same and favoured activation of a cascade of reactions through factor XII, the so-called intrinsic pathway. Each protein is a proenzyme numbered with a roman numeral and after activation, indicated with the suffix 'a', it activates the next proenzyme in the cascade (Davie & Ratnoff, 1964; Macfarlane, 1964). It was not known at the time that factor V and VIII were cofactors and not proenzymes.

Tissue factor was believed to form a complex with factor VII, phospholipids and calcium ions, which then convert factor X to active enzyme Xa (Nemerson, 1966; Williams &

Norris, 1966). There was evidence that one of the components of the tissue factor/VII complex was proteolytically active, however in the 1960s its identity was unknown.

Macfarlane wrote "The nature of "tissue factor" is still unknown, but there is some evidence that, in the presence of factor VII, it activates factor X enzymatically" (Macfarlane, 1972).

The details of the components of the extrinsic system and their interaction remained unclear and the question as to whether tissue factor existed remained unresolved. Was tissue factor one single protein or merely an unspecified property of different tissues? No human disease could be attributed to tissue factor deficiency; all other coagulation factors were described from a distinct deficiency state in humans. Furthermore, patients with the prominent bleeding tendencies of haemophilia A and haemophilia B had a normal PT, but clearly abnormal 'contact activation' with a prolonged 'partial thromboplastin time' or aPTT. The latter was introduced into clinical practice by Langdell *et al.* (1953). It was based on Langdell's observation that when tissue thromboplastin or 'complete thromoplastin' used in the PT was replaced with a crude cephalin or platelet suspension or with diluted tissue thromboplastin haemophilic plasma clotted less rapidly than normal plasma. Langdell used a crude cephalin preparation and advertised the 'partial thromoplastin time' as a simple one-stage clotting assay to screen for haemophilia. He stated in his discussion 'It is not possible with the data now available to do more than postulate possible underlying reactions responsible for the differences in clotting of normal and haemophilic bloods with various thromboplastins' (Langdell *et al.*, 1953).

Tissue factor pathway becomes the 'prima ballerina'

Only 30 years later, in 1995, Rapaport and Rao were able to write "Thus little reason exists today to doubt that the binding of factor VII to TF and the subsequent reactions so triggered play a "prima ballerina" role in the initiation of coagulation during haemostasis' (Rapaport & Rao, 1995). How was this firm statement possible after only 30 years?

Some observations did not fit in the classical waterfall or cascade theory which favoured the intrinsic system or contact activation. First, patients with factor XII deficiency (Hagman factor) have a prolonged aPTT and do not bleed. This deficiency was first observed when railway worker Mr Hagman sought surgical treatment for his peptic ulcer and was subsequently shown to have abnormal clotting tests. He had no bleeding complication following his stomach operation and died later in life of pulmonary embolism (Ratnoff & Colpy, 1955; Ratnoff *et al.*, 1968). Factor XI-deficient patients have only a mild haemorrhagic disorder and they rarely bleed into tissues after trauma but they may lose some blood post-operatively, predominantly in areas with high fibrinolytic activity. On the contrary, factor VII-deficient patients bleed significantly. These observations led to the conclusion that contact activation may not be the main *in vivo* initiator of clotting.

Factor VII was the only factor known to interact with

tissue factor. In 1977, it was shown that the complex of TF/factor VII activated both factor X and factor IX (Osterlund & Rapaport, 1977), corroborating earlier indirect evidence of factor IX activation in an animal study in which disseminated intravascular coagulation was induced by intravenous injection of tissue extracts (Rapaport *et al.*, 1966). This was seen as a possible explanation for bleeding resulting from cases of haemophilia A and B as these bleeding tendencies could not be sufficiently explained by clotting via the classic intrinsic pathway.

To solve some of the issues, it was essential that tissue factor be purified. Already in the 1890s, Alexander Schmidt knew the zymoplastic substance used for his experiments contained phospholipids. He described the substance as being thermostable and soluble in alcohol (Morawitz, 1905). Morawitz and co-workers extracted thrombokininase with water, described it as thermolabile and concluded the preparation contained protein (Morawitz, 1905). The first attempts to purify tissue factor were undertaken by Howell (1912) and Chargaff *et al.* (1944). Howell (1912) brought the two observations of Schmidt and Morawitz together and proposed that the thermolabile component was indeed a protein and that the thermostable component was a lipid. He extracted active material in a lipid fraction, however an aqueous emulsion of lipids and proteins showed more activity. Much later, Chargaff *et al.* (1944) demonstrated that tissue thromboplastin was associated with phospholipids. He sedimented a brain preparation with salt and demonstrated the activity to be in the sediment. After adding deoxycholate (bile salt), the active component was in the supernatant and could be recovered after removing the bile salts by dialysis. This clearly showed for the first time that tissue factor forms a complex with lipids and can be reversibly disrupted by deoxycholate. Thus, two components were therefore required for clot-promoting action: one a heat-stable phospholipid and the other a heat-labile protein. However, technology was not sufficiently advanced at that time to purify tissue factor to homogeneity. Between 1965 and 1969, Nemerson and Prydz independently solubilized a protein they believed to be tissue factor, separated it from other cell components and reassembled it with phospholipids (Nemerson & Spaet, 1964; Hvatum & Prydz, 1969; Nemerson, 1969; Nemerson & Pitlick, 1970).

The capacity for detergent solubilization of tissue factor lead ultimately to its purification in 1981. Two further essential observations facilitated the successful purification of tissue factor. The first was the discovery that tissue factor binds to concanavalin A-Sepharose as it is a glycoprotein (Zacharski *et al.*, 1974; Pitlick, 1975, 1976). The second observation was made by Bjorklid *et al.* (1973, 1975), who described sodium dodecyl sulphate (SDS) denaturation of tissue factor. This preparation could be renatured and regain full activity after reconstitution with phospholipids. Bach *et al.* (1981) purified bovine tissue factor to homogeneity for the first time with differential Triton X-100 extractions as a replacement for bile salts, combined with lecithin affinity chromatography and preparative SDS gels. Only 0.001% of the total protein content of the bovine brain is tissue factor; after starting

with 150 g bovine acetone brain powder, only 316 µg tissue factor was recovered. These minute quantities of pure protein facilitated the generation of a polyclonal antibody against bovine tissue factor. Coupling the antibody to an immunoadsorbent column allowed purification of more bovine tissue factor for subsequent generation of monoclonal antibody. With the immunoadsorbent column and the polyclonal antibody, 400 µg pure tissue factor was prepared in 5 d. The monoclonal antibody was used to enhance the efficiency of the purification procedure (Carson *et al.*, 1985a). Purification of human brain tissue factor was achieved in 1985 by affinity chromatography using human factor VII-agarose (Bronze *et al.*, 1985; Guha *et al.*, 1986). Tissue factor binds factor VII in a calcium-dependent manner and can thus be eluted with ethylene diamine tetra-acetic acid (EDTA) from factor VII-agarose. This allowed purification of 380 µg of human tissue factor from 49.36 g of brain acetone powder. In 1987, the efficiency of purification of human tissue factor from brain and placenta was greatly improved by using a monoclonal antibody against human tissue factor on immunoadsorbent columns. Monoclonal antibodies against human tissue factor allowed immunohistochemical localization of tissue factor in different organs and tissues for the first time. The endothelium lining the blood vessel and all the cellular components of the blood were negative, whereas the adventitia and the media showed predominantly fibroblast-associated localization. This formed the 'haemostatic envelope' around blood vessels. There was intense staining of the cortex of the brain, myocardium, respiratory mucosa, renal glomeruli and the epidermis (Drake *et al.*, 1989).

The next step was to isolate the tissue factor gene. Four different groups published the cDNA sequence of the tissue factor gene and its 5' and 3' flanking sequences concurrently in 1987 (Fisher *et al.*, 1987; Morrissey *et al.*, 1987; Scarpati *et al.*, 1987; Spicer *et al.*, 1987). These publications and the subsequent controversies between two groups is testimony to the renewed interest in tissue factor and the competition in the field at this time (Konigsberg & Nemerson, 1988). The complete genomic sequence spanning 12.4 kb was published in 1989 (Mackman *et al.*, 1989). The gene was localized to chromosome 1, specifically at 1p21-22 (Carson *et al.*, 1985b). The predicted primary translation product was a 295-residue polypeptide which included a leader sequence of 32 residues and a mature protein of 263 amino acids. A hydrophilic extracellular domain of 219 amino acids precedes a hydrophobic transmembrane domain (220-242) and a short cytoplasmic tail (243-263). There are two N-linked glycosylation sites ASN 11 and ASN 134. Deglycosylated *Escherichia coli*-derived recombinant tissue factor is fully active, so the significance of those glycosylated residues is unclear (Parbovsky *et al.*, 1989). Only 5 years later, two groups crystallized the extracellular domain of tissue factor and resolved the structure to 2.2 and 2.4 Å respectively (Harlos *et al.*, 1994; Muller *et al.*, 1994). It consists of two fibronectin type III modules and is very similar to that of the gamma interferon receptor. The two modules are orientated at an



Fig 2. Stereo view of the architecture of the tissue factor extracellular domain in a ribbon representation (courtesy of J. McVey).

angle of 125° , as shown in the ribbon representation in Fig 2.

It is essential that a potent procoagulant such as tissue factor or the tissue factor–factor VIIa (TF/VIIa) complex be tightly regulated, as indeed are other coagulation factors. Since the 1940s, there had been evidence of an inhibitor of tissue factor-initiated coagulation in plasma or serum (Schneider, 1947; Thomas, 1947). Again, animals were infused with a tissue factor-containing placental extract to induce disseminated intravascular coagulation. Preincubation of the placental preparation with serum prevented the death of the animals. In the late 1950s, the target of the putative inhibitor was demonstrated to be the TF/VIIa complex rather than tissue factor alone, and in the late 1980s the inhibitor was shown to be factor Xa dependent (Hjort, 1957; Rapaport, 1989). It has been named lipoprotein-associated coagulation inhibitor (LACI), extrinsic pathway inhibitor (EPI) and, since 1991, tissue factor pathway inhibitor (TFPI) (Rapaport & Rao, 1992).

The availability of highly purified natural or recombinant clotting factors and their inhibitors allowed study of their interaction *in vitro*. Different combinations of clotting factors were mixed and only by adding tissue factor did thrombin generation occur. This elegant work incorporating blood from individuals with genetic, acquired or induced coagulation deficiencies together with mathematical models enabled the establishment of tissue factor as the sole initiator of clotting *in vitro* and *in vivo* (Mann, 1999). Finally, the clotting cascade could be defined: upon attaching to its membrane-bound cofactor tissue factor, factor VII is activated to VIIa and subsequently the TF/VIIa complex activates factor X and factor IX. In this ‘initiation phase’, only a trace amount of thrombin is generated, which then back-activates factors X, V, VIII, IX and XI and ultimately generates sufficient thrombin to form a clot. This ‘propagation phase’ occurs independently of the TF/VIIa complex. More than 90 years after Morawitz formulated his theory, tissue factor or tissue thrombokinase is

once again recognized as the main initiator of clot formation.

Yet, despite the elucidation of TF structure and function, no human disease has been attributed to a deficiency of this principal factor. Recently targeted disruption of the tissue factor gene in mice was demonstrated to result in embryonic lethality (Bugge *et al.*, 1996; Carmeliet *et al.*, 1996; Toomey *et al.*, 1996). This is widely acknowledged as an indication that homozygotic disruption of the human tissue factor gene is incompatible with life. In contrast to a deficiency state, overexpression of tissue factor can be attributed to a variety of human diseases. Two examples are disseminated intravascular coagulation and Trousseau's syndrome. Activation of coagulation in septicemia is widely attributed to intravascular tissue factor expression on monocytes or endothelial cells (Ten Cate *et al.*, 1999). In animal models, infusion of Gram-negative bacteria or the causative toxin endotoxin is associated with high mortality and morbidity because of disseminated intravascular coagulation and septic shock. Infusion of tissue factor antibodies or TFPI prevents activation of coagulation and the animals survive lethal doses of endotoxin (Ten Cate *et al.*, 1999). *In vitro* studies have demonstrated that tissue factor expression can be induced in monocytes and endothelial cells upon stimulation with various inflammatory cytokines (endotoxin, tumour necrosis factor and interleukin 1). Historically, intravascular coagulation induced by pure tissue factor or tissue extracts rich in tissue factor was intensely studied and repeated many times since it was first reported by De Blaineville (1834).

Trousseau's syndrome is a complication of certain malignancies in which patients develop a chronic low-grade intravascular coagulation and repeated thromboembolic episodes (Sack *et al.*, 1977). This has been attributed at least partly to tissue factor expression on endothelial cells adjacent to tumour cells (Contrino *et al.*, 1996; Kakkar *et al.*, 1998). Tissue factor in tumours is not only expressed in endothelial cells but also in perivascular macrophages, fibroblasts and the tumour cells themselves. Increased expression of tissue factor in tumour cells correlates with poor prognosis and an increased rate of metastasis.

There have been significant advances in understanding tissue factor and its role in coagulation in the last 90 years, but there are still many unresolved issues. Its exact role in tumour development and dissemination, acute rejection of tissue transplants or arteriosclerosis are under investigation by several research teams. However, other questions remain to be answered. What is the role of the cytoplasmic tail of tissue factor in signal transduction or interaction with cytoskeletal proteins (Prydz *et al.*, 1999; Ruf & Mueller, 1999)? Is tissue factor expressed as dimers on the cell surfaces or within caveolae (Mulder *et al.*, 1996; Bach & Moldow, 1997)? Why does the disruption of the tissue factor gene result in embryonic lethality in mice? What is its role in embryonic development? Are there additional roles for tissue factor apart from initiation of thrombin generation in inflammation and arteriosclerosis?

Even after more than 90 years of research in tissue factor, many mysteries remain to be solved. Only one prediction is

accurate: within 20 years, an update of the 'History of Tissue Factor' will be necessary.

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