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Lipid-thrombosis interface

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Remnants produced on the lipolysis of triglyceride-rich lipoproteins provide a contact surface that activates the contact system of coagulation and therefrom factor VII. New evidence is reviewed suggesting that increased levels of circulating activated factor VII (VIIa) initiates coagulation and produces thrombin at higher rate at the site of an atheromatous lesion or at an injury site. This may have profound significance for the propagation of thrombus and for the thrombin-induced inflammatory and proliferative responses.

Vascular homeostasis is achieved by the regulated interaction of the coagulation and fibrinolytic systems. An imbalance in this equilibrium may lead to an increased risk of thrombosis or a bleeding diathesis. The role of PAI-1, a potent inhibitor of enzymes that generate plasmin, in the regulation of fibrinolytic activity, is discussed and the evidence linking the expression of its activity to hypertriglyceridaemia is reviewed. Moreover, the association between lipoprotein (a) and coronary heart disease is attributed to its interference in the normal activation of plasminogen to plasmin.

Blood clotting is generally viewed as a sequential cascade of zymogen activation where a proteolytic enzyme (thrombin) ultimately converts a soluble plasma protein (fibrinogen) into the insoluble cross-linked fibrin (thrombus or clot). Clotting pathways involve loops that amplify or attenuate enzyme generation as well as inhibitors of enzymes all contributing in the control of systemic enzyme levels and local thrombin concentration. Thrombus formation on precipitation of acute coronary events including unstable angina, myocardial infarction and sudden cardiac death, is now well recognized, and is the rationale for thrombolytic therapy. Fibrinolysis is also mediated by plasmin on activation of plasminogen by various enzymes and a number of inhibitors control the

local concentration of plasmin and therefrom fibrinolysis. Considerable advances have been made in understanding the biochemical events that control the rate of thrombin generation at the site of an atheromatous plaque or in response to vascular injury and the potential of the blood vessel wall as well as blood cells to participate actively in coagulation and fibrinolysis. Moreover, we are beginning to understand the role of thrombin as an orchestrator of the response to injury, i.e. thrombin may mediate not only haemostatic but also inflammatory and proliferative or reperative responses that can be relevant to the progression of atheroma. At the same time, an alternative wealth of knowledge has been accumulating from studies on cholesterol and lipoprotein metabolism that is attempting to explain the pathogenesis and development of atheroma by underlying mechanisms consistent with the 'lipid hypothesis'.[1,2] The present review focuses on new evidence that is beginning to expose the relationship between lipoprotein metabolism and initiation of coagulation. Evidence will also be presented suggesting that this link between lipids and the coagulation pathways may be important for normal haemostasis and thrombosis but also for the development and progression of atheroma. Moreover, our present knowledge on the relationship between fibrinolytic activity and plasma lipoprotein levels will be reviewed.

FACTOR VII COAGULANT ACTIVITY AND HYPERCOAGULABILITY

The Northwick Park Heart Study,[3] a prospective cardiovascular survey, has demonstrated that factor VII coagulant activity (VIIc) is, in middle aged men, a better predictor than the plasma concentration of cholesterol for subsequent precipitation of coronary heart disease (CHD) events. Although both variables were independent risk factors, there was a significant positive association between VIIc and the plasma concentration of cholesterol.[3] The relationship between coagulability and hypercholesterolaemia was explored in rabbits fed a cholesterol-supplemented diet. This resulted in a many-fold increase in the plasma concentration of large lipoprotein particles (chylomicron, very low (VLDL) and intermediate density (IDL) lipoprotein fractions) and a considerable increase in VIIc.[4] The major part of the increase in VIIc was due to an increase in the circulating activated factor VII (VIIa) whereas there was also a significant increase in the concentration of the zymogen (factor VII).[4,5] The increase in VIIc in the hypercholesterolaemic rabbit was also associated with an increase in the concentrations of prothrombin and of factor X that were due to an increase in their synthetic rate.[6] The increase in the synthetic rates of prothrombin and

of factor X in this animal model was attributed to an increased rate of thrombin generation (hypercoagulability).[7]

Factor VII is a vitamin K-dependent protein that circulates in human plasma at a concentration of about 450 ng/ml with about 4ng/ml present as the active enzyme factor VIIa.[8] Coagulant activity is expressed when factor VIIa forms a complex with its cofactor, tissue factor (TF) a cell membrane protein present on many cell types beneath the vascular endothelium.[9] The VIIa-TF complex cleaves factors IX and X to their active enzymes,[10] thereby initiating the 'common pathway' of coagulation (Fig.1). Factor Xa in the presence of TF will activate factor VII amplifying thus the prothrombotic signal on expression of TF. The contribution of the 'contact system of coagulation' in the activation of factors IX and VII will be dealt later. The VIIc assay is basically a modified quick prothrombin time assay in which the test plasma factor VIIa is made rate limiting.[11]

Our understanding on the regulation of the basal rate of thrombin generation in vivo and the contribution of various enzymes to haemostasis and thrombosis has improved in recent years with the introduction of plasma assays of prothrombin activation peptide (prothrombin fragment F1.2) and the peptides released on activation of factors IX and X (factors IX and X activation peptides). These activation peptides were assayed in the plasma from patients with various hereditary deficiencies, from normal subjects, and from experimental animals. The administration of purified protein C to protein C-deficient patients showed a sustained increase of protein C activation peptide, from reduced levels, whereas prothrombin fragment F1.2 gradually decreased into the normal range.[12] Protein C a vitamin K-dependent protein plays an important role in the regulation of the haemostatic system, since on its activation by thrombin[13] inhibits the conversion of prothrombin to thrombin via Xa by the inactivation of factors Va and VIIIa (Fig.1). In a 1 year old boy with protein C-deficiency combined with factor IX-deficiency, the prothrombin fragment F1.2 was elevated, before a single infusion of purified factor IX, and further increased during 8 h following the infusion.[14] The latter result can be consistent with the important role of factor IX in the activation of prothrombin.

A normal 'intrinsic coagulation pathway' including circulating factor IX/factor VIII is required for a normal haemostasis. This is evidenced by the severe bleeding diathesis in haemophilia A (deficiency in factor VIII) and haemophilia B (deficiency in factor IX). The administration of recombinant human factor VIIa (rFVIIa) to haemophiliacs is an alternative treatment and provides effective haemostasis,[15] presumably by-passing the intrinsic pathway. rFVIIa was infused into normal chimpanzees[16] and observed significant increases in the plasma lev-

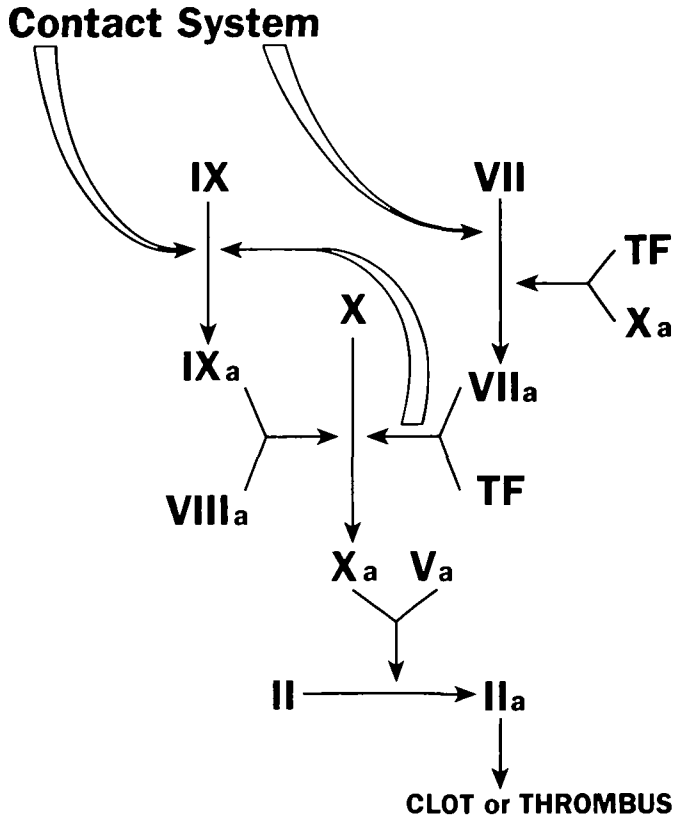

Fig. 1 A schematic model of factors IX and X activation by the extrinsic pathway of coagulation. The Xa generated allows for the formation of the prothrombinase enzyme complex resulting in the generation of thrombin (IIa) and the physiological coagulation response.

els of factor IX activation peptide (58%), factor X activation peptide (2-fold) and of prothrombin fragment F1.2 (114%). The concomitant administration of a potent monoclonal antibody of TF abolished the increase of factor X activation peptide and the increase in prothrombin fragment F1.2, but failed to reduce the increase in factor IX activation peptide. The same antibody administered to chimpanzees not infused with rFVIIa produced a decrease in IX activation peptide (26% at 24 h) and the factor X activation peptide (25% at 24 h) but no change in the levels of prothrombin fragment F1.2.[16] The above results overall suggest that the complex VIIa-TF may, under basal conditions, be im-

portant for the generation of thrombin, factor Xa and factor IXa, but that the circulating level of factor VIIa can provide a measure of the rate of thrombin generation. Consistent with the latter postulate a highly significant positive association between VIIc and prothrombin fragment F1.2 was demonstrated in an adult male population.[17]

THE ROLE OF CIRCULATING LIPOPROTEIN PARTICLES IN THE PRIMING OF THE EXTRINSIC PATHWAY OF COAGULATION

An increased VIIc is a frequent finding in patients with hypertriglyceridaemia[18–20] there being a positive association between VIIc and the concentration of triglycerides in the chylomicron and VLDL fractions of the circulating lipoproteins.[21] Plasma VIIc also increases after a fat-rich meal, a response that is accompanied by little or no change in factor VII antigen, and is due to the generation of circulating factor VIIa.[22–24] A striking exception to the general pattern of association between VIIc and hypertriglyceridaemia occurs, however, in patients lacking functional lipoprotein lipase (LPL). Despite the massive hypertriglyceridaemia in the chylomicron and VLDL fractions in this disorder,[25] neither VIIc nor VII antigen is elevated above normal,[26] suggesting that lipolysis of the triglyceride-rich lipoprotein particles has an important influence on the in vivo activation of factor VII. Activation of factor VII, and therefrom the priming of the extrinsic pathway of coagulation, can be achieved through activation of the contact system and the present evidence exposing the relationship between activation of the contact system of coagulation and lipolysis will be reviewed. Since factor VII (the zymogen) cannot activate factors X and IX, even in the presence of TF,[27,28] the scheme proposed may be important both for normal haemostasis and for thrombosis.

Four proteins, factor XII, prekallikrein, factor XI and high molecular weight kininogen (HMWK) comprise the contact system and a negatively charged surface (contact surface) is required for its activation.[29] Factor XII is unusual in its ability to autoactivate and thus is ideally poised to initiate proteolytic cascades. The best known substrates of activated factor XII (XIIa) include the other proteins of the contact system (prekallikrein, factor XI and HMWK), plasminogen, C1 (first component of complement) and factor VII. In the presence of contact surface, the activation of the contact system of coagulation can generate bradykinin, through the limited proteolysis of HMWK, and can activate the intrinsic pathway of coagulation through the activation of factors XI and IX. The genetically determined deficiencies of factor XII, prekallikrein and HMWK show abnormal in vitro plasma coagulation

but only factor XI deficiency is sometimes associated with a mild to moderate bleeding diathesis.

The observation that long-chain saturated fatty acids (FA) can provide a potent contact surface for the activation of human factor XII in a purified system suggested a physiologically relevant contact surface and a link between hypercoagulability and hyperlipidaemias.[30] Furthermore, a prompt and substantial activation of plasma factor XII was observed when citrated plasma from subjects without functional LPL or plasma from normal subjects that was supplemented with triglyceride-rich lipoprotein particles were treated with LPL.[26]

The relationship between VIIc and factor XIIa induced in citrated plasma was recently investigated.[31] In this experimental model the contact system of coagulation was initiated either by the contact surface present in the plasma from women in late pregnancy[32,33] or by micellar stearate[33] added to plasma diluted with an equal volume of buffer (plasma from normal healthy subjects or from women in late pregnancy). With either contact surface, increase in VIIc and levels of factor XIIa related to the potency of the contact surface. VIIc and XIIa failed to increase in prekallikrein- or factor XII-deficient plasma. The addition of purified human factor XII to factor XII-deficient plasma restored the stearate induced increase in VIIc.[31] The stearate induced VIIc observed in diluted plasmas was inhibited in the presence of anti-factor IX monoclonal antibody (60–70%). Moreover, in factor IX-deficient plasma, the stearate induced increase in VIIc was only 38% of that seen in normal plasma and was restored on addition of purified human factor IX.[31] Thus, in this in vitro system activated factor IX (generated through factor XIa) is responsible for the major part of factor VII activation, whereas the rest is probably through the direct activation by factor XIIa (Fig. 2). An alternative pathway that bypasses factor XIIa in the activation of factor XI was proposed which was based on the observation that in the presence of dextran sulphate (a contact surface) purified human factor XI could autoactivate and that the rate of this process increased many-fold in the presence of thrombin.[34] This pathway for the activation of factor XI was not apparent in the plasma system treated in the presence of stearate[31] and was not confirmed in plasma systems supplemented with dextran sulphate.[35,36]

The importance of factor IX for the in vivo activation of factor VII was demonstrated recently.[37] Employing a specific coagulant assay for factor VIIa[8], it was shown that in normal subjects about 1% of factor VII circulates as factor VIIa whereas in severe factor VIII-deficiency factor VIIa was about 60% and in severe factor IX-deficiency was less than 10% of the factor VIIa circulating in normal subjects.[37] These results are consistent with the postulate that, under basal conditions,

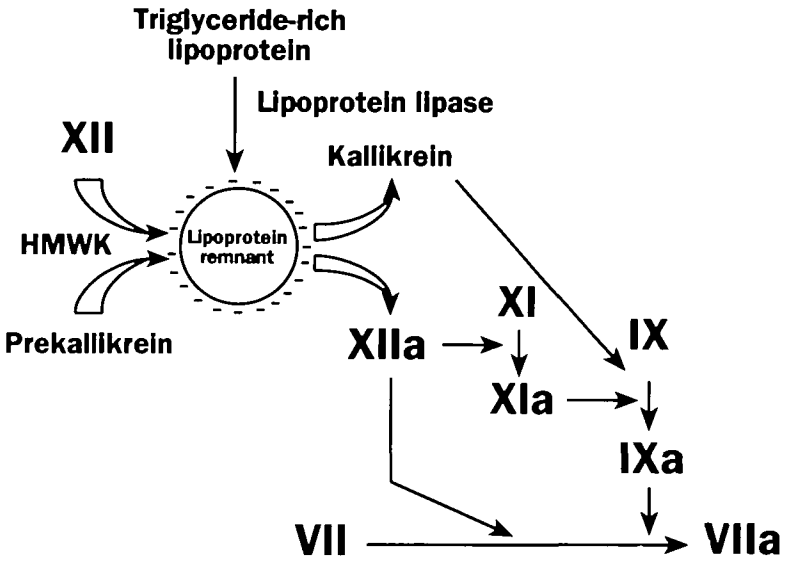

Fig. 2 A scheme linking the electronegative lipoprotein remnant surface to the activation of the contact and intrinsic pathways of coagulation as well as the priming of the extrinsic pathway through the activation of factor VII.

factor IXa is the principal activator of factor VII and that the bleeding diathesis in haemophilias can at least in part be due to the defective priming of the extrinsic pathway of coagulation.

Arachidic (C 20:0), behenic (C 22:0) or lignoceric (C 24:0) acids added to diluted citrated plasma also induced a FA concentration-dependent increase in VIIc.[38] Since oleic acid (C18:1) or other *cis*-unsaturated FA failed to induce increase in VIIc, it was suggested that a requirement for a potent contact surface is one containing immobile negatively charged groups with a critical charge density such as that found in micelles composed of FA in the crystalline phase, in membrane vesicles containing sulphatides,[38] or vesicles composed of negatively charged groups.[33] This requirement can be satisfied by FA in the interface of lipoprotein remnants. Thus, the addition to diluted citrated plasma of lipoprotein remnants, produced on incubation of plasma d<1.006 g/ml fraction with LPL, resulted in a post-incubation increase in the plasma VIIc.[38]

The above in vivo and in vitro observations can suggest that effects of dietary fat on VIIc are produced on release of long-chain saturated FA from the chylomicron and VLDL particles during postprandial lipolysis.

On this account, VIIc was examined in relation to plasma free FA levels in 5 healthy individuals during consumption of isocaloric high saturated fat, high unsaturated fat, and low fat diets, each taken for 4 weeks and separated by intervals of 12 weeks.[24] Plasma VIIc was respectively 6.5 and 13.1% of standard higher on the unsaturated and saturated fat diets than on the low fat diet. Furthermore the plasma concentration of free stearic acid was strongly associated with VIIc and this relationship remained statistically significant after allowance for the plasma concentrations on palmitic, oleic and linoleic acids.[24]

During lipolysis, FA are transferred from the core of the triglyceride-rich particle to the interface and are at physiological pH, in the ionized form, providing thus the negative charge[38] required for the activation of the contact system of coagulation and subsequently activation of factor VII. FA on the remnant interface inhibit LPL but can be transferred to albumin or other membranes to sustain lipolysis. The observations showing an increase in stearic acid and a decrease in oleic acid in the serum free FA fraction relative to the triacylglycerol fraction[24,26] may indicate that the relative residence time of stearic acid on remnant surfaces is longer than that of oleic acid. The segregation of saturated FA on remnant particles may arise from the physical properties of these FA that determine a relatively higher membrane partition coefficient[39] and/or lower affinity for the binding sites of albumin.[38]

THE ABNORMAL CONTROL OF THE CONTACT SYSTEM

At physiological concentration of the components of the contact system, rates of its activation are rather determined by the potency of the contact surface.[31] The major inhibitor of this cascade is C1-inhibitor (C1-INH), which contributes more than 90% of the plasma inhibitory activity towards XIIa. C1-INH is also the major inhibitor of kallikrein with less important contribution of α_2-macroglobulin. C1-INH-deficiency (hereditary angioedema), an autosomal dominant disorder, involves recurrent episodes of oedema of the gastrointestinal track, of the skin and subcutaneous tissues. The uncontrolled activation of the contact system and bradykinin generation during an attack may account for much of the clinical symptoms.[29]

Bradykinin is one of the most potent vasodilators known, which may contribute to the hypotension observed in septecaemia.[40] Endotoxin, a major cell wall component of Gram-negative bacteria or cell wall components of Gram-positive bacteria interact with the endothelium and inflammatory cells, releasing endogenous mediators such as cytokines, hydrolyses, prostaglandins and amines that contribute to the pathogenesis of septic shock. The expression of the TF gene in vascular endothelium mediated by the cytokine tissue necrosis factor may

be responsible for the disseminated intravascular coagulation and the severe failure of multiple organ systems. In contrast, changes in the contact system of coagulation are not seen in patients with disseminated intravascular coagulation in conditions such as malignancies or tissue injury, where endothelial cell mediated changes are not the primary mechanism. In an experimental animal model of lethal bacteraemia,[41] a significant correlation was shown between the decline of HMWK levels and the development of irreversible hypotension. In this experimental animal model an antibody against factor XII inhibited hypotension and prolonged survival.[42] Infectious and inflammatory diseases are frequently associated with hypertriglyceridaemia and increased serum free FA levels. This may be relevant to both the activation of the contact system of coagulation and the abnormal regulation of the fibrinolytic pathway. The mechanisms involved in endotoxin/cytokines-induced hypertriglyceridaemia in rodents have recently been investigated.[43]

Activation of proteins of the contact system may also play an important role in the pathogenesis of adult respiratory distress syndrome (ARDS) through stimulation of neutrophils that express and release substances that can cause endothelial and epithelial injury. Patients with the adult respiratory distress syndrome, due either to trauma or sepsis, have significantly reduced plasma levels of factors XII, prekallikrein and HMWK presumably due to their sustained activation.[29] In cardiopulmonary bypass or during simulated extracorporeal circulation, the contact and complement systems, platelets and neutrophils are activated. The generation of kallikrein leads to neutrophil degranulation and the release of elastase. The selective inhibition of kallikrein blocked its generation but also inhibited elastase release.[44] Products of the contact system,[45] the fibrinolytic system[46] and the complement system may also be involved in anaphylaxis in which mast cell degranulation and the release of a host of mediators signals the onset of symptoms.

THE EXPRESSION OF TF

The formation of the binary complex TF-VIIa results in efficient conversion of factors X and IX to factors Xa and IXa with an increase of the overall proteolytic efficiency, relative to free factor VIIa, by approximately 10^{7}.[47] TF-producing cells were recently identified in normal vessels and atheromatous plaques by *in situ* hybridization and immunohistochemistry. mRNA and protein were absent from endothelial cells lining normal artery and vein samples but were synthesized in scattered cells present in tunica media as well as fibroblast-like adventitial cells surrounding vessels.[48] In contrast, atheromatous plaques contain many cells synthesizing TF mRNA and protein suggesting that deposition

of TF protein on the matrix and the necrotic core of the plaque may contribute to the hyperthrombotic state of the diseased vessel.[48]

Experimental animal aortic baloon injury and cells in culture have recently provided information on the sequence of gene expression in response to stimulus. An early event in response to vessel wall injury is the induction of TF in vascular smooth muscle cells and consequently the propagation of thrombus.[49] TF activity was very high in uninjured adventitial cells and remained high following baloon injury. In contrast, TF activity was minimal in uninjured medial preparations, but rose markedly within 24 h of injury remaining high for 48 h.[49,50] TF mRNA was not detected in quiescent vascular smooth muscle cells but the presence of various agonists, including α-thrombin, induced a rapid and marked rise in TF mRNA and TF protein synthesis with a similar for all agonists time course.[50]

In endothelial cell cultures, TF mRNA and/or procoagulant activity is induced by α-thrombin, endotoxin or cytokines expressed by endotoxin.[51] Similar results were shown in monocytes where TF can be induced by a variety of mediators of inflamation and antigen-specific cellular immune responses.[52]

THROMBUS FORMATION AND ITS PROPAGATION

The activation of the contact system of coagulation in the interface of negatively charged lipoprotein remnants and the subsequent activation of factor VII is expected to increase the circulating levels of VIIa and consequently contribute to the basal rate of thrombin generation. The presence of increased levels of factor VIIa in circulation is probably of little significance to healthy individuals with minimal expression of TF at the vasculature. However, at a site of an atheromatous plaque or at a vascular injury site, where there is TF activity, increased levels of factor VIIa is expected to generate thrombin at a high rate. Moreover, the propagation of this thrombus may be augmented on expression of additional TF.

Coagulation was defined as a cascade of sequential zymogen activations that achieves the haemostatic balance. What has emerged in recent years to integrate the above definition is the participation of vascular and blood cells and of cell surface receptors, to the maintenance of haemostatic balance. Thus, in addition to its role in blood coagulation, thrombin is also a powerful agonist for a variety of cellular responses. Thrombin is a potent activator of platelet aggregation, is chemotactic for monocytes and mitogenic for lymphocytes, fibroblasts and vascular smooth muscle cells. Thrombin acts on the vascular endothelium to stimulate generation of prostacyclin, platelet-activating factor, plasminogen activator inhibitor and the potent smooth muscle cell mito-

gen, platelet derived growth factor. The recent discovery of thrombin receptors expressed by a variety of cells in culture and the use of experimental models of vessel wall injury are helping to rationalize these disparate functions and view thrombin as a mediator of inflammatory and proliferative responses such as those that occur in atherosclerosis and restenosis.[53] Within the receptor's amino terminal exodomain is a thrombin cleavage site and its proteolysis by thrombin generates a truncated form of the receptor, competent to initiate intracellular signalling and cell activation.[53] In normal-appearing human arteries, thrombin receptor was expressed almost exclusively in endothelial cells, whereas in atheroma was widely expressed in regions rich in macrophages and smooth muscle cells.[54] Like other signalling molecules, thrombin effects concentration-dependent and graded responses in its target cells.[55] The distant aim may be to by-pass the lipid-thrombus interface by developing new strategies that have as a goal the protection of the vessel wall.[56]

PROSTANOIDS AND DIETARY FAT

Prostacyclin (PGI_2) was the first prostanoid identified[57] and is derived mainly from the endothelium. It has potent antiaggregatory properties on platelets and can also disaggregate platelets which have already aggregated. PGI_2 is synthesized from arachidonic acid released from the plasma membrane after its hydrolysis from the *sn*-2 position of the membrane phospholipid by phospholipase A_2.[58] The free arachidonic acid is converted by the cyclo-oxygenase enzymes to endoperoxides and subsequently to prostanoid by the PGI_2synthase. Both these enzymes are also present in endothelial and smooth muscle cells. The omega-3 fatty acid, eicosapentaenoic acid, which is abundant in fatty fishes can also be substrate for cyclo-oxygenase and the resultant PGI_3 is equipotent to PGI_2.[59] In the platelet endoperoxides produced by cyclo-oxygenase are metabolized by thromboxane synthase[60] to thromboxane A_2 (TXA_2) which is a potent inducer of platelet aggregation.[61] The omega-3 fatty acid TXA_2 analogue, TXA_3, is less potent in inducing platelet activation[59] *(see below)*.

Prostacyclin (PGI_2) and thromboxane (TXA_2) have opposing effects on haemostasis and thrombosis. Aspirin irreversibly acetylates a serine residue of cyclo-oxygenase enzyme,[62] inhibiting thus TXA_2 and PGI_2 synthesis and secretion from the platelets and endothelial cells respectively. Platelets lack nuclei and are therefore unable to synthesize new cyclo-oxygenase so that the effect of aspirin persists for 7–10 days,[63] whereas endothelial cells, which are responsible for PGI_2 production, can synthesize new enzyme within a few hours.

PGI_2 production is stimulated by agonist occupation of endothelial cell receptors, e.g. by thrombin, bradykinin or adenine nucleotides which results in receptor coupled induction of phospholipases A_2.[64]. For instance potent cytokines, such as interleukin-1 or tumor necrosis factor-α have been shown to induce synthesis and secretion of phospholipase A_2 by rat mesangial cells and a parallel increase in PGE_2.[65] Classically, prostanoid synthesis is regulated by the availability of precursor fatty acid which is released by the induced phospholipase A_2. However, co-induction of the lipolytic enzyme and cyclo-oxygenase may result in a synergistically increase synthesis of the prostanoid.[65]

The low incidence of CHD in Eskimos is attributed to their high dietary intake of omega-3 fatty acids (eicosapentaenoic and docosahexaenoic acids) in fatty fish.[66] Eicasapentaenoic acid competes with arachidonic acid for the platelet cyclo-oxygenase resulting in synthesis of TXA_3, which is less potent platelet aggregatory agent than TXA_2.[59] Eicosopentaenoic acid can also be the substrate of endothelial cyclo-oxygenase resulting in synthesis of PGI_3 as inhibitor of platelet aggretation.[59]Therefore omega-3 fatty acids may promote an antithrombotic state by substituting potent TXA_2 for the less potent TXA_3. Consistent with the above, administration of a fish containing diet to survivors of myocardial infarction demonstrated significantly reduced mortality or reinfarction.[67]

The increased dietary intake of fat rich in omega-6 fatty acids, usually contributed by linoleic acid, is followed by an increased linoleic acid contribution in the platelet phospholipid together with a concomitant decrease in the contribution of arachidonic acid.[68] The relation of these changes to the platelet function in vivo and their contribution to the thrombotic state are not understood. However, the poineering studies of Bang and colleagues[69] comparing omega-3 with omega-6 polyunsaturated dietary fat to motality from CHD may be relevant to both the thrombotic and atherosclerotic elements of CHD. The Danes (high incidence of CHD) had twice as much linoleic acid in their food than the Eskimos (low incidence of CHD) and therefore the linoleic acid contribution to the platelet phospholipids in the Eskimos was less than half of that of the Danes. However, the Eskimos consumed nearly twice as much monounsaturates and 5-times as much omega-3 fatty acids as the Danes. The seven countries study[70] showed a negative correlation between monounsaturated (mainly oleic acid) fat consumption (and also the monounsaturated to saturated fatty acid ratio) and all causes death rate. The CHD death rates were lowest in cohorts with olive oil (mainly oleic acid) as the main dietary fat. The influence of dietary fat composition on total plasma cholesterol, lipoprotein cholesterol and plasma triglyceride levels has been the object of numerous epidemiological,

intervention and clinical trials. Conclusions from this research have provided the foundations of the lipid hypothesis, although many studies have arrived at conflicting evidence. Moreover, the study of platelets in vitro as an index of thrombogenicity has been unrewarding. Given our present state of knowledge, the most important question on the relationship between dietary lipids and CHD is to recommend appropriate diets that achieve maximum anti-atherosclerotic and anti-thrombotic effects already seen in populations that have reduced the contribution of saturated fatty acids and of cholesterol in the dietary fat. Recent reviews on the relationship betwen dietary fat and CHD provide a detailed account of the relevant research effort.[71–73]

HYPERLIPIDAEMIAS AND THE FIBRINOLYTIC PATHWAY

It is generally recognized that vascular homeostasis results from the regulated interaction of the coagulation and fibrinolytic pathways. These systems appear to be in dynamic equilibrium and any imbalance may lead to an increased risk of thrombosis or the tendency to develop bleeding diathesis. The fibrinolytic pathway is initiated on generation of the fibrin-degrading protease, plasmin, from plasminogen. The physiologically important enzymes that generate plasmin are tissue plasminogen activator (tPA) and urokinase (uPA), although some contribution of factor XIIa has been shown.[74] Several distinct types of plasminogen activator inhibitors (PAI) have been described but PAI-1 appears to be the physiological inhibitor of both tPA and uPA. PAI-1 is probably synthesized in the endothelium[75] but many other types of cells can express PAI-1. Cultured endothelial cells produce PAI-1 in response to a variety of stimuli including α-thrombin.[76]Although PAI-1 is present in trace amounts in plasma, its concentration may increase rapidly in response to a variety of agents or changes in the physiological state. Assay of PAI-1 is complicated by the presence in plasma of a latent form that can even be activated by negatively charged membranes.[77] However, the importance of PAI-1 in the regulation of fibrinolysis and its impact on thrombosis has recently been appreciated. For example, transgenic mice with overexpression of the human PAI-1 gene develop venous thrombosis[78] whereas human PAI-1 deficiency was reported as a cause of abnormal bleeding.[79,80] The coincident expression of PAI-1,[81] and of thrombin receptors[54] within an atheromatous plaque [48] may suggest that an imbalance in the fibrinolytic system may also contribute to thrombus propagation and the progression of atheroma.

Numerous studies have examined the relationship between plasma triglyceride levels and circulating PAI-1 in patients at risk for CHD as well as in hypertriglyceridaemic subjects with no symtoms of CHD (reviewed recently in [82, 83]). In the ECAT angina pectoris study[84] PAI-1

levels increased 2-to 3-fold between the lowest and the highest quintile of triglyceride, body mass index, and insulin. Multiple regression analysis has suggested that the relationships between PAI-1 and body mass index, body fat distribution, blood pressure or triglyceride levels are generally secondary to the relationship between PAI-1 and insulin.[84] The postulate that insulin resistence influences the synthesis of PAI-1 via its effects on lipoprotein metabolism was investigated using hepatocytes or endothelial cells in culture. VLDL isolated from hypertriglyceridaemic patients was shown to increase PAI-1 synthesis in endothelial cells[85,86] and the effect was abolished by an antibody against the apo B receptor.[85] LDL has no effect on endothelial cell synthesis of PAI-1[85, 87] or it induces increased synthesis by a process that is independent of the apo B receptor.[88] LDL minimally oxidized increased PAI-1 levels in the conditioned media of endothelial cells[87, 89] whereas extensive oxidation of LDL prevented and even reversed the effect of LDL on PAI-1 release by endothelial cells.[88]

Lipoprotein (a) (LP (a)) is a genetically determined lipoprotein particle that contains a specific apolipoprotein, apoprotein (a) (apo (a)), that is highly homologous to plasminogen. Essentially, the only difference between LP (a) and LDL is the presence of a single molecule of apo (a) that is attached to the apolipoprotein B of the particle by a disulphide bond. The concentration of LP (a) in the plasma varies widely between individuals and virtually all this variation can be attributed to the apo (a) gene.[90] Thus levels within an individual appear to remain remarkably constant. The association between LP (a) and CHD is well established and confirmatory studies continue to be published.[91,92] However, the molecular mechanism of the contribution of LP (a) in the pathogenesis of thrombosis and atherosclerosis has not been clarified. The similarity in structure between apo (a) and plasminogen has led to the suggestion that much of its thrombotic and atherosclerotic potential derives from its interference in fibrinolysis, notably by inhibiting the rate of plasmin generation. It was recently shown that during ongoing plasminogen activation, both plasminogen and apo (a) – in the form of LP (a) – bind to the fibrin surface and that the generation of plasmin depended on the concentration of both proteins.[93]

CONCLUSIONS

The molecular mechanism involved in the interaction of lipoprotein metabolic products and the coagulation pathways is only beginning to be understood. The activation of the contact system of coagulation at the interface of lipoprotein remnants and the subsequent activation of factor VII may have profound significance for haemostasis, thrombosis and the progression of atheromatous lesion. It is now generally accepted

that the initiation of the extrinsic pathway of coagulation requires the interaction of TF with factor VIIa and that factor VIIa in the absence of TF can be generated through enzymes derived on activation of the contact system. Activation of factor VII in vivo via the contact system is recognized and is the rationale for the use of factor VIIa in the clinical treatment of haemophilias (by passing the intrinsic pathway of coagulation). This priming of the extrinsic pathway of coagulation and thereby the levels of circulating factor VIIa may be of little immediate consequence for a healthy vessel wall with minimal expression of TF. However, at the site of an atheromatous plaque, or that of injury, an increased level of circulating factor VIIa will determine locally a higher rate of thrombin generation. Thrombin's concentration, at this site may be important not only for haemostasis but also for the inflammatory, proliferative or reparative responses. The propagation of thrombus and the progression of atheroma may be the outcome of these responses. The above may explain at least in part the interrelations between VIIc, hypercoagulability, plasma triglyceride concentration, LPL functional activity and risk of coronary thrombosis.[3] It is also relevant to the present thesis that patients with hypertriglyceridaemia and functional LPL are at increased risk of CHD,[94] whereas those with LPL deficiency appear to be at much lower risk.[95]

The exposed relationship between circulating lipoprotein remnants and the activation of the contact system may present new research opportunities aiming at increasing our understanding on the pathogenesis of numerous conditions that involve an abnormal regulation of the contact system (including those of atherosclerosis and CHD) and on the link between the various risk factors for CHD that clinical studies have been uncovering. The relevance of the contact system to normal haemostasis and thrombosis was neglected since patients deficient in component proteins show no bleeding diathesis and since the putative contact surface for its activation in vivo was not known. The new level of understanding may suggest strategies for the primary and secondary prevention of CHD or the treatment of other conditions that involve abnormal activation of the contact system.

An extensive biomedical literature attests to the controversy regarding the mechanism by which hyperlipidaemia influences fibrinolytic activity and particularly the expression of PAI-1. What is becoming very clear is that a balance between the rate of thrombin and fibrinolytic activity generation is achieved in normal haemostasis and that any factors that interfere with the normal control of the rate of plasmin generation can result in thrombosis or bleeding diathesis.

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