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Tissue Factor Pathways linking obesity and inflammation

Wolfram Ruf¹ and Fahumiya Samad²

¹Center for Thrombosis and Hemostasis, University Medical Center, Mainz, Germany; and Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA, USA

²San Diego Biomedical Research Institute, 10865 Road to the Cure, San Diego, CA 92121, USA

Abstract

Obesity is a major cause for a spectrum of metabolic syndrome-related diseases that include insulin resistance, type 2 diabetes, and steatosis of the liver. Inflammation elicited by macrophages and other immune cells contributes to the metabolic abnormalities in obesity. In addition, coagulation activation following tissue factor (TF) upregulation in adipose tissue is frequently found in obese patients and particularly associated with diabetic complications. Genetic and pharmacological evidence indicates that TF makes significant contributions to the development of the metabolic syndrome by signaling through G protein-coupled protease activated receptors (PARs). Adipocyte TF–PAR2 signaling contributes to diet-induced obesity by decreasing metabolism and energy expenditure, whereas hematopoietic TF–PAR2 signaling is a major cause for adipose tissue inflammation, hepatic steatosis and inflammation, as well as insulin resistance. In the liver of mice on a high fat diet, PAR2 signaling increases transcripts of key regulators of gluconeogenesis, lipogenesis and inflammatory cytokines. Increased markers of hepatic gluconeogenesis correlate with decreased activation of AMP-activated protein kinase (AMPK), a known regulator of these pathways and a target for PAR2 signaling. Clinical markers of a TF-induced prothrombotic state may thus indicate a risk in obese patient for developing complications of the metabolic syndrome.

Linkage of obesity to coagulation activation and thrombosis

Rising rates of overweight and obesity affect many industrialized and increasingly developing nations. Long term complications of obesity are expected to escalate accordingly, as epidemiological studies have documented the risk of obesity for the development of cardiovascular diseases and types 2 diabetes. The association of metabolic complications of obesity and cardiovascular mortality is specifically linked to central obesity, mirroring experimental data of increased susceptibility of rodent visceral adipose tissue to obesity-induced inflammation. Importantly, obesity is also a risk factor for developing arterial thrombosis and venous thromboembolism (reviewed in (1)). Weight loss by interventions such as diet, exercise, and gastric bypass surgery not only improves insulin sensitivity, but also reduces circulating levels of prothrombotic markers, including tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1), and reverse platelet dysfunction.

There is strong clinical evidence for activation of coagulation in both obese and diabetic individuals. Increased activity of the TF pathway is indicated by levels of surrogate markers of thrombin generation, such as prothrombin fragment F1.2 and thrombin–antithrombin (TAT) complexes, monocyte and plasma TF procoagulant activity, and circulating microparticle with TF activity (1). Importantly, activation of coagulation is observed in obese children and young adults, indicating an association of prothrombotic risk with obesity independent of age-related development of cardiovascular disease (2).

Prothrombotic abnormalities are recapitulated in animal models, making those highly valuable tools for deciphering mechanisms by which the hemostatic system contributes to the metabolic syndrome. TF is increased in the blood, adipose tissue, and adipose-infiltrating macrophages (3;4) and in accordance with human studies weight loss diminishes coagulation markers and adipose tissue inflammation (5). In addition, experimental studies, reviewed in more detail below, have defined pathogenic mechanisms by which TF and its cellular signaling functions promote weight gain and adipose tissue inflammation. Importantly, gene variants in protease activated receptor (PAR) 2 (F2RL1) are associated with individual variability in the body mass index (BMI) identified by population admixture mapping (6), providing initial epidemiological evidence for a crucial role of protease signaling in the development of obesity and resulting cardiovascular complications.

TF procoagulant function and signaling

TF initiates the coagulation cascade by binding and allosterically activating coagulation factor VIIa (FVIIa) (7). TF is structurally related to cytokine receptors, but unlike these receptors, TF lacks typical tyrosine kinase recruitment motifs in the cytoplasmic domain that instead is involved in the regulation of integrin function and MAP kinase p38 signaling (8–12). The extracellular domain of TF interacts with integrins (9). Ligation of endothelial cell expressed integrins is also the major function of the soluble, alternatively spliced isoform of TF (asTF) that promotes angiogenesis and monocyte recruitment (13;14). In contrast to direct integrin interaction of asTF, full-length, transmembrane anchored TF in addition forms a ligand FVIIa induced integrin complex supporting proteolytic activation and signaling of PAR2 (15). TF is therefore a functional receptor involved in both, cell signaling and coagulation.

Certain cell types, such as monocytes or macrophages, display little TF procoagulant activity, unless exposed to a secondary signal promoting rapid thiol-disulfide exchange- and protein disulfide isomerase- (PDI) dependent TF activation. TF becomes procoagulant, independent of de novo protein synthesis, following complement fixation to monocyte cell surfaces (16) or stimulation of the macrophage P2X7 receptor by the cell injury signal ATP (17). Of note, in the obese visceral adipose tissue, macrophages cluster around stressed and apoptotic adipocytes in so-called crown-like structures. The close proximity of these cells provides both free fatty acids that induce TF gene transcription downstream of toll like receptor signaling as well as injury mediators triggering subsequent TF procoagulant activation, as evidenced by local fibrin deposition detected by immunohistochemistry (1).

The binary TF-FVIIa signaling complex has no appreciable procoagulant activity, but fully supports proteolytic signaling specifically through PAR2 in a variety of pathological conditions (18). TF signaling also occurs in the initiation phase of coagulation by the nascent product FXa that cleaves either PAR1 or PAR2 (19). Importantly, signaling of the TF-FVIIa-FXa coagulation initiation complex requires stabilization and/or proper positioning of FXa by the endothelial protein C receptor (EPCR) (20). Additional complexity of protease signaling, in particular in the inflamed adipose tissue, arises from the sensitivity of PAR2 to a variety of extravascular proteases (21), including elastase that can promote inflammation and insulin resistance in the absence of tight inhibitory control (22). A better understanding of the contributions of coagulation proteases to PAR2-dependent pathologies will therefore require new mouse models with altered PAR2 sensitivity to specific proteolytic activation which can be developed guided by biochemical data (23).

TF contributions to the development of obesity

Inflammatory and metabolic changes increase TF expression in adipocytes and macrophages in the obese adipose tissue (3;4). Although TF is typically linked to thrombotic complications in cardiovascular diseases, genetic mouse models have defined roles for TF-dependent signaling in the development of obesity. The TF cytoplasmic domain is not relevant for TF procoagulant activity, but mice lacking this domain (TF^{CT} mice) display attenuated weight gain, increased energy expenditure, and improved glucose homeostasis under a high fat diet (3). Loss of thrombin signaling through PAR1 in a variety of tissues, excluding mouse platelets that do not express PAR1, has no influence on weight gain. However, mice deficient of PAR2, the major signaling receptor of TF protease complexes, show reduced high fat diet-induced obesity and insulin resistance. Bone marrow chimeric mice have demonstrated that the obesity-resistant phenotype of these mouse strains originated from non-hematopoietic cells. In a pharmacological proof of principle experiment with an antibody that interferes with FVIIa binding to TF, TF antibody blockade rapidly improves metabolism and fatty acid oxidation independently of weight reduction and changes in food intake or activity.

In vitro studies have delineated a TF signaling pathway regulating primary adipocyte function. TF cytoplasmic tail-dependent signaling induced by ligand FVIIa binding blunts both basal and insulin-mediated Akt activation and alters the expression of Akt target genes known to regulate weight gain. FVIIa-induced suppression of Akt downregulates adiponectin, known to be beneficial in weight regulation, and upregulates PAI-1, known to promote weight gain. These gene expression changes in adipocytes are prevented by an antibody that blocks FVIIa binding to TF, data confirmed by treatment experiments in vivo (3). Since weight control by adiponectin is linked to the regulation of AMP-activated protein kinase (AMPK) activation and PAR2 signals in a G protein-independent manner through cytosolic recruitment of β -arrestin 2 leading to inhibition of AMPK in adipocytes (24), suppression of AMPK signaling may be another component of the obesity promoting effects of TF-PAR2 signaling.

TF-initiated cell signaling in adipose tissue inflammation

Chronic inflammation significantly contributes to the development of insulin resistance. Inflammatory changes are particularly observed in the visceral adipose tissue that becomes infiltrated by macrophages changing to a proinflammatory phenotype in the context of free fatty acids and endogenous danger signals released from stressed adipocytes (25). Macrophages are key players in tissue homeostasis and can be activated by a variety of environmental cues to display either an inflammatory or a spectrum of alternatively activated phenotypes that regulate inflammation and support tissue repair and angiogenesis. Macrophages therefore control adipose tissue inflammation, but they are also embedded as a sensor for alterations in the local immune environment in obese adipose tissues. Indeed, the immune cell composition of the adipose tissue changes during the development of obesity with shifts to CD8⁺ T cells and interferon γ producing CD4⁺ T cells at the expense of regulatory CD4⁺ T cells. Eosinophils are rapidly outnumbered by mast cells and neutrophils, which participate in the chronic inflammatory response observed in obesity.

Weight loss or protection from diet-induced obesity, as seen in mice with abolished TF-PAR2 signaling (3), typically reduce the inflammatory macrophage population in the visceral adipose tissue and, importantly, genetic ablation of adipose tissue macrophages rapidly improves insulin sensitivity and ameliorates glucose intolerance in obese animals (26). Proinflammatory macrophages secrete tumor necrosis factor α and interleukin (IL) 1 β and 6 that reduce insulin sensitivity of adipocytes, hepatocytes, and muscle cells. Conversely, IL10 that can be produced by alternatively activated macrophages reverses the insulin-desensitizing effects of proinflammatory cytokines in target tissue.

The characterization of bone marrow chimeric mice showed that hematopoietic TF-PAR2 signaling made no contributions to the development of obesity, but unexpectedly the recruitment, maturation, or retention of inflammatory macrophages and consequently the development of insulin resistance were markedly attenuated (3). These data are consistent with evidence that TF contributes to endothelial transmigration of monocytes (27) and the regulation of integrins by TF cytoplasmic domain signaling. It is of interest that one of the integrins known to interact with TF (9), the leukocyte expressed integrin $\alpha 4 \beta 1$ integrin, also contributes to adipose tissue inflammation under similar high fat diet regimens (28).

In addition to their roles in macrophage recruitment, TF and PAR2 also regulate myeloid cell activation. Reactive oxygen species production by anti-phospholipid antibodies is abolished in TF^{CT} mice (29), PAR2-deficiency in microglia attenuates TNF α and IL6 production, while increasing IL10 (30), and PAR2 crosstalk with toll like receptors is contributing to the induction of the NF κ B pathway (31;32). However, PAR2 has clearly more complex roles in the regulation of inflammatory processes, since PAR2 contributes to alternative activated phenotypes of macrophages following endotoxin stimulation (33). Additional complexity arises from the variety of proteases that can activate PAR2 to modulate immune and non-immune cell functions (21). In the context of obesity, recruitment and activation of mast cells may trigger mast cell tryptase-dependent PAR2 activation. Neutrophil-derived elastase of importance for the development of obesity (22) may not only interrupt the control of TF-dependent coagulation (34) and signaling (35) by

TF pathway inhibitor (TFPI), but yield biased agonist PAR2 signaling through non-canonical PAR2 cleavage (36).

Despite these complexities, TF-dependent signaling of innate immune cells is strongly implicated in the development of adipose tissue inflammation and imbalances in glucose homeostasis. Indeed, short term targeting of TF signaling in the hematopoietic compartment with an antibody selectively blocking signaling, but not coagulation (15) resulted in a switch in inflammatory cytokine profile and improvement in insulin sensitivity (3). This rapid improvement of glucose homeostasis and changes in adipose tissue macrophage phenotypes resembled the effects of discontinuing high fat diet feeding of mice (37) or treatment of obese mice with omega-3 fatty acids that activate the G-protein coupled receptor 120 (38). Together with the genetic and pharmacological evidence for pathogenic roles of PAR2, these studies highlight the importance of G-protein coupled receptor signaling in the adipose tissue and obesity-associated metabolic disturbances.

Inflammatory TF-PAR2 signaling in hepatic gluconeogenesis and lipid metabolism

The liver plays a central role in lipid metabolism and maintaining glucose homeostasis. Obesity alters liver function and obese patients with metabolic complications may develop non-alcoholic fatty liver disease (NAFLD), a disease that ranges from fatty liver to the more severe condition of steatohepatitis that can progress to fibrosis and cirrhosis. Local inflammation induced by de novo recruitment of macrophages to the liver is an increasingly recognized contributor to both NAFLD and hepatic insulin resistance. We therefore evaluated with available pharmacological and genetic approaches the contributions of TF-PAR2 signaling to the development of hepatic steatosis and insulin resistance (39). From these experiments, additional details emerged on pathways of lipogenesis and gluconeogenesis that are specifically influenced by hematopoietic TF-PAR2 signaling (Figure 1).

Diet-induced obesity in mice produces a shift in innate immune cell populations from CD11b⁺/CD11c⁻ myeloid cell population to inflammatory CD11b⁺/CD11c⁺ macrophages. Cell tracing studies in bone marrow chimeric mice show that these cells derived from hematopoietic precursors and not from self-renewing liver resident macrophages (39). CD8⁺ T cells are concomitantly increased, while CD4⁺ T cells are decreased compared to lean mice. As previously seen in the visceral adipose tissue of obese mice (3), TF is expressed at high levels in liver CD11b⁺/CD11c⁺ macrophages from obese mice. Compared with obese controls, influx of CD11b⁺/CD11c⁺ macrophages is decreased in TF^{CT}, but not PAR2^{-/-} mice. However, both mutant mice display concordant reductions in CD8⁺ T cells and increases in CD4⁺ T cells compared to controls on a high fat diet. Moreover, pharmacological TF-PAR2 signaling blockade also predominantly reduces CD8⁺ T cell populations, identifying a major pathway that is specifically dependent on TF-dependent PAR2 signaling. The pharmacological and genetic evidence indicates that TF signaling in hepatic inflammation influences interferon γ expressing CD8⁺ immune cell populations, while anti-inflammatory effects of TF blockade are related to an upregulation of IL10 in CD11b⁺ myeloid cells (39).

TF and PAR2 signaling blockade concordantly affect pathways of hepatic gluconeogenesis that depends in the rate limiting expression of phosphoenolpyruvate carboxykinase 1 (PEPCK-1) and glucose-6-phosphatase (G6P). These elevated transcripts in obese mice are reduced along with signs of improved insulin sensitivity, for example increased insulin-induced Akt phosphorylation, in mice with defective hematopoietic TF-PAR2 signaling. Consistent with reduced hepatic steatosis in TF-PAR2 signaling deficient mice, increased gene expression of the lipogenic transcription factor, sterol regulatory element-binding protein 1c (SREBP1c) and its target fatty acid synthase (FAS) is attenuated compared to wild-type controls on the same high fat diet. Short term and prolonged blockade of TF also improves these measures of glucose homeostasis and reduces lipogenesis. Since plasma triglycerides levels are not significantly affected by long term anti-TF treatment, it appears that the primary effect of proinflammatory TF-PAR2 signaling in the liver is de novo lipogenesis leading to steatosis.

The upregulation of TF as a consequence of hypernutrition also causes diseases mediated by its procoagulant function. Genetically caused obesity due to leptin receptor mutation in db/db mice increases adipose tissue inflammation and insulin resistance that are improved by treatment with a selective thrombin inhibitor (40). In steatohepatitis induced by a diet deficient in methionine and choline or Western diet-induced hepatic steatosis, TF-dependent thrombin generation and PAR1 signaling drive macrophage and neutrophil accumulation and hepatic inflammation (41;42). Emerging evidence further indicates that hepatotoxic injury may upregulate TF in hepatocytes (43). While these data underscore the importance of TF-initiated pathologies in various aspects of the metabolic syndrome, much remains to be elucidated about cell-type specific roles of TF and its signaling receptors in regulating inflammation and metabolism.

Future perspectives and clinical implications

These preclinical studies provide proof-of-principle evidence that targeting TF-dependent PAR2 signaling (3;39), PAR2 antagonism (44), and pharmacological blockade of thrombin (40) improve metabolic complications of obesity. While there is also expanding clinical data supporting a close association of increased coagulation activation with diabetes and particularly the metabolic complications of obesity (1;45–47), little is known about the effects of anticoagulant therapy on the development of the metabolic syndrome in obese patients. Future studies should particularly focus on the nexus between coagulation, adipose inflammation and insulin resistance and how these pathways are influenced by conventional or new classes of protease selective oral anticoagulants.

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Figure 1.
Scheme of the cell type specific effects of TF-PAR2 signaling in obesity and metabolic syndrome.