TAM receptors, Gas6 and protein S: roles in inflammation and hemostasis

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Abstract
TAM receptors (Tyro3, Axl and Mer) belong to a family of Receptor Tyrosine Kinases that have important effects on hemostasis and inflammation. Also, they affect cell proliferation, survival, adhesion and migration. TAM receptors can be activated by the vitamin K-dependent proteins Gas6 and protein S. Protein S is more commonly known as an important co-factor for protein C, as well as a direct inhibitor of multiple coagulation factors. To our current knowledge the functions of Gas6 are limited to TAM receptor-activation. When activated, the TAM receptors have effects on primary hemostasis and coagulation, and they display an anti-inflammatory or a pro-inflammatory effect, depending on cell-type. To comprehend the effects that the TAM receptors and their ligands have on hemostasis and inflammation, we compare studies that report the different phenotypes displayed by mice with deficiencies in the genes of this receptor family and its ligands (protein S⁺⁻, Gas6⁻⁻, TAM⁻⁻, and variations to these). In this manner we aim to display which features are attributable to the different ligands. Because of the effects the TAM receptors have on hemostasis, inflammation and cancer growth, their modulation could make interesting therapeutic targets in thrombo-embolic disease, atherosclerosis, sepsis, autoimmune disease and cancer.

Introduction
In recent years, views on hemostasis and inflammation have shifted from a concept of two independent areas of biology, towards two closely related processes. It is now appreciated, that molecules that affect hemostasis often have an effect on inflammation and vice-versa. In this review we explore this dual role for Gas6, protein S and TAM receptors.
The TAM receptors are one of 20 subfamilies of Receptor Tyrosine Kinases (RTKs). First cloned in 1991, they were considered orphan receptors until 1995. In that year their ligands, protein S and Growth Arrest-Specific gene 6 (Gas6), were identified. Members of the TAM receptor family are Tyro3 (also called Brt, Dtk, Etk-2, Rek, Rse, Sky, Tif), Axl (also called Ark, Tyro7, Ufo) and Mer (also called c-Eyk, Mertk, Nyk, Tyro12). TAM receptors are composed of two immunoglobulin-like and two fibronectin Type III repeats in their extracellular domains, in tandem. This is connected to a single pass
transmembrane domain and a cytoplasmatic protein tyrosine kinase (Fig. 1A). Upon
ligand binding, the receptor dimerizes and the tyrosine kinase becomes activated.\textsuperscript{6}
In recent years, several signaling functions of the TAM receptors have been described,
such as stimulation of cell growth and proliferation, inhibition of apoptosis\textsuperscript{7,8}, mediation
of efferocytosis\textsuperscript{9}, stimulation of hemostasis\textsuperscript{10} and modulation of inflammation\textsuperscript{11}. In this
review we will focus on the functions of the TAM receptors pertaining to hemostasis and
inflammation. When activated by Gas6, the TAM receptors stimulate hemostasis by
facilitating platelet stabilization.\textsuperscript{10} The other ligand, protein S, has a TAM-independent
inhibitory effect on hemostasis.\textsuperscript{12-14} Activation of the TAM receptors was found to inhibit
TLR signaling, to induce phagocytosis and stimulate natural killer cell development,
leading to speculations about a role in preventing auto-immunity.

This review delineates the known functional similarities and differences between protein
S, Gas6 and the TAM receptors. The recently described knock-out mice for protein S,
Gas6 and the individual TAM receptors have strongly contributed to the new insights in
this field. By comparing the phenotypes of the different knock-out mice, we discuss the
functions of protein S that are attributable to TAM receptor activation and those functions
that are the effect of protein S alone.

**Protein S**

Protein S is a vitamin K-dependent protein encoded by the PROS1 gene in humans and
by Pros1 in mice. Unlike genes encoding for most vitamin K-dependent factors, the
PROS1 gene is also expressed in other tissues than the liver: transcription of PROS1 can
be found in the kidney, lungs or gonads. Protein S is produced by a variety of cell types,
e.g. hepatocytes, endothelial cells, megakaryocytes and osteoblasts.\textsuperscript{15} It contains an
amino terminal GLA domain, followed by a thrombin-sensitive loop region and four
EGF-like domains ending with the C-terminal, consisting of two laminin G repeats, that
together comprise the sex-hormone-binding globulin domain (Fig. 1B).\textsuperscript{16} The carboxy-
terminal region is sufficient for TAM-receptor binding and phosphorylation.\textsuperscript{17}
Protein S circulates in plasma at a concentration of 346 nmol/L\textsuperscript{18} and serves as an
anticoagulant by working as a non-enzymatic co-factor for activated protein C in the
breakdown of coagulation factors FVa and FVIIIa.\textsuperscript{12} It is further capable of binding FXa
and FVα directly, whereby it can autonomously inhibit coagulation. Factor Xa is also inhibited by protein S through acting as a co-factor for tissue factor pathway inhibitor (TFPI). In humans, it exists in a free active form (30-40%) and in an (almost) inactive form, bound to C4b-binding protein (60-70%). It is therefore plausible, that in humans protein S is apt to affect the complement system. In mice, however, protein S only exists in its free form, because the murine C4b-binding protein lacks the β-chain, which is essential for binding protein S. The functional consequences of this difference remain unknown.

Heterozygous deficiency of PROS1 is associated with an elevated risk for developing thrombosis, whereas homozygous deficiency is incompatible with life or leads to neonatal purpura fulminans in rare cases.

Lastly, protein S has been identified as a ligand for the TAM receptors in addition to Gas6. It has been shown capable of binding Tyro3, e.g. in osteoclasts. In retinal pigment epithelium protein S has been described to be equally important and interchangeable with Gas6 in vivo as a Mer ligand. Affinity between protein S and Axl, however, has never been shown. Protein S binding to Tyro3 and Mer shows a high degree of species specificity. Peculiar is that human protein S shows only weak or no affinity for the different human TAM-receptors, while bovine protein S displays good affinity to human Tyro3 (reviewed by Hafizi).

**Gas6**

Gas6 is a 75 kDa vitamin K-dependent protein, first discovered under conditions of growth arrest in embryonic mouse NIH 3T3 fibroblasts. It has high structural homology (~42%) with protein S and the modular composition is the same (as described above and shown in Fig. 1B). Unlike in protein S, the thrombin-sensitive region in Gas6 (a disulfide-bridged thumb loop) does not seem to be susceptible to cleavage by the action of serine proteases. The concentration of Gas6 is around 20-50 ng/mL (0.25 nmol/L) in plasma, and elevated to about 110 ng/mL in severe sepsis patients. These levels are much lower than those of the other vitamin K-dependent proteins of plasma. Another difference with other vitamin K-dependent proteins is that Gas6 is barely produced in the liver, but rather in heart, kidneys and lungs. Important tissues where Gas6
is expressed are endothelial cells, vascular smooth muscle cells and bone marrow. Gas6 has been shown to be present in murine platelets, but this presence in humans has been debated. Evidence does suggest that human platelets will aggregate upon TAM activation by Gas6.

There have been no reports in literature of cases with homo- or heterozygous deficiency of the GAS6 gene. Certain haplotypes of the GAS6 gene seem to have a protective role in the development of stroke.

Gas6 binds the TAM receptors with different affinities: Axl ≥ Tyro3 >> Mer. Functions of Gas6 seem to be limited to those caused by activation of the TAM receptors. These functions are dealt with in the next paragraphs.

**TAM receptors**

Expression of the individual TAM receptors can be found in many cell types, but the patterns vary. Tyro3 is mostly found in the central nervous system, kidneys, ovaries and testes. Axl is nearly ubiquitously expressed in most human cells originating from hematopoietic, epithelial and mesenchymal sources. Mer is predominantly expressed in ovaries, testes, prostate, lungs and kidneys and to a lesser extent in the thymus, spleen, liver, small intestine, colon and placenta.

Important cell types in which TAM receptors are active are e.g. antigen-presenting cells, monocytes and natural killer cells in the immune system, osteoclasts in bone, Sertoli cells in the testis, endothelial cells and vascular smooth muscle cells in the vasculature, and pigmental epithelium cells in the retina. In contrast, they are not expressed in granulocytes or blood lymphocytes. In tumor cells TAM-receptors are often up-regulated (reviewed by Linger).

Along with this wide expression of TAM receptors, many functions can be described. This review will not discuss all of these functions, but will focus on hemostasis and inflammation.

With respect to hemostasis, all three TAM receptors are located on platelets and mediate thrombogenesis and platelet stabilization. Platelet stabilization occurs after integrin activation, granule secretion and platelet aggregation, through platelet-to-platelet contact. Without this mechanism, platelet plugs disaggregate prematurely (reviewed by Prevost).
Important downstream mechanisms in platelets include increased granule secretion, activation of PI3K and phosphorylation of β3 integrin, leading to an increase in outside-in signaling via the αIIbβ3 integrin (Fig. 2).\textsuperscript{10,39} Also, vascular Gas6 up-regulates tissue factor in vascular cells when vessel injury occurs, leading to activation of the extrinsic coagulation pathway and thrombus formation.\textsuperscript{53} Gas6 is released from mouse platelets,\textsuperscript{37,38} but human platelets do not seem to contain Gas6.\textsuperscript{34,35} Besides, only expression of the Mer receptor on human platelets has been shown.\textsuperscript{54} Still, Gas6 levels in plasma were higher in patients with venous thromboembolic disease, as compared to healthy volunteers.\textsuperscript{55} Also, genetic evidence shows an association between certain single nucleotide polymorphisms in the GAS6 gene and stroke,\textsuperscript{56} making involvement of TAM-receptor in human, and not only murine, hemostasis likely.

Activation of the TAM receptors by Gas6 amplifies pro-inflammatory endothelial cell (EC) activation, leading to expression of VCAM-1 and ICAM-1. In platelets and ECs, TAM receptor phosphorylation leads to increased expression of P-selectin. PSGL-1 on leukocytes binds to P-selectin. The enhanced expression of adhesion molecules induces sequestration of platelets and leukocytes to ECs and each other. Hereby, the TAM receptors support leukocyte extravasation and inflammation, and adhesion of platelets to endothelial cells.\textsuperscript{57} Protein S levels are increased in artherosclerotic vessels. By activation of Mer, protein S inhibits macrophage scavenger receptor A-mediated acetylated low-density lipoprotein (AcLDL) uptake in macrophages, thereby reducing the formation of foam cells.\textsuperscript{58} These mechanisms can possibly explain part of the recent findings that SNP mutations in TAM receptor genes are correlated with the formation of atherosclerotic plaques.\textsuperscript{39,60}

Protein S is upregulated by IL-4 in primary T-cells.\textsuperscript{61} Natural killer T cells require Mer to induce the transcription of IL-4 and IFN-γ.\textsuperscript{62} Whether this results in a feed-back loop in vivo is unknown. A cell-proliferative function of TAM signaling that aids inflammation is induced differentiation in the maturation process of natural killer cells.\textsuperscript{63} Similarly in the kidney, activation of Axl promotes inflammation through increased proliferation of mesangial cells.\textsuperscript{64}

In contrast to supporting the inflammatory response as described above, TAM receptor signaling inhibits inflammation by multiple mechanisms. Activation of Mer, in contrast
to Axl, inhibits glomerular inflammation during glomerulonephritis.\textsuperscript{65} In antigen-presenting cells, TAM receptor signaling inhibits LPS-induced cytokine production (e.g. TNF-\textgreek{a}).\textsuperscript{11} Activation of cytokine receptors leads to an IFNAR/STAT1 upregulation of Axl (Fig. 3). Together with the IFNAR/STAT1 signaling cassette, the TAM receptors induce the transcription of the anti-inflammatory SOCS1 and SOCS3, and inhibit both cytokine receptors and TLR signaling pathways.\textsuperscript{66,67} The decreased expression of these anti-inflammatory mediators in TAM\textsuperscript{−/−} cells seems to point to a crucial role of SOCS proteins in the anti-inflammatory action of TAM receptors. However, a direct dependence of TAM anti-inflammatory function on SOCS has not yet been proven. TAM activation also induces Twist transcriptional repressors that suppress nuclear factor-\textkappa B (NF\textkappa B)-dependent transcription.\textsuperscript{68} TLR signaling in its turn suppresses Gas6 and protein S expression via NF\textkappa B in macrophages.\textsuperscript{69} Administration of recombinant Gas6 in a murine sepsis model, results in less mortality due to reduced neutrophil migration.\textsuperscript{70}

Another important anti-inflammatory mechanism is that TAM receptors enhance phagocytosis of apoptotic cells, also known as efferocytosis (Fig. 4).\textsuperscript{71} Gas6 and protein S bind to phosphatidylserine-positive moieties with their N-terminal GLA-domain (gamma-carboxyglutamic domain).\textsuperscript{9,72,73} The C-terminal binds to Mer on macrophages and Axl and Tyro3 on dendritic cells,\textsuperscript{74} causing the intracellular kinase to phosphorylate.\textsuperscript{75-77}

Although the exact signaling cascades remain unknown, a variety of signaling molecules has been shown to be relevant, such as PI3K, phospholipase C\textgreek{y}2, Src family kinases and interactions with the \textalpha\textnu\textbeta5 integrin. Rac1 is responsible for cytoskeletal rearrangement.\textsuperscript{78,79} Protection of the blood-brain barrier integrity also occurs through cytoskeletal rearrangement of brain endothelium by Rac1. It has been shown that this can be mediated by ligation of protein S to Tyro3, after which the protective sphingosine 1-phosphate receptor is activated.\textsuperscript{80}

Accumulated apoptotic cell debris that exists when efferocytosis is impaired, contains a variety of autoantigens that may cause lupus-like autoimmunity. Whether TAM receptors are involved in the etiology of lupus erythematosus remains unknown. An association between decreased protein S levels and SLE has been described,\textsuperscript{81} whereas elevated Gas6 levels are associated with disease activity in SLE.\textsuperscript{82} Mutations in the murine and human genes coding for Mer lead to impaired phagocytosis by retinal pigment epithelial cells.
This leads to cumulation of photoreceptor outer segments and retinal degeneration, causing retinitis pigmentosa in humans.\textsuperscript{83} It should be noted that this Mer-dependent signaling is mediated by both Gas6 and protein S as equally important ligands.\textsuperscript{30} The TAM receptors do not mediate phagocytosis of bacteria, yeast or latex particles.\textsuperscript{75,84} However, viral entry into target cells is facilitated by Axl by apoptotic mimicry of the viral envelope.\textsuperscript{85}

Recently, new ligands for TAM receptor-mediated efferocytosis have been described: Tubby, tubby-like protein 1 (Tulp1)\textsuperscript{86} and galectin-3.\textsuperscript{87} Tubby and galectin-3 specifically bind to Mer, whereas Tulp1 can activate all three of the TAM receptors. Whether these new ligands can affect other mechanisms besides phagocytosis through TAM-activation, remains to be elucidated.

**Knock-out mice**

Single, dual and triple knock-out mice for the Tyro3/Axl/Mer receptors have been described extensively, as well as Gas6-deficient mice. Protein S knock-out mice however had not been described until recently. The various phenotypes, exhibited by the different knock-out mice, with effects on hemostasis, inflammation and other systems, are summarized in table 1. As can be seen in this table, knock-out mice for protein S, Gas6 and TAM receptors show a large variety of phenotypes. Protein S\textsuperscript{−/−} mice have a lethal coagulopathy and vascular malformation. The latter is most probably due to the impaired blood flow caused by thrombi and – since the malformation is more severe than one would expect caused just by impaired blood flow – one could speculate it to be due to insufficient Axl signaling. However, protein S/Axl binding has never been determined. Meanwhile, protein S has been shown to induce proliferation of vascular smooth muscle cells by receptor activation, a function known to be attributed to Axl when activated by Gas6.\textsuperscript{88}

Protein S knock-outs with cell-specific Cre drivers give new insights into protein S production: endothelial and hematopoietic cells produce 43\% of the circulating protein S and local production of protein S in vascular smooth muscle cells is important for the vascular formation, as Sm22-Cre/protein S\textsuperscript{0/0} knock-out mice show increased vascular permeability.
Gas6 knock-out mice exhibit a reduction of thrombus formation, improved survival when challenged with a thrombotic stimulus, vascular defects and reduced liver inflammation. Knocking out any of the TAM receptors separately also protects mice against thrombosis. When only Tyro3 is knocked out, the mice develop neurological disorders. Axl−/− mice have vascular defects (like protein S and Gas6 knock-outs), impaired vascular remodeling after hemodynamic stress and an increased inflammatory response in the central nervous system, caused by reduced debris removal. Mer knock-out mice show many autoimmune-like features. This is probably due to the loss of the inhibitory effect of Mer on the NF-κB pathway, leading to excessive amounts of pro-inflammatory cytokines and because of the loss of efferocytosis. Triple TAM knock-out mice seem to combine all individual phenotypes (Tyro3−/−, Axl−/− and Mer−/−) and show a more severe phenotype (especially regarding inflammation).

**Discussion**

In this review of the literature, we have systematically explored the phenotypes of TAM/protein S/Gas6-deficient mice. Gas6, protein S and the TAM receptors have effects on primary hemostasis and coagulation, and they display an anti-inflammatory or a pro-inflammatory effect, depending on cell-type and even receptor. Besides describing these effects and their underlying mechanisms, the other goal of this review is to try hypothesize which functions of the TAM receptors are attributable to protein S. Because of the severe impairment of the haemostatic function in protein S knock-outs, it is difficult to assess the TAM-mediated effects in these mice. The disorders in vascular development seen, are too severe to only be caused by the coagulopathy. Although functional phenotypes in protein S-deficient mice may be hidden by redundant actions of Gas6, the local synthesis of protein S seems to have a role in the vascular development. Since mice lacking Axl suffer from similar underdeveloped blood vessels, this could imply locally produced protein S is required for activating Axl. Since affinity between protein S and Axl has never been shown, it might be caused by protein S via a different mechanism than Axl activation. A recent study describes how protein S can inhibit VEGF-Α–dependent EC migration, mitosis, and signaling via activation of Mer. Although by this mechanism protein S deficient mice would have
increased vascular proliferation, one could hypothesize that an imbalance of proliferative
and anti-proliferative mechanisms could result in vascular malformation. Especially since
the Gas6/Axl stimulatory effect and the protein S/Mer inhibitory effect on vascular
proliferation are both mediated by phosphorylation of SHP2. Apart from the vascular
malformation, no other potential TAM receptor-mediated effects could be identified,
based on the experiments with protein S knock-out mice.
Comparing Gas6 knock-out mice with TAM receptor knock-outs is another way to
analyze the effects of Gas6-independent TAM activation. Specifically the lupus-like auto-
immune syndrome present in triple TAM knock-out mice is less pronounced in Gas6^{-/-}
mice. This indicates that, in Gas6 knock-out mice, another ligand fulfills the inhibitory
task within the immune system, possibly protein S. This concurs with the findings that
protein S plays an equivalent role to Gas6 in the efferocytosis stimulated by the TAM
receptors, and that SLE patients may show lower protein S levels and/or anti-protein S
antibodies. However, the newly described ligands Tubby, Tulp1 and Galectin-3 are
also likely candidates that could explain this difference between the phenotypes.
The physiological role of Gas6 has been deducted mainly in GAS6^{-/-} mice, which are
viable and able to reproduce. Until now no humans have been identified with a total Gas6
deficiency, so either Gas6 deficiency has no major clinical consequences in man, or -
although less probable - total Gas6 deficiency is not compatible with life in humans.
Unlike human protein S, human Gas6 is a high-affinity ligand for all human TAM
receptors with K_d in the nM range (reviewed by Hafizi). Redundancy with protein S in
its function to activate Mer may actually mask important functions of Gas6, as has been
demonstrated in the retina, in which locally produced protein S is as potent as Gas6 in
activating Mer. Therefore, much remains to be elucidated on protein S-mediated TAM-
receptor activation.
Furthermore, certain molecular properties of the ligands require attention. The effect of
multimerization of Gas6 and protein S on TAM receptor binding and activation is not
completely clear. Upon purification, a major part of protein S is in a multimerized form
with increased phospholipid binding properties. Similar high molecular weight
multimers of protein S have been observed in plasma and reported to posses equal
anticoagulant properties as the monomers. It has been shown that membranes that
contain phosphatidylserine serve as a scaffold for the auto-oxidation of Cys residues in protein S, which promotes the oligomerization of protein S that is required for Mer dependent apoptotic cell clearance. This oligomerization may also provide a mechanism that allows the abundant protein S to activate Mer when necessary and not constitutively. Whether oligomerization of protein S increases the affinity (single bond interaction) or the avidity (combined strength of multiple bond interactions) with TAM receptors is still to be determined, as well as whether murine protein S oligomerizes like human protein S. Further research should be performed to elucidate when and how oligomerization is required for TAM-mediated functions of protein S and Gas6.

The importance of systemic circulating protein S to TAM receptor activation is unclear. Complete deletion of protein S results in embryonic malformation and thrombosis in protein S knock-out mice. Furthermore, deletion of either hepatic or endothelial/hematopoietic production of protein S leads to ~50% reduction in systemic levels and fibrin formation in blood vessels. Thus, it seems that to clarify the role of circulatory protein S as a TAM-receptor ligand, it will be necessary to generate an animal with mutant protein S, which only displays anticoagulant activity and no TAM receptor-activating activity. This could potentially be obtained by the introduction of sequences in the PROS1 gene that would delete or mutate the SHBG-like region of protein S. Another way to generate an animal devoid of systemic TAM activating protein S would be to silence the residual hepatic protein S production by siRNA in tie2-Cre/protein Sfl/fl animals which would than have to be supplemented pharmacologically with protein S that lacks the SHBG-domain or protein S with an inactive SHBG-domain to adequate anticoagulant levels. To generate such mice would be a major task and the relevance with regard to human disease can be questioned. However, such an approach may provide proof-of-principle data regarding the overall importance of non-redundant TAM-related protein S functions. A similar approach would be needed with a Gas6−/− background to investigate the TAM-related functions of protein S that are redundant with Gas6.

From a clinical perspective, further research in this field could provide therapeutical options for important diseases. A TAM receptor-antagonist or a Gas6-antagonist, could provide protection against thrombosis without a bleeding diathesis. Since vitamin K-antagonists also inhibit Gas6 (and protein S), beside factors II, VII, IX and X, anti-
coagulant effects of these drugs might partially also be caused by reduced stimulation of TAM receptors. The recent findings of a reduction of mortality in septic mice given recombinant Gas6, even after onset of the disease, brings a new potential supportive therapy for the critically ill. This also raises the question whether usage of vitamin K-antagonists in septic patients might increase mortality. Further research could also provide more insight and possible therapeutic options for auto-immune diseases like SLE, and, although not discussed in this review, cancer growth. The fact that the described receptors and their ligands’ functions lie within three general groups of disease (hemostasis, inflammation and cancer growth), should alert researchers of creating possible adverse effects when developing drugs. However, considering the above, it will be of great interest to see what the coming two decades of TAM receptor research will bring to medicine.

Authorship
Contribution: J.H. van der Meer, T. van der Poll and C. van ’t Veer wrote the manuscript.
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References


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<th>Knock-out type</th>
<th>Effects on</th>
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<td><strong>Hemostasis</strong></td>
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| Protein S\(^{-/-}\)                                                          | • Death by coagulopathy with macroscopic blood clots and extensive hemorrhages between E15.5 and E17.5.  
  • Intravascular and interstitial fibrin depositions.  
  • Increased amounts of megakaryocytes in the liver, suggesting peripheral thrombocytopenia.\(^{95,96}\)  
  • Intravascular and interstitial fibrin depositions.  
  • Increased amounts of megakaryocytes in the liver, suggesting peripheral thrombocytopenia.\(^{95,96}\) | Not described                                                                                                                                                                                                                                                                                                                                                                                        |
| Protein S\(^{+/-}\)                                                          | • 44\% decrease in protein S levels.  
  • 53\% decrease in APC cofactor activity.  
  • FVa based clotting time is shortened, thrombin generation is elevated, the lag time for thrombin generation is shortened.\(^{89,94}\)  
  • 44\% decrease in protein S levels.  
  • 53\% decrease in APC cofactor activity.  
  • FVa based clotting time is shortened, thrombin generation is elevated, the lag time for thrombin generation is shortened.\(^{89,94}\) | Not described                                                                                                                                                                                                                                                                                                                                                                                        |
| Protein S\(^{-/-}\) in hepatocytes (Alb-Cre/protein S\(^{fl/fl}\))            | • 15\% show fibrin depositions in blood vessels.  
  • 55\% decrease in protein S levels.  
  • 47\% decrease in APC cofactor activity.\(^{89}\)  
  • Fibrin depositions in blood vessels (but less severe than in Alb-Cre/protein S\(^{1/2}\)).  
  • 43\% decrease in protein S levels.  
  • 49\% decrease in APC cofactor activity.\(^{89}\) | Not described                                                                                                                                                                                                                                                                                                                                                                                        |
| Protein S\(^{-/-}\) in endothelial and hematopoietic cells (tie2-Cre/protein S\(^{fl/fl}\)) | • 15\% show fibrin depositions in blood vessels.  
  • 55\% decrease in protein S levels.  
  • 47\% decrease in APC cofactor activity.\(^{89}\)  
  • Fibrin depositions in blood vessels (but less severe than in Alb-Cre/protein S\(^{1/2}\)).  
  • 43\% decrease in protein S levels.  
  • 49\% decrease in APC cofactor activity.\(^{89}\) | Not described                                                                                                                                                                                                                                                                                                                                                                                        |
| Protein S\(^{-/-}\) in vascular smooth muscle cells (Sm22-Cre/protein S\(^{fl/fl}/\text{Gas6}^{-/-}\)) | Not described                                                                                                                                                                                                                                                                                                                                                                                       | Not described                                                                                                                                                                                                                                                                                                                                                                                        |
| **Gas6\(^{-/-}\)**                                                           | • Reduced inflammation and reduced myofibroblast activation in the steatotic liver, reducing liver fibrosis.\(^{95}\)  
  • Endothelial cells express less VCAM-1 and ICAM-1 when stimulated with TNF-\(\alpha\) than WT.  
  • Reduced sequestration of platelets onto endothelium, of leukocytes onto endothelium and of platelets to leukocytes.  
  • Reduced leukocyte extravasation and inflammation in endotoxemia, vasculitis and heart transplantation.\(^{39}\)  
  • More hypoxia-induced cell death and higher II-1β and TNF-\(\alpha\) expression in murine macrophages.\(^{96}\)  
  • More graft-versus-host disease when receiving liver transplantation.\(^{97}\)  
  • Less mortality and proteinuria in accelerated nephrotic nephritis than in WT mice.\(^{64}\)  
  • More stable atherosclerotic plaques by increased fibrosis and fewer macrophages.\(^{98}\)  
  • Reduced sequestration of platelets onto endothelium, of leukocytes onto endothelium and of platelets to leukocytes.  
  • Reduced leukocyte extravasation and inflammation in endotoxemia, vasculitis and heart transplantation.\(^{39}\)  
  • More hypoxia-induced cell death and higher II-1β and TNF-\(\alpha\) expression in murine macrophages.\(^{96}\)  
  • More graft-versus-host disease when receiving liver transplantation.\(^{97}\)  
  • Less mortality and proteinuria in accelerated nephrotic nephritis than in WT mice.\(^{64}\)  
  • More stable atherosclerotic plaques by increased fibrosis and fewer macrophages.\(^{98}\)  
  • Elevated vascular permeability.\(^{38}\)  
  • Less oligodendrocytes and microglial activation after demyelination.\(^{99}\) | Vascular defects leading to permeation into liver parenchyma.\(^{38}\)                                                                                                                                                                                                                                                                                                               |
| Gas6\(^{-/-}\), protein S\(^{1/2}\) in retinal cells Prost\(^{1/2}/\text{Nes-}\)Cre/Gas6\(^{-/-}\) | Not described                                                                                                                                                                                                                                                                                                                                                                                      | Not described                                                                                                                                                                                                                                                                                                                                                                                        |
| Tyro3/Axl/Mer\(^{-/-}\)                                                       | • Recurrent thrombosis and hemorrhages in several tissues (including the brain), associated with the presence of antibodies to phospholipids as seen in auto-immune syndromes.\(^{39}\)  
  • Impaired hemostasis, thrombocytopenia due to platelet dysfunction and megakaryocytopenia.\(^{100}\)  
  • After ~4 weeks spleens and lymph nodes enlarge.  
  • After one year the spleens are about ten times the normal size.  
  • Hyperproliferation of constitutively activated B and T-cells (the latter slightly more).  
  • Ectopic lymphocytes in every researched organ.  
  • Clinical manifestations mimic autoimmune diseases similar to rheumatoid arthritis, pempigus vulgaris and systemic lupus erythematosus.  
  • Spermatogenesis in males defected.  
  • Testes one third of wild-type size.  
  • Blindness due to impaired phagocytosis of photoreceptor outer segments by retinal pigment epithelial cells.\(^{31}\) | Blindness due to impaired phagocytosis of photoreceptor outer segments by retinal pigment epithelial cells.\(^{31}\)                                                                                                                                                                                                                       |
T cells express elevated amounts of IL-2 receptor and lectin CD69.
• B cells express Fas, CD44 and IFN-γ.
• Vascular endothelia express ICAM-I. Increased antibody titers can be found to dsDNA, collagen, cardiolipin, phosphatidyltyrosine, phosphatidylethanolamine and phosphatidylinositol.
• Macrophages produce high levels of IL-12 and MHCII is strongly increased.
• When given LPS intraperitoneally, LPS induced TNF-α response doubles in comparison to WT.
• Inactivation of Mer contributes the most to the above. 

• Young adults: diminished hippocampal long-term potential.
• Aged: neural degeneration with seizures and paralysis.

• Increase in apoptosis in response to flow reduction in carotid artery.
• Impaired vascular remodeling: Increase in CD45+ cells and decrease in VSMC, macrophages, and neutrophils.
• Enhanced inflammation in the CNS because of delayed removal of myelin debris during experimental autoimmune encephalomyelitis.

• Blindness due to impaired phagocytosis of photoreceptor outer segments by retinal pigment epithelial cells.

• Decreased induction of c-Src and STAT3.

Table 1. Summary of phenotypical effects on hemostasis, inflammation and other systems as seen in various knock-out mice.
Fig 1. A: **The structure of the Tyro3/Axl/Mer receptor**: the N-terminal starts with two immunoglobulin-like (Ig) domains, followed by two fibronectin type 3 domains, followed by a single pass transmembrane domain and a protein tyrosine kinase at the C-terminal.

**B: The structure of the TAM ligands protein S and Gas6**: The N-terminal contains a gamma carboxyglutamic acid (GLA) domain, followed by a thrombin-sensitive region (TSR), followed by four epidermal growth factor (EGF)-like domains, followed by a C-terminal sex hormone-binding globulin (SHBG)-like domain, consisting of two laminin G (LG) repeats.

**Fig. 2 TAM-mediated platelet stabilization and leukocyte adhesion.** ADP and Gas6 increase expression of $\alpha_{IIb}\beta_3$ integrin via PI3K/Akt. After binding to fibrinogen, granule secretion is elevated by outside-in signaling. TAM receptor phosphorylation also leads to increased expression of P-selectin, which binds to PSGL-1 on leukocytes, and increased expression of adhesion molecules ICAM-1 and VCAM-1 by endothelial cells, also stimulating sequestration of leukocytes. Gas6 up-regulates tissue factor in endothelial cells upon vessel injury (not depicted), leading to activation of the extrinsic coagulation pathway. ADP, adenosine diphosphate; ICAM-1, intercellular adhesion molecule 1; PI3K, phosphatidylinositol 3-kinases; PSGL-1, P-selectin glycoprotein ligand-1; VCAM-1, vascular cell adhesion molecule-1.

**Fig. 3 Effects of TAM receptors on inflammation.** IFN-α induces TAM receptor expression. TAM signaling usurps the IFNAR/STAT1 cassette to inhibit TLR and JAK signaling via SOCS1 and SOCS3. TAM activation induces Twist, which suppresses NFκB-dependent transcription reducing pro-inflammatory cytokine production. NFκB inhibits GAS6 and protein S expression. ASK, apoptosis signal-regulating kinase; IFN-α, interferon-α; IFNAR, interferon-α/β receptor; IRAK, interleukin-1 receptor-associated kinase; IRF, interferon regulatory factor; JAK, Janus kinase; LPS, lipopolysaccharide; MyD88, myeloid differentiation primary response gene 88; NFκB, nuclear factor κB; SOCS, suppressor of cytokine signaling proteins; STAT, signal transducer and activator of transcription.
of transcription; TLR, Toll-like receptor; TRAF, TNF receptor-associated factor; TRIF, TIR-domain-containing adapter-inducing interferon-β.

**Fig 4. Putative model for TAM-mediated efferocytosis.** The GLA-domains of protein S and Gas6 bind to the phosphatidylserine-positive cell membrane of an apoptotic moiety. The SHBG-domains bind to TAM receptors, which causes phosphorylation of the intracellular protein tyrosine kinase. Phosphorylated by the kinase, PI3K induces phosphorylation of PIP$_2$ to PIP$_3$, which facilitates phagocytosis. TAM receptor activation stimulates phospholipase C$_γ$2, leading to enhanced Protein Kinase C (PKC) activity. It has also been suggested that a Src family kinase is activated, resulting in recruitment of FAK, functionally cross-talking with αvβ5 integrin. It has also been suggested that a complex consisting of c-Src, PI3K, and STAT3 is established by Mer phosphorylation. This complex then inhibits inflammation in DCs. DAG, diacylglycerol; FAK, focal adhesion kinase; IP$_3$, inositol trisphosphate; MFGE8, milk fat globule-EGF factor 8; PI3K, phosphatidylinositol 3-kinases; PIP$_2$, phosphatidylinositol (4,5)-bisphosphate; PIP$_3$, phosphatidylinositol (3,4,5)-triphosphate; PKC, protein kinase C; PLC, phospholipase C; STAT, signal transducer and activator of transcription.
Fig. 1

A

N

Ig domains

Fibronectin type 3 domains

Protein Tyrosine Kinase domain

B

N

TSR

GLA domain

EGF-like domains

LG1

LG2

SHBG-like domain
Fig. 2

Extrinsic coagulation pathway

Tissue Factor

α-granule

Outside-in signaling

Platelet

Gas6

Platelet

α<sub>IIb</sub>β<sub>3</sub> integrin

P-selectin

Endothelial Cell

Platelet

TAM receptor dimer

ICAM-1

VCAM-1

ADP

Fibrinogen

PSGL-1

PI3K

Akt

Fig. 2

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Fig. 3

Cytokines

Cytokine receptor

JAK

STAT1

SOCS1

SOCS3

TLR3

TLR9

MyD88

TRIF

LPS

IFNAR

IFN-α

Gas6

Protein S

BTwist

ASK1

IRF7

IRAKs

IRF3

TRAF6

TRAF

IRF3

Twist

NFκB
TAM receptors, Gas6 and protein S: roles in inflammation and hemostasis

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