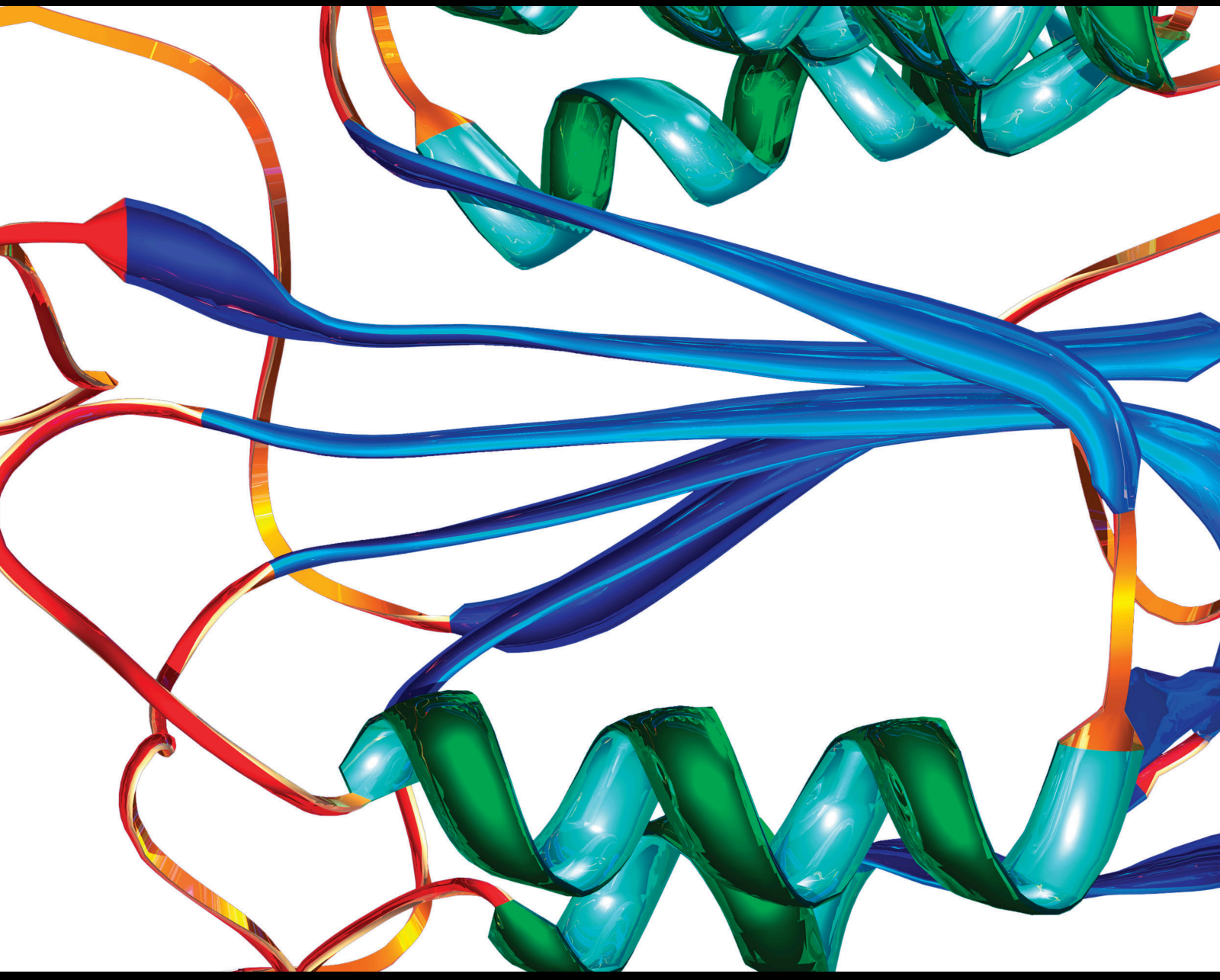
Disease Markers

Role of the Gas6/TAM System as a Disease Marker and Potential Drug Target

Lead Guest Editor: Pier P. Sainaghi
Guest Editors: Mattia Bellan and Alessandra Nerviani



Role of the Gas6/TAM System as a Disease Marker and Potential Drug Target

Disease Markers

Role of the Gas6/TAM System as a Disease Marker and Potential Drug Target

Lead Guest Editor: Pier P. Sainaghi
Guest Editors: Mattia Bellan and Alessandra Nerviani

Contents

Hindawi
Disease Markers
Volume 2021, Article ID 2854925, 3 pages
https://doi.org/10.1155/2021/2854925

Editorial

Role of the Gas6/TAM System as a Disease Marker and Potential Drug Target

Pier Paolo Sainaghi,[1,2,3] **Mattia Bellan**,[1,2,3] **and Alessandra Nerviani**[3,4]

[1]*Department of Translational Medicine, Università del Piemonte Orientale UPO, Novara, Italy*
[2]*Division of Rheumatology, CAAD (Center for Translational Research on Autoimmune and Allergic Diseases) "AOU Maggiore della Carità", Novara, Italy*
[3]*IRCAD, Interdisciplinary Research Center of Autoimmune Diseases, Novara, Italy*
[4]*Experimental Medicine and Rheumatology, William Harvey Research Institute and Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK*

Correspondence should be addressed to Mattia Bellan; mattia.bellan@med.uniupo.it

Received 22 June 2020; Accepted 22 June 2020; Published 18 January 2021

Growth arrest-specific 6 (Gas6) is a gene cloned in 1993 [1] encoding for a vitamin K-dependent protein expressed in different tissues [1–3]. Its biological activities are mediated by the interaction with three tyrosine kinase receptors: Tyro3, Axl, and MerTK, which are commonly and collectively abbreviated as TAM [4]. These receptors share a common feature: their extracellular domain is proteolytically cleaved and released in a soluble form (sTyro3, sAxl, and sMer; sTAM collectively) and acts as a decoy receptor; consistently, the shedding of the ectodomain entails the reduction of transmembrane receptors available for the ligands [5, 6].

Different activities have been attributed to Gas6/TAM interaction: it has been shown to act on platelet function [7], to regulate cell growth [8], to mediate the phagocytosis of apoptotic bodies [9], and to switch off inflammatory response [10].

On these bases, Gas6 and sTAM role as biomarkers has been explored and partially validated in several human diseases, particularly in those where fibrosis and inflammation are relevant [11]. In this context, the diagnostic performance of Gas6 has been evaluated in neuroinflammatory [12–14] and neurodegenerative disorders [15]; moreover, Gas6 and the circulating forms of TAM receptors have been observed to be increased in the plasma of patients affected by systemic lupus erythematosus, being predictive of disease severity [16, 17]. Among other inflammatory disorders, Gas6 and TAM system receptors have been also proposed as disease biomarkers in rheumatoid arthritis [18] and Sjogren's syndrome [19].

Consistently, being related to fibrosis and inflammation, Gas6 and sAxl have been found increased in the plasma of patients affected by liver cirrhosis [20], a condition in which the overly exuberant accumulation of extracellular matrix proteins commonly triggered by chronic injury of the hepatic parenchyma with an inflammatory component leads to hepatic fibrosis with a structural and functional disruption. In this context, since their plasmatic levels are predictive of the development of complications of chronic liver diseases such as hepatocellular carcinoma [21] and oesophageal varices [22], they may be proposed as biomarkers of disease severity [23].

We should not neglect that Gas6 has a great structural homology with protein S, an important regulator of coagulative cascade, which also shares the same receptors. Therefore, the system has been explored in the context of thromboembolic but its role in clinical setting is under investigation [24, 25].

Finally, an overactivation of the system has been associated to several solid and hematological neoplastic conditions and identified as a potential negative prognostic biomarker [26–29].

However, in all these conditions, the altered plasmatic concentration of Gas6 and its receptors does not seem to be only an epiphenomenon, but rather to contribute to disease pathogenesis. This is why, targeting TAM is a novel strategy proposed for different human diseases. Recently, Espindola et al. [30] have demonstrated that both Gas6 and Axl expressions are enhanced in patients with idiopathic pulmonary fibrosis (IPF); interestingly, specifically targeting Gas6/Axl interaction significantly inhibited the synthetic, migratory, and proliferative properties of IPF fibroblasts and prevented the development of pulmonary fibrosis in a murine model. Consistently, the blockade of Gas6/Axl axis is associated to a reduced collagen deposition and liver fibrosis in a murine model of Ccl-4-induced liver disease [31]. These findings support the idea that Gas6/TAM system is a promising target of antifibrotic treatments. This is not surprising, considering that tyrosine kinase inhibition is a common strategy in oncology as well; consistently, TAM receptor blockade has been already proposed for different neoplastic conditions [32–35].

In conclusion, a deeper knowledge of this relatively novel and unexplored system might contribute to clarify the pathogenetic mechanisms underlying the development of different human diseases and, potentially, to make available novel promising therapeutic tools.

Conflicts of Interest

The authors have no conflict of interest related to this publication.

Pier Paolo Sainaghi
Mattia Bellan
Alessandra Nerviani

References

[1] G. Manfioletti, C. Brancolini, G. Avanzi, and C. Schneider, "The protein encoded by a growth arrest-specific gene (Gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade," *Molecular and Cellular Biology*, vol. 13, no. 8, pp. 4976–4985, 1993.

[2] G. C. Avanzi, M. Gallicchio, G. Cavalloni et al., "Gas6, the ligand of Axl and Rse receptors, is expressed in hematopoietic tissue but lacks mitogenic activity," *Experimental Hematology*, vol. 25, no. 12, pp. 1219–1226, 1997.

[3] M. G. Melaragno, D. A. Wuthrich, V. Poppa et al., "Increased expression of Axl tyrosine kinase after vascular injury and regulation by G protein-coupled receptor agonists in rats," *Circulation Research*, vol. 83, no. 7, pp. 697–704, 1998.

[4] K. Nagata, K. Ohashi, T. Nakano et al., "Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases," *Journal of Biological Chemistry*, vol. 271, no. 47, pp. 30022–30027, 1996.

[5] J. P. O'Bryan, Y. W. Fridell, R. Koski, B. Varnum, and E. T. Liu, "The transforming receptor tyrosine kinase, Axl, is post-translationally regulated by proteolytic cleavage," *Journal of Biological Chemistry*, vol. 270, no. 2, pp. 551–557, 1995.

[6] E. Thorp, T. Vaisar, M. Subramanian, L. Mautner, C. Blobel, and I. Tabas, "Shedding of the Mer tyrosine kinase receptor is mediated by ADAM17 protein through a pathway involving reactive oxygen species, protein kinase Cδ, and p38 mitogen-activated protein kinase (MAPK)," *Journal of Biological Chemistry*, vol. 286, no. 38, pp. 33335–33344, 2011.

[7] J. H. M. van der Meer, T. van der Poll, and C. van 't Veer, "TAM receptors, Gas6, and protein S: roles in inflammation and hemostasis," *Blood*, vol. 123, no. 16, pp. 2460–2469, 2014.

[8] C. Schneider, R. M. King, and L. Philipson, "Genes specifically expressed at growth arrest of mammalian cells," *Cell*, vol. 54, no. 6, pp. 787–793, 1988.

[9] G. Zizzo, B. A. Hilliard, M. Monestier, and P. L. Cohen, "Efficient clearance of early apoptotic cells by human macrophages requires M2c polarization and MerTK induction," *Journal of Immunology*, vol. 189, no. 7, pp. 3508–3520, 2012.

[10] F. Alciato, P. P. Sainaghi, D. Sola, L. Castello, and G. C. Avanzi, "TNF-α, IL-6, and IL-1 expression is inhibited by GAS6 in monocytes/macrophages," *Journal of Leukocyte Biology*, vol. 87, no. 5, pp. 869–875, 2010.

[11] M. Bellan, M. G. Cittone, S. Tonello et al., "Gas6/TAM system: a key modulator of the interplay between inflammation and fibrosis," *International Journal of Molecular Sciences*, vol. 20, no. 20, p. 5070, 2019.

[12] M. Bellan, M. Pirisi, and P. P. Sainaghi, "The Gas6/TAM system and multiple sclerosis," *International Journal of Molecular Sciences*, vol. 17, article 1807, 2016.

[13] P. P. Sainaghi, L. Collimedaglia, F. Alciato et al., "Growth arrest specific gene 6 protein concentration in cerebrospinal fluid correlates with relapse severity in multiple sclerosis," *Mediators of Inflammation*, vol. 2013, Article ID 406483, 7 pages, 2013.

[14] P. P. Sainaghi, L. Collimedaglia, F. Alciato et al., "Elevation of Gas6 protein concentration in cerebrospinal fluid of patients with chronic inflammatory demyelinating polyneuropathy (CIDP)," *Journal of the Neurological Sciences*, vol. 269, no. 1-2, pp. 138–142, 2008.

[15] P. P. Sainaghi, M. Bellan, F. Lombino et al., "Growth arrest specific 6 concentration is increased in the cerebrospinal fluid of patients with Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 55, no. 1, pp. 59–65, 2017.

[16] C. S. Wu, C. Y. Hu, H. F. Tsai et al., "Elevated serum level of growth arrest-specific protein 6 (Gas6) in systemic lupus erythematosus patients is associated with nephritis and cutaneous vasculitis," *Rheumatology International*, vol. 34, no. 5, pp. 625–629, 2014.

[17] L. Ballantine, A. Midgley, D. Harris, E. Richards, S. Burgess, and M. W. Beresford, "Increased soluble phagocytic receptors sMer, sTyro3 and sAxl and reduced phagocytosis in juvenile-onset systemic lupus erythematosus," *Pediatric Rheumatology Online Journal*, vol. 13, no. 1, p. 10, 2015.

[18] L. Xu, F. Hu, H. Zhu et al., "Soluble TAM receptor tyrosine kinases in rheumatoid arthritis: correlation with disease activity and bone destruction," *Clinical and Experimental Immunology*, vol. 192, no. 1, pp. 95–103, 2018.

[19] B. Qin, J. Wang, N. Ma et al., "The association of Tyro3/Axl/-Mer signaling with inflammatory response, disease activity in patients with primary Sjogren's syndrome," *Joint, Bone, Spine*, vol. 82, no. 4, pp. 258–263, 2015.

[20] M. Bellan, G. Pogliani, C. Marconi et al., "Gas6 as a putative noninvasive biomarker of hepatic fibrosis," *Biomarkers in Medicine*, vol. 10, no. 12, pp. 1241–1249, 2016.

[21] K. Staufer, M. Dengler, H. Huber et al., "The non-invasive serum biomarker soluble Axl accurately detects advanced liver fibrosis and cirrhosis," *Cell Death & Disease*, vol. 8, no. 10, article e3135, 2017.

[22] M. Bellan, P. P. Sainaghi, M. T. Minh et al., "Gas6 as a predictor of esophageal varices in patients affected by hepatitis C virus related-chronic liver disease," *Biomarkers in Medicine*, vol. 12, no. 1, pp. 27–34, 2018.

[23] M. Bellan, L. M. Castello, and M. Pirisi, "Candidate biomarkers of liver fibrosis: a concise, Pathophysiology-oriented Review," *Journal of Clinical and Translational Hepatology*, vol. 6, no. 3, pp. 317–325, 2018.

[24] A. Schnegg-Kaufmann, S. Calzavarini, A. Limacher et al., "A high Gas6 level in plasma predicts venous thromboembolism recurrence, major bleeding and mortality in the elderly: a prospective multicenter cohort study," *Journal of Thrombosis and Haemostasis*, vol. 17, no. 2, pp. 306–318, 2019.

[25] P. P. Sainaghi, F. Alciato, S. Carnieletto et al., "Gas6 evaluation in patients with acute dyspnea due to suspected pulmonary embolism," *Respiratory Medicine*, vol. 103, no. 4, pp. 589–594, 2009.

[26] P. P. Sainaghi, L. Castello, L. Bergamasco, M. Galletti, P. Bellosta, and G. C. Avanzi, "Gas6 induces proliferation in prostate carcinoma cell lines expressing the Axl receptor," *Journal of Cellular Physiology*, vol. 204, no. 1, pp. 36–44, 2005.

[27] J. Han, R. Tian, B. Yong et al., "Gas6/Axl mediates tumor cell apoptosis, migration and invasion and predicts the clinical outcome of osteosarcoma patients," *Biochemical and Biophysical Research Communications*, vol. 435, no. 3, pp. 493–500, 2013.

[28] J. S. Waizenegger, I. Ben-Batalla, N. Weinhold et al., "Role of growth arrest-specific gene 6-Mer axis in multiple myeloma," *Leukemia*, vol. 29, no. 3, pp. 696–704, 2015.

[29] H. J. Lee, Y. M. Jeng, Y. L. Chen, L. Chung, and R. H. Yuan, "Gas6/Axl pathway promotes tumor invasion through the transcriptional activation of Slug in hepatocellular carcinoma," *Carcinogenesis*, vol. 35, no. 4, pp. 769–775, 2014.

[30] M. S. Espindola, D. M. Habiel, R. Narayanan et al., "Targeting of TAM receptors ameliorates fibrotic mechanisms in idiopathic pulmonary fibrosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 197, no. 11, pp. 1443–1456, 2018.

[31] C. Bárcena, M. Stefanovic, A. Tutusaus et al., "Gas6/Axl pathway is activated in chronic liver disease and its targeting reduces fibrosis via hepatic stellate cell inactivation," *Journal of Hepatology*, vol. 63, no. 3, pp. 670–678, 2015.

[32] I. Ben-Batalla, A. Schultze, M. Wroblewski et al., "Axl, a prognostic and therapeutic target in acute myeloid leukemia mediates paracrine crosstalk of leukemia cells with bone marrow stroma," *Blood*, vol. 122, no. 14, pp. 2443–2452, 2013.

[33] C. D. May, J. Garnett, X. Ma et al., "AXL is a potential therapeutic target in dedifferentiated and pleomorphic liposarcomas," *BMC Cancer*, vol. 15, no. 1, p. 901, 2015.

[34] E. Martinelli, G. Martini, C. Cardone et al., "AXL is an oncotarget in human colorectal cancer," *Oncotarget*, vol. 6, no. 27, pp. 23281–23296, 2015.

[35] D. J. Pinato, M. W. Brown, S. Trousil et al., "Integrated analysis of multiple receptor tyrosine kinases identifies Axl as a therapeutic target and mediator of resistance to sorafenib in hepatocellular carcinoma," *British Journal of Cancer*, vol. 120, no. 5, pp. 512–521, 2019.

Hindawi
Disease Markers
Volume 2020, Article ID 1614627, 9 pages
https://doi.org/10.1155/2020/1614627

Review Article

New Insights into the Role of Tyro3, Axl, and Mer Receptors in Rheumatoid Arthritis

Sara Pagani,[1] Mattia Bellan,[2,3,4] Daniele Mauro,[1] Luigi Mario Castello,[2] Gian Carlo Avanzi,[2] Myles J. Lewis,[1] Pier Paolo Sainaghi,[2,3,4] Costantino Pitzalis,[1] and Alessandra Nerviani[1]

[1]*Centre for Experimental Medicine & Rheumatology, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK*
[2]*Department of Translational Medicine, Università del Piemonte Orientale (UPO), Novara, Italy*
[3]*Internal Medicine Division, Immunorheumatology Unit, CAAD (Center for Autoimmune and Allergic Diseases), "Maggiore della Carità" Hospital, Novara, Italy*
[4]*IRCAD (Interdisciplinary Research Center of Autoimmune Diseases), Novara, Italy*

Correspondence should be addressed to Alessandra Nerviani; a.nerviani@qmul.ac.uk

Received 5 April 2019; Revised 1 December 2019; Accepted 4 January 2020; Published 21 January 2020

Academic Editor: Johannes Honekopp

Rheumatoid Arthritis (RA) is the most common chronic inflammatory autoimmune disease involving joints. Among several pathogenic mechanisms, the impairment of homeostatic regulators of inflammation seems to be critically important to sustain persistent infiltration and activation of immune and stromal cells within the diseased synovium. Tyrosine kinase receptors Tyro3, Axl, and Mer are members of the TAM family. Upon binding their ligands Growth Arrest-Specific gene 6 (Gas6) and Protein S (ProS1), TAM receptors (TAMs) exert numerous and diverse biologic functions. Activated Axl and Mer, for instance, can negatively regulate the inflammatory cascade and mediate phagocytosis of apoptotic cells, contributing to prevent the development of autoimmunity. Thus, a role for TAMs has been hypothesized in RA. In this review, we will summarise unmet clinical needs in RA, depict the biology of TAMs and TAM ligands, focussing on their ability to regulate the immune system and inflammation cascade, and finally offer an overview of the state-of-the-art literature about the putative role of TAM axis in RA.

1. Introduction

Rheumatoid Arthritis (RA) is a chronic inflammatory autoimmune disease characterised by persistent inflammation of diarthrodial joints [1]. Despite significant advances in the understanding and management of RA, further studies evaluating novel pathogenic pathways and therapeutic targets are needed to improve the clinical outcome of patients. Among several mechanisms, impairment of homeostatic regulators of inflammation seems to be critically important to sustain the persistent cellular infiltration and activation of immune and stromal cells within the diseased synovium [2]. Tyro3, Axl, and Mer are three tyrosine kinase receptor (TKR) members of the TAM family, which can be activated by binding their cognate ligands Growth Arrest-Specific gene 6 (Gas6) and Protein S (ProS1) [3]. TAM receptors (TAMs) have been implicated in several biological processes such as inhibition of apoptosis and promotion of cell survival and proliferation [4, 5], inhibition of granulocytes adhesion to the endothelium [6], and stabilisation of blood clots [7]. Furthermore, and of particular importance in the context of RA, TAMs can also finely regulate the inflammatory cascade [8] and mediate the engulfment of apoptotic corpses [9], contributing to prevent the development of autoimmune reactions.

Here, we will initially summarise unmet clinical needs in RA (Section 2) and describe the biology of TAMs and TAM ligands (Section 3). We will then focus on TAMs' ability to

control the immune system and inhibit the inflammatory cascade (Section 4). Finally, we will offer an overview of the state-of-the-art literature about the putative role of the TAM axis in RA (Section 5).

2. Unmet Needs in Rheumatoid Arthritis

RA is the most common chronic inflammatory autoimmune disease affecting joints. If not adequately treated, RA eventually causes long-term disabilities and poor quality of life [1]. RA pathogenesis is multifactorial and only partially understood. In the prearticular phase of the disease, characterised by systemic loss of the immune tolerance, autoantibodies directed against arthritogenic peptides are generated in genetically susceptible subjects [10]. Subsequently, multiple factors such as viral infections, microvascular defects, and local microtraumas likely contribute to shifting the pathogenic process from the periphery to the joints, hence initiating the articular phase of the disease [2].

Within the affected joint, autoantibodies bind their cognate antigens and activate the complement cascade, ultimately triggering proinflammatory reactions mediated by resident synovial cells and immune cells recruited from peripheral blood. This persistent infiltration of the synovial membrane by inflammatory cells is, at least partially, self-sustained by intrinsic and/or acquired defects of homeostatic regulatory mechanisms operating a negative feedback on the inflammatory cascade [2, 11].

Over the last two decades, thanks to the introduction of biologic agents into the therapeutic scenario, the clinical outcome of RA patients has critically improved. Nevertheless, substantial unmet clinical needs remain to be addressed for further refining the diagnosis and ameliorating the prognosis of patients. For instance, biomarkers able to accurately predict the diagnosis, severity, and progression of RA have yet to be defined. Moreover, a still significant percentage of patients, despite being aggressively treated with multiple agents, fail to reach a low-disease activity or remission status [12]. In the era of precision medicine, the identification of predictors able to guide the choice of the best drug for the right patient represents one of the most important goals of ongoing trials. Even if exciting news is currently coming from the analysis of the cellular and molecular content of the diseased synovial tissue [13], further investigations are still required. To date, a few studies have explored TAMs' pathogenic role and potential diagnostic and prognostic value in RA. As described below, the biological features of TAMs and TAM ligands make this system a promising candidate biomarker and a future therapeutic target in RA.

3. Biology of TAM Receptors and Ligands

3.1. Structure, Expression, and Activation of TAM Receptors and Ligands. The acronym TAM is derived from the names of the three RTK members of the family: Tyro3, Axl, and Mer [14]. Structurally, all TAMs are considerably similar and contain the following: an extracellular amino-terminal region carrying tandem immunoglobulin-related domains, which mediate ligands' binding, followed by two fibronectin type III repeats; a single-pass transmembrane domain; and a catalytically competent tyrosine kinase intracellular domain [15, 16]. TAMs had been considered "orphan" receptors until 1995 when their ligands ProS1 and Gas6 were identified [17]. Gas6 can bind and activate all three TAMs, however, with different degrees of affinity (Axl>Tyro3>>Mer); conversely, ProS1 is the preferential ligand for Tyro3 and Mer but has a significantly lower affinity for Axl [17, 18].

Although Axl, Mer, and Tyro3 mRNA can be detected in embryonic tissues [19], TAMs are dispensable for embryonic growth and nonessential for the viability of the foetus as demonstrated by the healthy birth of triple TAM knockout (KO) mice [20]. In adult tissues, TAMs are broadly expressed but can be primarily found in the nervous and reproductive systems, retinal cells, and hematopoietic lineages [21]. Myeloid cells (i.e., monocytes/macrophages and dendritic cells (DCs)), in particular, display TAMs on their surface [8, 22] though with distinctive features. On the one hand, Axl and Tyro3 are usually upregulated by monocyte-derived DCs [23] and, among them, Axl is preferentially induced by GM-CSF and IFN-α stimulation [24]. On the other hand, Mer is a typical macrophage receptor predominantly expressed by anti-inflammatory macrophage M2c, obtained *in vitro* by treating monocytes with M-CSF and IL-10 [25]. Overall, both Axl and Mer seem to be gradually acquired as monocytes differentiate into DCs and macrophages, respectively. Interestingly, despite being expressed by several neoplastic lymphocytes, TAMs are almost undetectable in nonpathologic B and T cells [21], except for specific subsets of B cells [26] and CD4+CD25+ regulatory T cells [27].

Depending on which cells or tissues are expressed by, TAMs can activate different intracellular pathways and mediate a wide range of biological functions [28]. In most nonsentinel cells, activation of TAM tyrosine kinases is coupled to the downstream activation of the phosphoinositide-3-kinase (PI3K)/AKT pathway. Conversely, in antigen-presenting cells (APCs) and other immune cells harbouring the Type I Interferon Receptor (IFNAR), the JAK/STAT signalling becomes the preferential downstream pathway [29]. As mentioned above, TAMs are activated upon binding their cognate ligands Gas6 and ProS1. However, besides this conventional ligand-dependent stimulation, in nonphysiological circumstances of overexpression, Axl activation can also occur without binding its ligand but through the aggregation of extracellular domains and subsequent reciprocal autophosphorylation [30].

3.2. Regulation of TAM Receptors: Shedding Mechanisms and Epigenetic Modulation. Several mechanisms can critically regulate TAM protein expression, including cleavage of extracellular domains and epigenetic control of mRNA translation. Concerning the former, two A disintegrin and metalloproteinases (ADAM), namely ADAM10 and 17, are the principal enzymes involved in the generation of soluble Axl (sAxl) and soluble Mer (sMer) extracellular domains [31, 32]. TAMs shedding may have important physiological and pathological implications: in fact, because of Axl high affinity for its ligand Gas6, sAxl behaves as a potent decoy receptor

for circulating Gas6. Hence, in the presence of excessive cleavage, not only the amount of functional transmembrane receptors is reduced but also the availability of Gas6 is impaired as it is sequestered by sAxl [33]. Interestingly, proinflammatory stimuli such as phorbol 12-myristate 13-acetate (PMA) [31] and lipopolysaccharide (LPS) [32] are inducers of Axl and Mer shedding, respectively. In this context, the highly inflamed articular microenvironment during RA might play an essential role by enhancing TAM cleavage and, eventually, altering their homeostatic regulation. Furthermore, it has been shown that rheumatoid synovium expresses higher levels of both ADAM-10 and ADAM-17 compared with osteoarthritic and healthy joints [34]. Besides, RA-derived synovial fibroblasts further upregulate ADAMs' expression upon stimulation with proinflammatory cytokines in comparison with resting cells [35]. Shedding of the ectodomain can unmask secondary cleavage sites that, if activated, release soluble intracellular domains; recently, it has been suggested that all three TAMs have intramembrane cleavage sites potentially targeted by gamma-secretase shedding complexes [36].

Importantly, since soluble TAM ectodomains can be easily quantified, they may become valuable diagnostic and/or prognostic biomarkers in the context of inflammatory and autoimmune conditions. Indeed, significant variations of plasmatic levels of TAMs and ligands have been described in numerous pathological conditions. For instance, raised concentrations of soluble TAMs associate with lupus [37–39], Sjogren's syndrome [40, 41], and RA [42, 43]; Gas6 is heightened in a multitude of diseases, such as inflammatory autoimmune demyelinating diseases [44], Alzheimer's disease [45], and hepatic fibrosis [46]. Higher levels of Gas6 also predict oesophageal varices in patients affected by hepatitis C virus (HCV) liver disease [47] and correlate with disease severity in multiple sclerosis [48] and renal involvement in systemic lupus erythematosus (SLE) [49]. Conversely, other authors have found lower Gas6 plasmatic concentrations in lupus [50], Behcet's disease [51], and inflammatory bowel diseases [52] in comparison with healthy controls. Heterogeneity of the cohorts included in the studies might account for these discrepancies since different ethnicity, stage of the disease, previous treatments, and comorbidities can influence the level of expression of both soluble TAMs and TAM ligands.

Epigenetic control, which is acquiring increasing importance, is another mechanism able to regulate TAM protein expression. Briefly, miRs are small noncoding RNA that can modulate the mRNA translation of target genes, hence altering their effector pathways. Research of Axl-modulating miRs was initially performed in malignant cells and tissues and provided a fascinating list of candidates [53]: among them, miR-34a has been selected and studied also in the context of inflammation. Interestingly, it was found that the inhibition of miR-34a in macrophages caused the downregulation of proinflammatory cytokines' release [54] and, in line with these results, that RA DCs were characterised by unrestrained activation of miR-34a driving the uncontrolled production of inflammatory molecules secondary to Axl repression [55].

4. TAM Receptors as Regulators of the Immune System

TAMs' ability to maintain immune system homeostasis and control inflammatory responses in adult tissues was firstly suggested by the phenotype of Mer kinase-dead (MerKD) mice, characterised by an excessive production of Tumor Necrosis Factor (TNF) α upon LPS stimulation and death by endotoxic shock caused by less-lethal doses of LPS [56]. Later on, it was also shown that mutants lacking all three TAMs (known as $TAM^{-/-}$ mice) developed multiorgan signs and symptoms typical of autoimmune inflammatory diseases [20, 21]. $TAM^{-/-}$ mice became progressively blind and sterile and showed gradual enlargement of secondary lymphoid organs caused by an uncontrolled proliferation of B/T lymphocytes [21]; at about six months of age, they displayed a wide range of full-blown clinical, serological, and histological manifestations including immunoglobulin deposits in glomeruli, circulating autoantibodies, vasculitic skin lesions, alopecia, and swollen joints [20, 21].

In the attempt to explain these broad pathological manifestations, two essential TAM-regulated functions were identified and described: the inhibition of Toll-Like-Receptors (TLRs) induced inflammatory cascade and the uptake of apoptotic cells by APCs. The impairment of these mechanisms in the absence of TAMs could, at least partially, recapitulate and explain $TAM^{-/-}$ phenotype.

4.1. Inhibition of Toll-Like Receptor- (TLR-) Mediated Inflammation. Upon being bound by their ligands, TLRs respond by enhancing the release of proinflammatory cytokines, which are crucial for host defence mechanisms against microbial pathogens. On the other hand, failure of TLR fine-tuning causing their unrestrained activation may generate an inflamed environment promoting autoimmunity [57]. APCs like DCs and macrophages use TAMs to regulate and switch off inflammatory reactions secondary to TLR stimulation, thus preventing the chronic activation of TAM-expressing cells [22].

Molecular mechanisms by which TAMs exert this inhibitory function have been particularly well studied in DCs expressing Axl. The initial inflammatory rush provoked by TLR activation and typically exploiting the IFNAR/STAT1 as downstream activator signal can, in turn, also prompt Axl upregulation. Once Axl has been exposed on the cell membrane and activated by its ligand, it can complex with the IFNAR and usurp the IFNAR/STAT1 machinery from TLRs, eventually determining the switch from a pro- to an anti-inflammatory phenotype of the cell. Coupling of Axl with IFNAR upregulates the transcription of inhibitory factors, for instance, the suppressors of cytokine signalling family 1/3 (SOCS1/3) [22]. Mer is likewise important for the inhibition of inflammation in macrophages [58] and macrophage-like cell lines [8]. As reported by Alciato et al., Mer activation by its ligand Gas6 drives the downregulation of LPS-induced production of TNF-α and IL-6 in monocyte-derived macrophages and U937-derived macrophage-like cells by triggering PI3K/AKT and NF-kappa B pathways [8]. Furthermore, as suggested by Zizzo et al., the Mer/Gas6 axis

not only can prevent proinflammatory cytokines' release but also induce the expression of anti-inflammatory mediators (i.e., IL-10) by M2c anti-inflammatory macrophages. Ultimately, Mer/Gas6-induced IL-10 represents a positive feedback loop for M2c cell homeostasis, and it is critical for maintaining an anti-inflammatory and immune-tolerant environment [25]. TAMs' ability to contain the overproduction of TNFα and IL-6 is particularly important in the context of RA since both of these cytokines are abundantly produced within the rheumatoid synovial tissue and sustain the chronic inflammatory process [59, 60]. Clinical efficacy of biologic agents targeting TNFα and IL-6 (e.g., infliximab [61] and tocilizumab [62], respectively) further confirms the detrimental effects played by these molecules in RA.

4.2. Phagocytosis of Apoptotic Cells. The second TAM-mediated mechanism relevant to the immune system regulation is the phagocytosis of apoptotic cells, also called efferocytosis. Removal of apoptotic debris is crucial for maintaining adult tissues healthy and functional. In mice lacking TAMs, initial evidence of defective efferocytosis can be observed in tissues and organs characterised by high cellular turnover, for instance, retina, reproductive, and immune system. Failure to phagocyte apoptotic residues in these tissues clinically manifests with blindness, sterility, and pathological enlargement of secondary lymphoid organs, respectively [20]. Unremoved apoptotic cells are a source of autoantigens and can drive the development of autoimmunity [63, 64], thus underpinning a strong link between the absence of TAMs and the broad-spectrum autoimmune manifestations observed in the triple KO.

The mechanism of efferocytosis used by TAMs is peculiar and carefully regulated. During apoptosis, dying cells expose phosphatidylserine (PtDSer) on their membrane as an "eat me" signal, which makes phagocytes able to discriminate them from other necrotic or healthy cells. TAM ligands Gas6 and Pros1 allow TAM-mediated efferocytosis by binding the PtDSer residues on apoptotic cells via their Gla domains and TAMs on APCs via their amino-terminal region. In this way, TAM ligands function as a "bridge" between apoptotic cells and TAM-expressing phagocytes [65]. Mer was the first TAM receptor discovered to mediate efferocytosis thanks to early experiments performed using MerKD mice. MerKD-derived macrophages were indeed unable to adequately clear thymocytes, but fascinatingly, their phagocytosis deficiency was restricted to apoptotic cells and independent of Fc receptor. Altogether, these findings suggested a critical and exclusive role of Mer in the clearance of apoptotic bodies [66].

Even though Mer has been historically considered the only TAM responsible for the efferocytosis process, recent data highlighted that, under certain circumstances, also other members of the TAM family can acquire phagocytic activity [33]. Depending on the surrounding microenvironment, the same cell type can upregulate either Mer or Axl: in the presence of tolerogenic or immunosuppressive stimuli, Mer is the principal mediator of efferocytosis, and its final aim is maintaining normal tissue cellularity in physiological conditions or upon anti-inflammatory treatment; conversely, following proinflammatory activation of phagocytes, Mer is downregulated and, in turn, Axl takes control of the process [33]. Notably, in RA synovial tissue, NF-κB is strongly activated and provides a robust prosurvival signal and sustains the resistance to apoptosis [67]. Thus, once again, a strong link between one of TAM-mediated functions and the development of RA exists, suggesting that TAMs may be involved in the pathogenesis of the disease.

4.3. TAM Receptors Link the Innate and Adaptive Immunity. Once activated, cells of the adaptive immune system should feedback to innate immune cells to avoid their chronic and uncontrolled activation. Due to their characteristics, including the relatively late appearance in evolution, TAMs seem designated to represent this important connection.

In favour of this hypothesis, it has been recently showed that TAM ligand ProS1 is upregulated exclusively by activated (not resting) T cells and can inhibit their proliferation [68]. The mechanism proposed for explaining this process involves ProS1 ability to create a bridge between PtDSer, exposed by T cells only transitorily after being activated, and TAMs expressed by APCs [69]. ProS1, by binding PtDSer on T cells with its Gla domain and TAMs harboured by DCs with its SHBG domain, favours the connection between these two cell types from the adaptive and innate immune systems. The interaction between TAM/PtDSer drives an inhibitory signal that restrains the proinflammatory activation of DCs, hence limiting the production of cytokines such as IL-6 and TNFα, and will also ultimately inhibit T cells. As a proof of concept, preventing ProS1 to bind activated T cells triggered a rapid increase of activated DCs and proinflammatory molecule release [68].

Virtually, all TAM activities listed so far occur because of their expression by innate immune cells (either monocytes/-macrophages or DCs). However, as an exception, a new TAM function involving CD4+CD25+ T regulatory (T-reg) cells has recently been described. T-reg cells exert their regulatory role largely by preventing the immune cell-induced organ damage. On the one hand, by suppressing autoreactive lymphocytes, T-reg cells are fundamental to avoid autoimmunity; on the other hand, however, an excessive activation of T-reg cells would lead to unhealthy immunosuppression. Defective expression, functionality, and generation of T-reg cells have been described in several autoimmune conditions including RA, in which they are highly present within the inflamed synovial tissue but reduced in the periphery [70]. Surprisingly, Axl and Mer have been detected on the surface of T-reg cells; once activated, Axl/Gas6 enhances the suppressive capacity of T-reg, supporting, once again, Gas6 anti-inflammatory abilities [27].

Overall, the interaction between TAMs, Gas6/ProS1 and innate/adaptive cells is a complex and finely-tuned process. Small changes to this delicate balance could favour the development of autoimmunity and chronic inflammation. Little is known at this regard in RA, but compelling evidence is growing, and future studies will hopefully further elucidate these critical aspects.

5. TAM Receptors Implications in Rheumatoid Arthritis

As mentioned above, the relevance of TAMs in the development and progression of inflammatory arthritis was initially hinted by the phenotype of $TAM^{-/-}$ mice, characterised by broad-spectrum autoimmune manifestations, predominantly resembling SLE but also including inflammatory arthritis [21]. So far, human studies mainly focussed on TAMs' role in SLE showing that impairment in this receptor system is associated with lupus development, and soluble TAMs/ligands may be valuable diagnostic and/or prognostic biomarkers in this condition [3, 71]. Additional and new evidence about TAMs in RA has recently become available and is continuously growing. Over the last decades, several studies have investigated different models of arthritis in TAM single, double, and triple KO mice. One of the most accredited hypotheses that researchers are trying to prove implicates that dysregulation of the TAM axis triggers autoimmune reactions and the development of chronic inflammation within the synovial tissue. If this is correct, adjustments of the "aberrant" TAM system could represent a promising therapeutic target in arthritis.

Following the initial report of the triple TAM KO phenotype, a recent work on the same mice quite surprisingly showed that, in comparison with wild types (WT), KO littermates had neither macroscopic nor histological evidence of inflammatory arthritis in ankle joints until the age of 52 weeks [72]. As suggested by the authors, a different phenotype observed in a genotypically identical animal model may be justified by changes in the interplay between genetic and environmental factors, including, for example, improved cleanliness of facilities and modifications of the microbiota. The latter, in particular, could represent an exciting link with RA as the dysbiosis seems to be a promoter of inflammatory arthritis [73]. Despite not showing clinically evident arthritis, however, both adolescent and adult $TAM^{-/-}$ mice had significantly more marked bone marrow oedema, which is an early sign of arthritis [72].

Further studies from the same group also showed that in a KRN serum transfer model of arthritis, the absence of Axl ($Axl^{-/-}$) or Mer ($Mer^{-/-}$) caused more severe disease in comparison with WT [74, 75]. Of note, the exacerbated pathology was observed only in ankles of $Axl^{-/-}$ mice, whereas no effect was seen in the knees of Axl KO mice [75]. The histological analysis of the synovial tissue enabled a potential interpretation for this clinical outcome. While ankle synovium was characterised by high expression of Axl and a predominance of anti-inflammatory M2 macrophages, synovial tissue sampled from the knees had scant M2 macrophages and virtually absent Axl. Mer-deficient mice had instead aggravated disease in all the joints assessed [75].

The first *in vivo* evidence that TAMs might be therapeutically exploited to improve arthritis was provided in CIA mice treated with adenoviruses overexpressing ProS1 or Gas6. Intra-articular delivery of both TAM ligands Gas6 and ProS1 caused clinical and histological improvement by decreasing the production and release of Th1- and Th17-related proinflammatory cytokines (e.g., IL12/IFNγ and IL-23/IL-17, respectively) [76]. In contrast, only ProS1-overexpressing virus administered via a systemic route was able to improve the disease and reduce the number of splenic Th1-cells, leaving Th17 levels unaffected [76]. TAM ligands' effects may, therefore, depend on the delivery route and be "broader" when given locally. In line with these findings, it has been reported that the cytokine profile of *in vitro* stimulated peripheral blood CD4+ T cells isolated from $Axl^{-/-}$/$Mer^{-/-}$ mice is characterised by higher IFNγ but normal IL-17 [77].

The protective role played by Mer activation by its ligand ProS1 has been lately further confirmed in a KRN serum transfer arthritis model [74] and in a three-dimensional model of human synovium [74], hence enhancing the translational value of this discovery. On the other hand, Mer agonist antibodies were shown to have instead a detrimental effect on arthritis, which can be explained by their capacity of inhibiting Mer-mediated efferocytosis, proving that apoptotic cells removal is fundamental for homeostasis of the synovial tissue.

More recently, Culemann et al. found that Axl is expressed by a distinct subset of CX3CR1+ tissue-resident macrophages forming an immunological barrier at the synovial lining. These peculiar macrophages do not derive from circulating monocytes, proliferate locally, and share features with epithelial cells. By creating tight junctions and expressing anti-inflammatory receptors, these lining-layer macrophages tend to isolate the synovium and prevent the infiltration of inflammatory cells [78].

In contrast with the protective role hypothesised for Axl and Mer, induction of arthritis in $Tyro3^{-/-}$ mice revealed that the third member of TAMs might instead play a proarthritic role. In particular, Tyro3 KO mice had less marked synovial fibroblast proliferation and osteoclast activation and were protected from bone damage in comparison with WT controls [79]. Furthermore, circulating levels of soluble Tyro3 positively correlated with disease activity and erosive burden in patients with RA [80]. It seems, therefore, that activated Tyro3 may be responsible for stimulating synovial hypertrophy, cartilage destruction, and bone erosion, suggesting a dual antithetic role for the TAM axis in arthritis depending on which receptor is activated, i.e., an anti-inflammatory effect in case of Axl or Mer but proerosive if Tyro3 is triggered. Of course, these observations should be taken into account when hypothesising a therapeutic exploitation of TAMs in inflammatory arthritis.

In contrast with a rather high number of studies in animal models, investigations of the TAM system in patients with RA have only recently returned a hot topic of research after an opening report published in 1999 when O'Donnel et al. found that Axl was expressed by a discrete subset of synoviocytes and vascular smooth muscle cells [43]. Our preliminary unpublished data have confirmed that Axl seems preferentially expressed by a subset of synovial lining macrophages, suggesting that it might play a similar "barrier" role as described in animal models of experimental arthritis.

It has been hypothesized that impaired TAM functioning prevents synovial cells to properly switch the inflammatory reactions off, thus triggering the development of chronic

arthritis. The assumption of a defective expression of Axl in patients with RA was elegantly demonstrated in 2017 by Kurowska-Stolarska et al., who showed that CD1c+ DCs isolated from patients with RA have constitutively high levels of miR-34a and, subsequently, inhibited Axl expression in comparison with healthy donors [55]. Importantly, by inhibiting miR-34a, mice become resistant to arthritis, and DCs acquire back the ability to limit proinflammatory cytokine production.

As mentioned above, the Mer/Gas6 axis mediates anti-inflammatory effects in CD206+ CD163+ M2c macrophages by reducing the release of proinflammatory molecules like TNF or IL-6 [8] and, at the same time, by inducing anti-inflammatory mediators such as IL-10, which, in turn, can also positively regulate Gas6 continued secretion [25]. Interestingly, monocyte-derived macrophages isolated from RA patients treated with TNF-inhibitors showed downregulation of surface markers typically associated with inflammation (e.g., CD40 and CD80) but also upregulation of Mer, hence suggesting that, upon treatment, cells acquire the same anti-inflammatory properties as other Mer-positive macrophages. In line with this, *in vitro* studies confirmed that anti-TNF agents were able to inhibit proinflammatory cytokines and upregulate IL-10, activating a positive feedback mechanism involving the Gas6/Mer axis that, ultimately, limited the inflammatory cascade [81].

Recently, single-cell transcriptomic profiling of synovial tissue allowed the identification of several distinct subsets of synovial macrophages, differently expressed based on the nature and stage of the disease. In keeping with its postulated anti-inflammatory role, Mer was significantly highly expressed in osteoarthritic tissue compared to RA; moreover, among RA-specific macrophage subsets, Mer was upregulated in the so-called "anti-inflammatory" group [82]. Not surprisingly, therefore, emerging data suggest that synovial macrophages isolated from RA patients in remission are characterised by a CD163/CD206/Mer-positive signature [83].

The critical regulatory role played by TAM shedding and soluble TAM generation has gathered growing evidence. As mentioned above, indeed, quantification of circulating soluble TAMs and TAM ligands may represent a novel interesting biomarker system. For instance, in RA, sTyro3 serum levels were found elevated compared to healthy controls and correlated with rheumatoid factor titre, the number of swollen joints, and joint erosion scores [80]. The role and interpretation of sMer plasma levels, instead, are still controversial. In one of the available reports, sMer was significantly lower in comparison with healthy controls, with no correlation observed between sMer and disease activity scores; conversely, a different study reported increased levels of circulating sMer in RA, however, reiterating the absence of significant correlations with clinical parameters [84].

Lower levels of Gas6, ProS1, and sAxl in RA have also been documented [42, 43]. Gas6 and sAxl, both significantly decreased in patients compared to healthy controls, positively correlated between them; Gas6 also negatively correlated with the presence of erosions and positively with disease activity scores [42]. Because in RA several disease processes occur at the joint site, the discovery that sAxl is one of the most abundant proteins detected in synovial fluid of RA patients suggests that dysregulation of Axl synovial expression may be a pathogenic pathway worth to be explored in future studies [85].

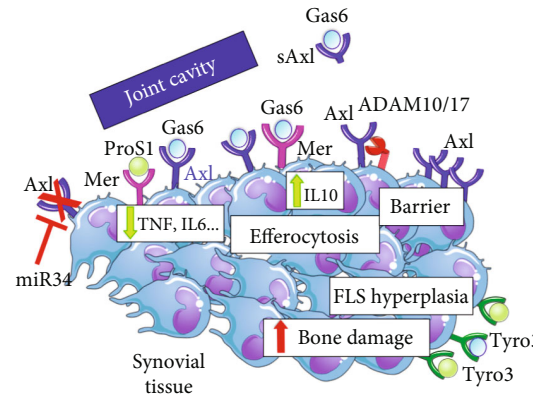


Figure 1: Model of TAM receptors and ligands' effects in synovial tissue. Axl and Mer, once activated by their cognate ligands, exert a protective role within the joint by reducing the production of proinflammatory cytokines, such as TNF and IL-6, and triggering the phagocytosis of apoptotic cells. Axl, specifically, also contributes to form a barrier on the synovial lining while Mer further enhances the anti-inflammatory response by upregulating IL-10. Axl is negatively regulated by miR-34a, which is constitutively activated in RA DCs, and can be cleaved and released as soluble (s) Axl in the joint space by proteinases like ADAM10/17. In contrast, Tyro3 may foster synovial hypertrophy of fibroblast-like-synoviocytes (FLS) and increase bone loss.

6. Conclusion

RA is a chronic inflammatory autoimmune disease affecting joints. Impairment of homeostatic regulators of inflammation likely contributes to the development of persistent inflammatory infiltration of the diseased synovium. Because the defective functionality of TKRs Tyro3, Axl, and Mer (TAM) results in the abnormal activation of the immune system, it has been postulated that these receptors may be implicated in the development of autoimmune diseases including RA.

A protective role for Axl and Mer is supported by finding that induced arthritis is significantly more severe in mice lacking these two receptors. Moreover, Axl likely contributes to physically protecting the joint as it has been found expressed by a special subset of CX3CR1+ lining macrophages originating from synovial precursors and able to form a tight function-mediated barrier. Interestingly, RA-derived DCs have defective Axl expression secondary to the upregulation of its inhibitory micro-RNA miR-34a. Mer, which is typically expressed by anti-inflammatory M2c-polarised macrophages, is upregulated in noninflammatory arthritis like osteoarthritis and RA in remission. Plausibly, Mer plays a crucial role in the synovium by enhancing IL-10, inhibiting proinflammatory cytokines production, and preventing the accumulation of apoptotic cells. In contrast with these results, data about the role of Tyro3 in arthritis showed that its activation is detrimental for the joints as it mediates synovial hypertrophy and increases the erosive burden. Overall, however, the

exogenous administration of TAM ligands seems to ameliorate the disease in experimental models of arthritis. Finally, there is growing attention to the quantification of soluble circulating TAM receptors/ligands and its relationship with clinical phenotypes and disease progression.

In conclusion, available evidence suggests that Axl, Mer, and Tyro3 might play an important and multifaceted role in RA (Figure 1), and further studies on this topic are called to clarify TAMs' role and therapeutic potential.

Conflicts of Interest

The authors declare no conflicts of interests.

References

[1] D. L. Scott, F. Wolfe, and T. W. J. Huizinga, "Rheumatoid arthritis," *The Lancet*, vol. 376, no. 9746, pp. 1094–1108, 2010.

[2] G. S. Firestein and I. B. Mcinnes, "Immunopathogenesis of rheumatoid arthritis," *Immunity*, vol. 46, no. 2, pp. 183–196, 2017.

[3] C. V. Rothlin and G. Lemke, "TAM receptor signaling and autoimmune disease," *Current Opinion in Immunology*, vol. 22, no. 6, pp. 740–746, 2010.

[4] S. Goruppi, E. Ruaro, and C. Schneider, "Gas6, the ligand of Axl tyrosine kinase receptor, has mitogenic and survival activities for serum starved NIH3T3 fibroblasts," *Oncogene*, vol. 12, no. 3, pp. 471–480, 1996.

[5] P. P. Sainaghi, L. Castello, L. Bergamasco, M. Galletti, P. Bellosta, and G. C. Avanzi, "Gas6 induces proliferation in prostate carcinoma cell lines expressing the Axl receptor," *Journal of Cellular Physiology*, vol. 204, no. 1, pp. 36–44, 2005.

[6] G. C. Avanzi, M. Gallicchio, F. Bottarel et al., "GAS6 inhibits granulocyte adhesion to endothelial cells," *Blood*, vol. 91, no. 7, pp. 2334–2340, 1998.

[7] A. Angelillo-Scherrer, L. Burnier, N. Flores et al., "Role of Gas6 receptors in platelet signaling during thrombus stabilization and implications for antithrombotic therapy," *The Journal of Clinical Investigation*, vol. 115, no. 2, pp. 237–246, 2005.

[8] F. Alciato, P. P. Sainaghi, D. Sola, L. Castello, and G. C. Avanzi, "TNF-alpha, IL-6, and IL-1 expression is inhibited by GAS6 in monocytes/macrophages," *Journal of Leukocyte Biology.*, vol. 87, no. 5, pp. 869–875, 2010.

[9] H. A. Anderson, C. A. Maylock, J. A. Williams, C. P. Paweletz, H. Shu, and E. Shacter, "Serum-derived protein S binds to phosphatidylserine and stimulates the phagocytosis of apoptotic cells," *Nature Immunology*, vol. 4, no. 1, pp. 87–91, 2003.

[10] I. B. Mcinnes and G. Schett, "The pathogenesis of rheumatoid arthritis," *The New England Journal of Medicine*, vol. 365, no. 23, pp. 2205–2219, 2011.

[11] E. M. Tan and J. S. Smolen, "Historical observations contributing insights on etiopathogenesis of rheumatoid arthritis and role of rheumatoid factor," *The Journal of Experimental Medicine*, vol. 213, no. 10, pp. 1937–1950, 2016.

[12] E. Astorri, A. Nerviani, M. Bombardieri, and C. Pitzalis, "Towards a stratified targeted approach with biologic treatments in rheumatoid arthritis: role of synovial pathobiology," *Current Pharmaceutical Design*, vol. 21, no. 17, pp. 2216–2224, 2015.

[13] F. Humby, M. Lewis, N. Ramamoorthi et al., "Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients," *Annals of the Rheumatic Diseases*, vol. 78, pp. 761–772, 2019.

[14] D. Prasad, C. V. Rothlin, P. Burrola et al., "TAM receptor function in the retinal pigment epithelium," *Molecular and Cellular Neurosciences*, vol. 33, no. 1, pp. 96–108, 2006.

[15] X. Huang, P. Finerty, J. R. Walker et al., "Structural insights into the inhibited states of the Mer receptor tyrosine kinase," *Journal of Structural Biology*, vol. 165, no. 2, pp. 88–96, 2009.

[16] C. Heiring, B. Dahlbäck, and Y. A. Muller, "Ligand recognition and homophilic interactions in Tyro3: structural insights into the Axl/Tyro3 receptor tyrosine kinase family," *Journal of Biological Chemistry*, vol. 279, no. 8, pp. 6952–6958, 2004.

[17] T. N. Stitt, G. Conn, M. Gore et al., "The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases," *Cell*, vol. 80, no. 4, pp. 661–670, 1995.

[18] K. Ohashi, K. Nagata, J. Toshima et al., "Stimulation of sky receptor tyrosine kinase by the product of growth arrest-specific gene 6," *Journal of Biological Chemistry*, vol. 270, no. 39, pp. 22681–22684, 1995.

[19] C. Lai and G. Lemke, "An extended family of protein-tyrosine kinase genes differentially expressed in the vertebrate nervous system," *Neuron*, vol. 6, no. 5, pp. 691–704, 1991.

[20] Q. Lu, M. Gore, Q. Zhang et al., "Tyro-3 family receptors are essential regulators of mammalian spermatogenesis," *Nature*, vol. 398, no. 6729, pp. 723–728, 1999.

[21] Q. Lu and G. Lemke, "Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family," *Science*, vol. 293, no. 5528, pp. 306–311, 2001.

[22] C. V. Rothlin, S. Ghosh, E. I. Zuniga, M. B. A. Oldstone, and G. Lemke, "TAM receptors are pleiotropic inhibitors of the innate immune response," *Cell*, vol. 131, no. 6, pp. 1124–1136, 2007.

[23] H. M. Seitz, T. D. Camenisch, G. Lemke, H. S. Earp, and G. K. Matsushima, "Macrophages and dendritic cells use different Axl/Mertk/Tyro3 receptors in clearance of apoptotic cells," *The Journal of Immunology*, vol. 178, no. 9, pp. 5635–5642, 2007.

[24] S. Scutera, T. Fraone, T. Musso et al., "Survival and migration of human dendritic cells are regulated by an IFN-α-inducible Axl/Gas6 pathway," *Journal of Immunology*, vol. 183, no. 5, pp. 3004–3013, 2009.

[25] G. Zizzo, B. A. Hilliard, M. Monestier, and P. L. Cohen, "Efficient clearance of early apoptotic cells by human macrophages requires M2c polarization and MerTK induction," *Journal of Immunology*, vol. 189, no. 7, pp. 3508–3520, 2012.

[26] W.-H. Shao, R. A. Eisenberg, and P. L. Cohen, "The Mer receptor tyrosine kinase is required for the loss of B cell tolerance in the chronic graft-versus-host disease model of systemic lupus erythematosus," *The Journal of Immunology*, vol. 180, no. 11, pp. 7728–7735, 2008.

[27] G.-J. Zhao, J.-Y. Zheng, J.-L. Bian et al., "Growth Arrest-Specific 6 Enhances the Suppressive Function of $CD4^+CD25^+$ Regulatory T Cells Mainly through Axl Receptor," *Mediators of Inflammation*, vol. 2017, Article ID 6848430, 13 pages, 2017.

[28] M. Schoumacher and M. Burbridge, "Key roles of AXL and MER receptor tyrosine kinases in resistance to multiple anticancer therapies," *Current Oncology Reports*, vol. 19, no. 3, p. 19, 2017.

[29] G. Lemke and C. V. Rothlin, "Immunobiology of the TAM receptors," *Nature Reviews Immunology*, vol. 8, no. 5, pp. 327–336, 2008.

[30] P. Bellosta, M. Costa, D. A. Lin, and C. Basilico, "The receptor tyrosine kinase ARK mediates cell aggregation by homophilic binding," *Molecular and Cellular Biology*, vol. 15, no. 2, pp. 614–625, 1995.

[31] J. P. O'Bryan, Y. W. Fridell, R. Koski, B. Varnum, and E. T. Liu, "The transforming receptor tyrosine kinase, Axl, is post-translationally regulated by proteolytic cleavage," *Journal of Biological Chemistry*, vol. 270, no. 2, pp. 551–557, 1995.

[32] E. Thorp, T. Vaisar, M. Subramanian, L. Mautner, C. Blobel, and I. Tabas, "Shedding of the Mer tyrosine kinase receptor is mediated by ADAM17 protein through a pathway involving reactive oxygen species, protein kinase Cδ, and p38 mitogen-activated protein kinase (MAPK)," *Journal of Biological Chemistry*, vol. 286, no. 38, pp. 33335–33344, 2011.

[33] A. Zagórska, P. G. Través, E. D. Lew, I. Dransfield, and G. Lemke, "Diversification of TAM receptor tyrosine kinase function," *Nature Immunology*, vol. 15, no. 10, pp. 920–928, 2014.

[34] T. Isozaki, B. J. Rabquer, J. H. Ruth, G. K. Haines, and A. E. Koch, "ADAM-10 is overexpressed in rheumatoid arthritis synovial tissue and mediates angiogenesis," *Arthritis & Rheumatology*, vol. 65, no. 1, pp. 98–108, 2013.

[35] S. Ishii, T. Isozaki, H. Furuya et al., "ADAM-17 is expressed on rheumatoid arthritis fibroblast-like synoviocytes and regulates proinflammatory mediator expression and monocyte adhesion," *Arthritis Research & Therapy*, vol. 20, no. 1, article 159, 2018.

[36] J. A. M. Merilahti, V. K. Ojala, A. M. Knittle, A. T. Pulliainen, and K. Elenius, "Genome-wide screen of gamma-secretase-mediated intramembrane cleavage of receptor tyrosine kinases," *Molecular Biology of the Cell*, vol. 28, no. 22, pp. 3123–3131, 2017.

[37] J. J. Orme, Y. Du, K. Vanarsa, J. Mayeux, and L. Li, "Heightened cleavage of Axl receptor tyrosine kinase by ADAM metalloproteases may contribute to disease pathogenesis in SLE," *Clinical Immunology*, vol. 169, pp. 58–68, 2016.

[38] G. Zizzo, J. Guerrieri, L. M. Dittman, J. T. Merrill, and P. L. Cohen, "Circulating levels of soluble MER in lupus reflect M2c activation of monocytes/macrophages, autoantibody specificities and disease activity," *Arthritis Research & Therapy*, vol. 15, no. 6, p. R212, 2013.

[39] H. Zhu, X. Sun, L. Zhu et al., "The expression and clinical significance of different forms of Mer receptor tyrosine kinase in systemic lupus erythematosus," *Journal of Immunology Research*, vol. 2014, Article ID 431896, 12 pages, 2014.

[40] B. Qin, J. Wang, N. Ma et al., "The association of Tyro3/Axl/-Mer signaling with inflammatory response, disease activity in patients with primary Sjogren's syndrome," *Joint, Bone, Spine*, vol. 82, no. 4, pp. 258–263, 2015.

[41] C.-H. Chen, H.-C. Chen, C.-C. Chang et al., "Growth arrest-specific 6 protein in patients with Sjögren syndrome: determination of the plasma level and expression in the labial salivary gland," *PLoS One*, vol. 10, no. 10, article e0139955, 2015.

[42] I. H. Bassyouni, M. M. El-Wakd, N. A. Azab, and R. H. Bassyouni, "Diminished soluble levels of growth arrest specific protein 6 and tyrosine kinase receptor Axl in patients with rheumatoid arthritis," *International Journal of Rheumatic Diseases*, vol. 20, no. 1, pp. 53–59, 2017.

[43] K. O'Donnell, I. C. Harkes, L. Dougherty, and I. P. Wicks, "Expression of receptor tyrosine kinase Axl and its ligand Gas6 in rheumatoid arthritis: evidence for a novel endothelial cell survival pathway," *The American Journal of Pathology*, vol. 154, no. 4, pp. 1171–1180, 1999.

[44] P. P. Sainaghi, L. Collimedaglia, F. Alciato et al., "Elevation of Gas6 protein concentration in cerebrospinal fluid of patients with chronic inflammatory demyelinating polyneuropathy (CIDP)," *Journal of the Neurological Sciences*, vol. 269, no. 1-2, pp. 138–142, 2008.

[45] P. P. Sainaghi, M. Bellan, F. Lombino et al., "Growth arrest specific 6 concentration is increased in the cerebrospinal fluid of patients with Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 55, no. 1, pp. 59–65, 2017.

[46] M. Bellan, G. Pogliani, C. Marconi et al., "Gas6 as a putative noninvasive biomarker of hepatic fibrosis," *Biomarkers in Medicine*, vol. 10, no. 12, pp. 1241–1249, 2016.

[47] M. Bellan, P. P. Sainaghi, M. T. Minh et al., "Gas6 as a predictor of esophageal varices in patients affected by hepatitis C virus related-chronic liver disease," *Biomarkers in Medicine*, vol. 12, no. 1, pp. 27–34, 2018.

[48] P. P. Sainaghi, L. Collimedaglia, F. Alciato et al., "Growth arrest specific gene 6 protein concentration in cerebrospinal fluid correlates with relapse severity in multiple sclerosis," *Mediators of Inflammation*, vol. 2013, Article ID 406483, 7 pages, 2013.

[49] C. Ekman, A. Jönsen, G. Sturfelt, A. A. Bengtsson, and B. Dahlbäck, "Plasma concentrations of Gas6 and sAxl correlate with disease activity in systemic lupus erythematosus," *Rheumatology*, vol. 50, no. 6, pp. 1064–1069, 2011.

[50] H. Zhu, X. Sun, L. Zhu et al., "Different expression patterns and clinical significance of mAxl and sAxl in systemic lupus erythematosus," *Lupus*, vol. 23, no. 7, pp. 624–634, 2014.

[51] S. Guermazi, M. Hamza, and K. Dellagi, "Protein S deficiency and antibodies to protein S in patients with Behçet's disease," *Thrombosis Research*, vol. 86, no. 3, pp. 197–204, 1997.

[52] E. Aadland, O. R. Odegaard, A. Røseth, and K. Try, "Free protein S deficiency in patients with chronic inflammatory bowel disease," *Scandinavian Journal of Gastroenterology*, vol. 27, no. 11, pp. 957–960, 1992.

[53] G. Mudduluru, P. Ceppi, R. Kumarswamy, G. V. Scagliotti, M. Papotti, and H. Allgayer, "Regulation of Axl receptor tyrosine kinase expression by miR-34a and miR-199a/b in solid cancer," *Oncogene*, vol. 30, no. 25, pp. 2888–2899, 2011.

[54] P. Jiang, R. Liu, Y. Zheng et al., "MiR-34a inhibits lipopolysaccharide-induced inflammatory response through targeting Notch1 in murine macrophages," *Experimental Cell Research*, vol. 318, no. 10, pp. 1175–1184, 2012.

[55] M. Kurowska-Stolarska, S. Alivernini, E. G. Melchor et al., "Micro RNA-34a dependent regulation of AXL controls the activation of dendritic cells in inflammatory arthritis," *Nature Communications*, vol. 8, article 15877, 2017.

[56] T. D. Camenisch, B. H. Koller, H. S. Earp, and G. K. Matsushima, "A novel receptor tyrosine kinase, Mer, inhibits TNF-alpha production and lipopolysaccharide-induced endotoxic shock," *The Journal of Immunology*, vol. 162, no. 6, pp. 3498–3503, 1999.

[57] J. Wang, Y. Hu, W. W. Deng, and B. Sun, "Negative regulation of Toll-like receptor signaling pathway," *Microbes and Infection*, vol. 11, no. 3, pp. 321–327, 2009.

[58] T. Deng, Y. Zhang, Q. Chen, K. Yan, and D. Han, "Toll-like receptor-mediated inhibition of Gas6 and ProS expression

facilitates inflammatory cytokine production in mouse macrophages," *Immunology*, vol. 135, no. 1, pp. 40–50, 2012.

[59] C. Q. Chu, M. Field, M. Feldmann, and R. N. Maini, "Localization of tumor necrosis factor alpha in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis," *Arthritis & Rheumatism*, vol. 34, no. 9, pp. 1125–1132, 1991.

[60] A. Kennedy, U. Fearon, D. J. Veale, and C. Godson, "Macrophages in synovial inflammation," *Front Immun Frontiers*, vol. 2, p. 52, 2011.

[61] M. Feldmann and R. N. Maini, "Anti-TNFα therapy of rheumatoid arthritis: what have we learned?," *Annual Review of Immunology*, vol. 19, no. 1, pp. 163–196, 2001.

[62] M. Mircic and A. Kavanaugh, "The clinical efficacy of tocilizumab in rheumatoid arthritis," *Drugs Today.*, vol. 45, no. 3, pp. 189–197, 2009.

[63] U. S. Gaipl, L. E. Munoz, G. Grossmayer et al., "Clearance deficiency and systemic lupus erythematosus (SLE)," *Journal of Autoimmunity*, vol. 28, no. 2-3, pp. 114–121, 2007.

[64] I. Baumann, W. Kolowos, R. E. Voll et al., "Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 46, no. 1, pp. 191–201, 2002.

[65] E. D. Lew, J. Oh, P. G. Burrola et al., "Differential TAM receptor-ligand-phospholipid interactions delimit differential TAM bioactivities," *Elife*, vol. 3, p. 87, 2014.

[66] R. S. Scott, E. J. McMahon, S. M. Pop et al., "Phagocytosis and clearance of apoptotic cells is mediated by MER," *Nature*, vol. 411, no. 6834, pp. 207–211, 2001.

[67] B. Bartok and G. S. Firestein, "Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis," *Immunological Reviews*, vol. 233, no. 1, pp. 233–255, 2010.

[68] E. A. Carrera-Silva, P. Y. Chan, L. Joannas et al., "T cell-derived protein S engages TAM receptor signaling in dendritic cells to control the magnitude of the immune response," *Immunity*, vol. 39, no. 1, pp. 160–170, 2013.

[69] K. Fischer, S. Voelkl, J. Berger, R. Andreesen, T. Pomorski, and A. Mackensen, "Antigen recognition induces phosphatidylserine exposure on the cell surface of human $CD8^+$ T cells," *Blood*, vol. 108, no. 13, pp. 4094–4101, 2006.

[70] H. Kelchtermans, L. Geboes, T. Mitera, D. Huskens, G. Leclercq, and P. Matthys, "Activated $CD4^+CD25^+$ regulatory T cells inhibit osteoclastogenesis and collagen-induced arthritis," *Annals of the Rheumatic Diseases*, vol. 68, no. 5, pp. 744–750, 2009.

[71] P. L. Cohen and W.-H. Shao, "Gas6/TAM receptors in systemic lupus erythematosus," *Disease Markers*, vol. 2019, Article ID 7838195, 9 pages, 2019.

[72] C. E. J. Waterborg, M. I. Koenders, P. L. E. M. van Lent, P. M. van der Kraan, and F. A. J. van de Loo, "*Tyro3*/*Axl*/*Mertk*-deficient mice develop bone marrow edema which is an early pathological marker in rheumatoid arthritis," *PLoS One*, vol. 13, no. 10, article e0205902, 2018.

[73] A. Picchianti-Diamanti, C. Panebianco, S. Salemi et al., "Analysis of gut microbiota in rheumatoid arthritis patients: disease-related dysbiosis and modifications induced by etanercept," *International Journal of Molecular Sciences*, vol. 19, no. 10, article 2938, 2018.

[74] C. E. J. Waterborg, S. Beermann, M. G. A. Broeren et al., "Protective role of the MER tyrosine kinase *via* Efferocytosis in rheumatoid arthritis models," *Frontiers in Immunology*, vol. 9, p. 742, 2018.

[75] C. E. J. Waterborg, M. G. A. Broeren, E. N. Blaney Davidson et al., "The level of synovial AXL expression determines the outcome of inflammatory arthritis, possibly depending on the upstream role of TGF-β1," *Rheumatology*, vol. 58, no. 3, pp. 536–546, 2018.

[76] B. T. van den Brand, S. Abdollahi-Roodsaz, E. A. Vermeij et al., "Therapeutic efficacy of Tyro3, Axl, and Mer tyrosine kinase agonists in collagen-induced arthritis," *Arthritis and Rheumatism*, vol. 65, no. 3, pp. 671–680, 2013.

[77] F. Ye, L. Han, Q. Lu et al., "Retinal self-antigen induces a predominantly Th1 effector response in Axl and Mertk double-knockout mice," *The Journal of Immunology*, vol. 187, no. 8, pp. 4178–4186, 2011.

[78] S. Culemann, A. Grüneboom, J. Á. Nicolás-Ávila et al., "Locally renewing resident synovial macrophages provide a protective barrier for the joint," *Nature*, vol. 572, no. 7771, pp. 670–675, 2019.

[79] G. Ruiz-Heiland, Y. Zhao, A. Derer et al., "Deletion of the receptor tyrosine kinase Tyro3 inhibits synovial hyperplasia and bone damage in arthritis," *Annals of the Rheumatic Diseases*, vol. 73, no. 4, pp. 771–779, 2014.

[80] L. Xu, F. Hu, H. Zhu et al., "Soluble TAM receptor tyrosine kinases in rheumatoid arthritis: correlation with disease activity and bone destruction," *Clinical and Experimental Immunology*, vol. 192, no. 1, pp. 95–103, 2018.

[81] Y. Degboé, B. Rauwel, M. Baron et al., "Polarization of rheumatoid macrophages by TNF targeting through an IL-10/STAT3 mechanism," *Frontiers in Immunology*, vol. 10, p. 3, 2019.

[82] F. Zhang, K. Wei, K. Slowikowski et al., "Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry," *Nature Immunology*, vol. 20, no. 7, pp. 928–942, 2019.

[83] S. R. Finlay, S. Alivernini, A. Elmesmari et al., "OP0269 Activation of mertk+cd206+ subpopulation of human synovial tissue-resident macrophages limits inflammatory response," *Annals of the Rheumatic Diseases*, vol. 77, Supplement 2, pp. 183–183, 2018.

[84] J. Wu, C. Ekman, A. Jönsen et al., "Increased plasma levels of the soluble Mer tyrosine kinase receptor in systemic lupus erythematosus relate to disease activity and nephritis," *Arthritis Research & Therapy*, vol. 13, no. 2, article R62, 2011.

[85] M. Bhattacharjee, L. Balakrishnan, S. Renuse et al., "Synovial fluid proteome in rheumatoid arthritis," *Clinical Proteomics*, vol. 13, no. 1, article 12, 2016.

Hindawi
Disease Markers
Volume 2019, Article ID 2387614, 13 pages
https://doi.org/10.1155/2019/2387614

Review Article

TAM Receptor Pathways at the Crossroads of Neuroinflammation and Neurodegeneration

Giacomo Tondo,[1,2] **Daniela Perani**,[1,2] **and Cristoforo Comi**[3,4]

[1]*Vita-Salute San Raffaele University, Milan, Italy*
[2]*In Vivo Human Molecular and Structural Neuroimaging Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy*
[3]*Neurology Unit, Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy*
[4]*Interdisciplinary Research Center of Autoimmune Diseases, Università del Piemonte Orientale, Novara, Italy*

Correspondence should be addressed to Cristoforo Comi; cristoforo.comi@med.uniupo.it

Received 14 March 2019; Revised 4 June 2019; Accepted 12 August 2019; Published 15 September 2019

Academic Editor: Massimiliano M. Corsi Romanelli

Increasing evidence suggests that pathogenic mechanisms underlying neurodegeneration are strongly linked with neuroinflammatory responses. Tyro3, Axl, and Mertk (TAM receptors) constitute a subgroup of the receptor tyrosine kinase family, cell surface receptors which transmit signals from the extracellular space to the cytoplasm and nucleus. TAM receptors and the corresponding ligands, Growth Arrest Specific 6 and Protein S, are expressed in different tissues, including the nervous system, playing complex roles in tissue repair, inflammation and cell survival, proliferation, and migration. In the nervous system, TAM receptor signalling modulates neurogenesis and neuronal migration, synaptic plasticity, microglial activation, phagocytosis, myelination, and peripheral nerve repair, resulting in potential interest in neuroinflammatory and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Multiple Sclerosis. In Alzheimer and Parkinson diseases, a role of TAM receptors in neuronal survival and pathological protein aggregate clearance has been suggested, while in Multiple Sclerosis TAM receptors are involved in myelination and demyelination processes. To better clarify roles and pathways involving TAM receptors may have important therapeutic implications, given the fine modulation of multiple molecular processes which could be reached. In this review, we summarise the roles of TAM receptors in the central nervous system, focusing on the regulation of immune responses and microglial activities and analysing in vitro and in vivo studies regarding TAM signalling involvement in neurodegeneration.

1. Introduction

Receptor tyrosine kinases (RTKs) are a large group of cell surface receptors which transmit signals from the extracellular space to the cytoplasm and nucleus. RTKs regulate several cellular processes, including cellular growth, differentiation, proliferation, motility, and apoptosis in multiple organs and systems [1]. The TAM receptor subgroup has received growing attention due to the crucial role in the preservation of homeostatic balance through the modulation of immune, nervous, vascular, and reproductive functions [2–6].

TAMs include three receptors: Tyro3, Axl, and Mertk, which are differentially expressed in different tissues. Among TAMs, Tyro3 is the most widely expressed in the adult central nervous system (CNS) [7, 8]. In rats, its expression is very low at embryonic stages, while it dramatically increases during early postnatal stages reaching high, stable levels in the adult CNS, thus revealing a temporal correlation with synaptogenesis [9, 10]. Tyro3 has been found in the olfactory bulbs, the piriform cortex, the amygdala, the cerebellum, the cerebral cortex, and the hippocampus [11, 12], being expressed in neurons, gonadotrophin-releasing hormone (GnRH) neurons, radial glia, astrocytes, and oligodendroglia [8, 11, 13]. Tyro3 is also expressed in the peripheral nervous system (PNS), specifically in dorsal root ganglion (DRG) neurons and Schwann cell [14, 15]. Outside the nervous system,

Tyro3 is expressed in the breast, kidney, lung, testis, ovary, retina, and hematopoietic cell lines including platelets and monocytes/macrophages [16–25]. Axl and Mertk expression in the nervous system is lower than Tyro3 but relatively constant throughout development [11]. Axl expression has been revealed in the hippocampus and in the cerebellum [26, 27]. It has been found in oligodendroglia [11, 28], astrocytes [29, 30], microglia [9, 31], and Schwann cells [14]. Axl is highly expressed by migrating GnRH neurons [32]. It is also present in cells of the heart, breast, skeletal muscle, liver, kidney, testis, and bone marrow, in platelets, and in monocytes/macrophages [16, 20, 21, 26, 33–37]. Mertk expression has been detected in low levels in the brain, oligodendrocytes, astrocytes, and microglia [11, 27, 29, 31]. Low levels of Mertk are reported in the heart and skeletal muscle, while high levels are reported in the ovary, prostate, testis, lung, kidney, and retina [17, 23, 36, 38–41]. Mertk is also expressed in monocytes/macrophages, dendritic cells, natural killer cells, megakaryocytes, and platelets [16, 42–45].

The most characterised TAM ligands are two vitamin k-dependent proteins, the Growth Arrest Specific 6 (Gas6) and Protein S (Pros1), which are widely expressed in different human tissues including the brain [12, 46]. Gas6 gene was firstly identified in embryonic mouse fibroblast [47]. It has been reported a production in the heart, kidney, lungs, liver, endothelial cells, vascular smooth muscle cells, bone marrow, and murine platelets [4, 16, 48–51]. Gas6 is extensively expressed in both the CNS and PNS, and its production increases from the embryonic stage to the adult stage [12]. It is secreted by neurons and endothelial cells [28, 32, 52], being produced in several brain regions including cerebral cortex, hippocampus, cerebellum, midbrain, and thalamus [12]. Gas6 mRNA has been also identified in spinal motor neurons and dorsal root ganglion neurons [14]. Pros1 is a protein expressed by hepatocytes, osteoblast, megakaryocytes, and endothelial cells [4, 53]. Pros1 can be detected in high levels in plasma, where it plays an anticoagulant activity, both autonomously and acting as a cofactor in the breakdown of the coagulation factors [54, 55]. In the CNS, Pros1 is expressed at a low level, mainly in the locus coeruleus and in choroid plexus [12].

The wide distribution of TAMs accounts for their multiple functions. TAM signalling can affect cell proliferation, survival/apoptosis, and migration, and it is involved in the modulation of homeostasis, phagocytosis, and inflammatory responses [2]. Consequently, a dysregulation of TAMs can be related to a plethora of pathological processes, such as chronic inflammation and autoimmune diseases [5], cancer progression [56], defects of spermatogenesis [57], retinal degeneration [58], brain neuroinflammation, myelination abnormalities, cancer, and neurodegeneration [2]. For these reasons, TAMs represent an interesting potential therapeutic target in different conditions.

The development of the promising therapeutic strategy able to modulate TAM actions relies on the complete understanding of TAM signalling. The variety of the physiopathological responses related to TAM activation firstly depends on the interaction between TAMs and their ligands. All three TAMs are activated by Gas6, which binds Axl with the highest affinity [59–62]. Pros1 binds Mertk and Tyro3 but not Axl [23, 52]. This implies that Axl may be the most important Gas6 receptor in different tissues [2]. Axl and Mertk have a soluble form in human plasma, which derives from the cleavage of the full-length receptor by a metalloproteinase, with consequent inactivation [63]. The soluble circulating TAMs capture the corresponding ligands inhibiting the full-length receptor action [64]. The presence of a soluble form with biological effects further increases the clinic and therapeutic potential of TAMs.

Different cellular responses can be generate depending on the activated receptor, pathway, and cellular type. Gas6/Axl signal promotes cell survival, growth, and proliferation via activation of the PI3K- (phosphatidylinositol-3-kinase-) Akt, extracellular signal-regulated kinase 1/2 (ERK1/2), and MAPK (mitogen-activated protein kinase) [28, 32, 65]. Axl acts limiting inflammatory responses by inducing the suppressor of cytokine signalling (SOCS) proteins, which in turn inhibit toll-like receptors and cytokine receptor signalling in dendritic cells [66, 67]. Pros1/Tyro3 also activates the PI3K-Akt pathway, protecting neurons from excitotoxicity-induced apoptosis in the mouse [68]. Mertk supports cell survival reducing apoptosis induced by different stimuli [69, 70]. Mertk regulates phagocytosis and clearance of apoptotic cells in different tissues, such as testis [71] and retina, where phagocytosis prevents retinal degeneration via Gas6/Pros1-mediated Mertk activation [23, 58, 72]. Promoting survival, chemoresistance, and motility, TAMs may have an oncogenic potential, depending on the cell type and tissue context [73]. Indeed, TAMs are overexpressed in different cancers, including hematopoietic malignancy, skin, lung, breast, prostatic, and CNS cancers [2]. Inhibition of TAMs may reduce tumor cell survival and stimulate antitumoral immunity, implying a remarkable therapeutic potential [1].

In the nervous system, TAMs play many different relevant functions in modulating cell survival, proliferation, and migration, regulating synaptogenesis, myelination, and neurotrophic and neuroimmune responses [2, 3, 5, 8, 74].

In this review, we will summarise the role of TAMs in the CNS and PNS, especially focusing on the modulation of microglial activation and myelination. We will explore the current findings on the role of inflammation in neurodegeneration, specifically what has been reported in animal models and in human studies. We will highlight the main findings on the role of TAM signalling in common neurodegenerative diseases: Alzheimer's disease (AD), Parkinson's disease (PD), and Multiple Sclerosis (MS).

2. Multiple Roles for TAMs in the Peripheral and Central Nervous Systems

TAMs are important modulators of neurogenesis, the process by which neurons are generated by neural stem cells (NSCs). NSCs can be found in the adult mammalian brain, namely, in the subventricular zone of the lateral ventricles and in the hippocampal subgranular zone. Neurons generated from NSCs are able to integrate into preexisting neural circuitries, which make them one of the most interesting

candidates as the target for therapeutic strategies in neurodegeneration [75]. TAMs regulate survival, proliferation, and differentiation of NSCs. *In vitro* studies showed that cultured NSCs lacking TAMs have reduced growth and proliferation, delayed differentiation, and increased apoptosis [27]. Specifically, the loss of Gas6 caused a reduction in the number of NSCs in the subventricular zone [76]. TAMs showed to protect hippocampal neurons and stimulate NSC proliferation negatively influencing the production of microglia proinflammatory cytokines [9, 27, 30]. Recent works revealed a role of Pros1 in regulating NSC quiescence and differentiation [77, 78]. Pros1-deficient murine NSCs had a marked increase in proliferation, with a dramatic decrease in newborn neurons and a corresponding increase of astrocytes, suggesting that Pros1 is necessary for maintaining NSC quiescence and generating new neurons [78]. Furthermore, Pros1 has been shown to finely regulate self-renewal of NSCs, since its genetic ablation increased self-renewal by 50%, favouring the maintenance of the NSC pool [77].

The abundant expression of TAMs in the hippocampus [11], the inhibition of apoptosis in Gas6-mediated cultured hippocampal cells [9], and the interaction with proteins involved in synaptic enlargement [8] suggest a role of TAMs in synaptogenesis and modulation of synaptic plasticity. Tyro3 is highly expressed in hippocampal neurons, specifically in the CA1 field [11], which is involved in long-term potentiation (LTP). LTP underlies synaptic plasticity, representing a persistent strengthening of synapses and controlling learning and memory [79]. Gas6 can induce Tyro3 phosphorylation activating the MAPK and the PI3K pathways, which play a crucial role in the induction of hippocampal LTP. TAMs may affect synaptic plasticity also in an alternative way. Synapsis are dynamic structures which can undergo both rapid generation and elimination, to reinforce essential circuits and to eliminate redundant connections [80]. Astrocytes, which express TAMs, actively participate to synaptic formation, function, and elimination [81]. Astrocytes were found to eliminate synapses and neural debris through Mertk and another phagocytic receptor, the multiple EGF-like domains 10 protein (MEGF10). The loss of these receptors caused a 50% reduction in the astrocyte modulation of synapsis elimination processes, thus affecting the capacity to refine excess functional synapsis [82].

TAMs regulate neuronal migration, especially GnRH neurons during development [83]. Normal sexual maturation depends on the migration of GnRH neurons from the olfactory placode to the hypothalamus, which is regulated by a Gas6/Axl signalling [84]. GnRH neurons also express Tyro3, which is equally important to preserve reproductive function: Axl/Tyro3 null mice, in fact, show reproductive abnormalities such as delayed and abnormal cyclicity [13]. Gas6 expression seems to be related to survival of GnRH neurons, which are reduced in Gas6 null mice, leading to delayed sexual maturation [85].

Pros1/Tyro3 are involved in protection from N-methyl-D-Aspartate-receptor- (NMDAr-) mediated neurotoxicity. [68, 86]. Pros1 showed to protect mouse cortical neurons from apoptosis in an *in vivo* model of NMDA-induced excitotoxic lesions, requiring Tyro3 as a receptor but not Axl or Mertk [86]. This finding was confirmed in vitro [68].

TAMs are involved in cancer genesis in both the CNS and PNS [87–89]. Gas6 and Axl are overexpressed in human glioma and in glioblastoma multiforme, predicting a poor prognosis [90]. Axl and Mertk are often coexpressed in astrocytoma [91]. Mesenchymal glioma can express high levels of Mertk [88], whose increased expression has been also related to infiltration into the CNS by acute lymphoblastic leukemia cells [92]. Tyro3 was found downregulated in diffuse astrocytomas, consistently with a loss of differentiation in tumoral cells [93]. Gas6 and Axl activation was reported in schwannoma, correlating with pathological survival and proliferation of tumoral cells [94], and Axl expression was increased also in a malignant peripheral nerve sheath tumor [95]. Inhibition of TAMs stimulates antitumor immunity and inhibits tumor cell survival [91, 96], representing a potential antitumoral therapeutic approach.

In the PNS, TAMs modulate myelination and peripheral nerve repair [9, 14, 15, 97]. Gas6 is a growth factor for Schwann cells, which are responsible for myelination in the PNS, also exhibiting an antiapoptotic effect on these cells [14]. Gas6 and Tyro3 are highly expressed in dorsal root ganglion (DRG) neurons [14]; Gas6 and Axl are also expressed in the sciatic nerve [9]. Physiological peripheral nerve myelination, mediated by Schwann cells, may be related to Tyro3 activation by its binding partner Fyn-nonreceptor cytoplasmic tyrosine kinase, since Tyro3 knockout mice show reduced myelin thickness and Fyn knockout mouse DRG cultures exhibit decreased myelin formation [15]. Previous studies suggested a fine regulation of TAM signalling during nerve injury/repair. Gas6 showed regulated expression in the sciatic nerve after nerve transection, decreasing six hours after nerve injury and progressively increasing within two weeks [9]. Furthermore, nerve injury increased Axl/Tyro3 expression in dorsal root ganglion Schwann cells [14]. Schwann cell activity is also related to the interaction with proregenerative macrophages, which may produce Gas6 in response to remyelinating stimuli; Gas6 loss within monocyte lineage cells negatively affects remyelination after nerve injury [97].

3. TAMs in Immune Regulation and Microglial Activation

TAMs play a central role in immune modulation, regulating the coordination between innate and adaptive immune responses [66]. TAM triple knockout (TKO) mice provided a model to define this role [21]. After few weeks of apparently normal development, TKO mice showed aberrant proliferation of active T and B lymphocytes, with diffuse tissue infiltration which led to autoimmune symptoms similar to those of human autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus [21, 98]. Since TAMs have been isolated in monocytes/macrophages but not in T and B lymphocytes, the immune dysregulation in TKO mice is due primarily to the loss of TAMs in macrophages and, in general, in antigen-presenting cells (APCs), which are also constitutively active in TKO mice, enhancing

the production of proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin- (IL-) 12 [21, 67]. Further studies showed that TAMs may contribute to the negative regulation of immune responses [56]. The stimulation of innate immune response leads to type I interferon production, which activates the type I interferon receptor (IFNAR)/Janus kinase (JAK)/signal transducer and activator of transcription protein (STAT) pathway. The IFNAR/JAK/-STAT pathway enhances the production cytokines but also the expression of Axl [99]. The association of the TAM/ligand complex with IFNAR switches off the inflammatory response activating the transcription of suppressor of cytokine signalling (SOCS) 1 and SOCS3, which in turn inhibit toll-like receptors (TLRs) and cytokine receptors to resolve inflammation [67, 100, 101].

TAM modulation in inflammatory responses may be a strategical process to reduce brain disorders induced by chronic inflammation. Systemic chronic inflammation in TKO mice has been shown to provoke direct brain damage and neuronal death through multiple mechanisms, including hyperproduction of TNF-α and autoantibodies, increased permeability of the brain-blood barrier, T lymphocyte infiltration, and abnormal protein aggregate deposition [102].

Another crucial anti-inflammatory mechanism is that TAMs regulate phagocytosis in several tissues [103, 104]. Gas6 plays an essential role in this mechanism [105, 106]. Apoptotic cells expose on the external cell membrane the phosphatidylserine, which is recognised by Gas6 [106]. Thus, Gas6, bridging the phosphatidylserine to TAMs, drives macrophages to the apoptotic cells favouring phagocytosis [98, 103]. Phagocytosis, allowing the removal of apoptotic cells and cell debris, results critical in reducing potential stimuli to autoimmunity, such as the release of intracellular content from necrotic cells, and to degeneration, such as protein deposits and apoptotic cell accumulation. TAM modulation of the clearance of apoptotic cell may vary in different conditions. In the retina, Mertk and Tyro3 play a pivotal role in maintaining normal functioning [105, 107]. In fact, Mertk knockout mice showed retinal degeneration mainly due to the deficit of phagocytosis of residual rods and cones [105]. Tyro3 may compensate for Mertk loss promoting phagocytosis, thus reducing the severity of Mertk-associated photoreceptor degeneration [107]. Conversely, in a focal brain ischemia model, Mertk knockdown mice showed reduction of local phagocytic activity with a resulting less pronounced postischemic atrophy [108].

In the CNS, phagocytosis is an essential mechanism to regulate synapsis and myelination and to prevent neuroinflammation and neurodegeneration. Microglia are resident macrophages in the brain and spinal cord, recognised as the major phagocytic element; however, astrocytes participate to the phagocytic activity in the elimination of synapses and neuronal debris from the brain [82]. Microglia are an important regulator of brain homeostasis and immunity, potentially acting both in neuroinflammatory and anti-inflammatory responses. The polarization of microglia is mainly driven by cytokines. A resting microglia phenotype, which produces anti-inflammatory mediators and neurotrophic factors, is stimulated by interleukin- (IL-) 4 and IL-13, while an activated proinflammatory phenotype is stimulated by IL-1, tumor necrosis factor α, and toll-like receptor ligands [109]. Different kinds of insults can trigger the switch into the activated phenotype, promoting the engagement of the immune system. In physiologic conditions, neuroinflammatory response is self-limiting, aiming to eliminate pathogens and stimulating tissue repair. In neurodegenerative conditions, a sustained inflammatory response, due to the failure of the resolution of the insult, generates detrimental effects, self-maintaining a vicious circle with the production of proinflammatory molecules which, again, sustains the inflammatory response. Individual genetic background can predispose to the overproduction of proinflammatory mediators. Such mechanism has been extensively studied in neurodegenerative and neuroinflammatory diseases [110–112].

Several reports have shown that TAMs regulate multiple microglial functions, acting on both quiescent and activated microglia and facilitating phagocytosis and clearance of apoptotic cells and cellular debris [31]. Mertk and Axl are expressed in microglia, whereas Tyro3 is highly present in neurons [31]. In adult mice, the result of a deficient activity of both Axl and Mertk is the impairment of apoptotic cell clearance, along with a reduced activity of microglial cells [31]. While Mertk regulate resting microglia, Axl actions are predominant in proinflammatory environments [31, 44]. Coherently, Mertk expression is stimulated by immunosuppressive drugs, such as dexamethasone, whereas proinflammatory stimuli increase Axl expression and inhibit Mertk expression [44]. Notably, Axl defects have been related to delayed phagocytosis and prolonged induced axonal damage [60] and significant induction of Gas6, Axl, and Mertk was revealed in a mouse model of experimental autoimmune encephalomyelitis [113], confirming the complex roles of TAMs in different situations.

4. TAMs in Myelination, Demyelination, and Remyelination

Oligodendrocytes and Schwann cells are responsible for myelination in the CNS and PNS, respectively. The loss of oligodendrocytes and Schwann cells can cause inefficient myelination. Different kinds of insults (trauma, compression, and infections), immune hyperactivation, or autoimmune-inflammatory diseases (e.g., MS and CIDP) may cause demyelination, which in some cases could be repaired. Clearance of myelin debris is a crucial step in the process of remyelination, which can be reduced by inefficient phagocytosis. TAMs play potential roles in all these processes, which consequently may be affected by TAM signalling dysregulation [52].

Tyro3, highly expressed in oligodendrocytes, has been proposed as the principal candidate for traducing promyelinating effects of Gas6 during developmental myelination [114]. The loss of Tyro3 provoked, both *in vitro* and *in vivo*, delayed myelination and reduced myelin thickness. This effect was not due to changes in proliferation/differentiation of oligodendrocytes but to an impaired myelin production potentially related to the oligodendrocytes Tyro3, although the involvement of other cells expressing Tyro3

could not be excluded [114]. A recent study expanded these findings, confirming that a loss of Tyro3 reduced myelin thickness independently from oligodendroglia or microglia changes in response to a demyelinating insult and that Tyro3 regulated the nature of myelin repair influencing its radial expansion [115].

Gas/TAM signalling finely modulate remyelination after myelin damage. Gas6 is an important promoter of both oligodendrocytes and Schwann cell survival [14, 15, 28]. Gas6 stimulates remyelination both in vitro and in mouse models of demyelination induced by the toxic cuprizone [116, 117]. Administration of Gas6 into the CNS after cuprizone-induced demyelination results in more efficient remyelination [118]. After a peripheral injury, Schwann cells operate the clearance of myelin debris, stimulated by Axl/-Mertk-dependent pathways [119].

Immune hyperactivation also contributes to impair remyelination as shown in a mouse model of experimental autoimmune encephalomyelitis (EAE), where loss of Axl increases CNS inflammation, delaying the removal of myelin debris [120]. In addition, Gas6 knockout mice show remyelination abnormalities due to increased microglial activation [116]. The specific contribution in remyelinating processes of the Gas6/Axl signalling has been showed in a study in Gas6/Axl double knockout mice [121]. The toxic cuprizone provoked extensive axonal damage in mutant mice, also associated with an abnormal inflammatory response due to a reduced expression of SOCS, suggesting that Gas6/Axl signalling may be important in reducing CNS inflammation and maintaining axonal integrity after demyelinating/proinflammatory stimuli [121].

5. The Role of TAMs in Neurodegenerative and Neuroinflammatory Diseases

Evidence on the role of TAMs in neurodegenerative/neuroinflammatory diseases is rapidly growing. At present, there is a significant bulk of data in animal models of AD, PD, and MS, while very few data are reported in patients.

Chronic neuroinflammation, mediated by microglia and astrocytes, is a crucial player in neurodegeneration [122]. The pathological hallmark of many neurodegenerative diseases is a specific protein deposit; it is the case of beta-amyloid and tau accumulation in Alzheimer's disease (AD) and alpha-synuclein in Parkinson's disease (PD). In vitro and animal model studies showed that pathological protein deposits can stimulate a chronic neuroimmune response, with in turn releases proinflammatory cytokines and reactive oxygen species, contributing to degeneration. On the other hand, an ineffective microglial phagocytosis is an early finding in the disease process, impairing clearance of abnormal proteins [123].

The role of microglia has been extensively studied in animal models of neurodegenerative disease. The development of mouse models of amyloid deposition allowed testing the effects of amyloid-activated microglia in AD in vivo [124]. The polarization of microglia into a proinflammatory phenotype is likely to be a key step in neurodegeneration. In an AD model, a pathogenic stimulus such as hypoxia, able to promote amyloid deposition and neurodegeneration, triggered the polarization of microglia into an activated phenotype [125]; consistently, the suppression of proinflammatory responses produced protective effects in a lipopolysaccharide inflammation-induced AD model [126]. Results of studies in PD mouse models confirmed the role of activated microglia in neurodegeneration: MPTP administered to mice induced a consistent gliosis in the substantia nigra pars compacta associated with significant upregulation of inducible nitric oxide synthase [127]. In MS, proinflammatory T helper lymphocytes are classically considered the main players in lesion generation. Nevertheless, it is proved that MS development is associated with microglial activation and, notably, this was observed both in active demyelinating lesions and inflammatory nondemyelinating areas [128]. Other studies in AD models, however, showed that the experimental increase of microglial activation could also enhance clearance of the amyloid deposits [124]. This effect could be maximum in the very early stage of neurodegeneration, when a "protective" inflammation develops with the aim of contrasting and clearing the pathological process [109]. Differently, in other conditions microglial activation may be detrimental. A recent study showed that microglia-mediated phagocytosis can be activated by phosphatidylserine, which is externalised by live neurons containing tau deposits, and an analogous phagocytic signal exists in human tauopathies [129]. These different results suggest that the classification of microglia into an activated and a resting phenotype is only a simplification, since various microglial populations exist with a specific role, detrimental or beneficial in different stages of disease.

PET imaging for neuroinflammation is a valid approach for in vivo quantification of dynamic changes in neuroinflammatory processes. Increasing data provided by several PET studies, especially in AD, confirm that microglial activation accompanies neurodegeneration, particularly in the early phase, where a therapeutic approach might be beneficial [130].

Studies were performed to investigate the role of TAMs in AD, overall point to a protective effect against progression, probably acting on both neuronal survival and amyloid deposition. It was showed that the nerve growth factor, which may counteract AD-related neurodegeneration of cholinergic neurons [131], induced both Tyro3 and Axl expression in differentiating embryogenic cells and protecting them against apoptosis [132]. Moreover, a study on the role of Tyro3 in amyloid precursor protein (APP) processing and amyloid deposition in the hippocampus of AD models, showed that the overexpression of Tyro3 significantly decreased amyloid beta plaques burden from cell lines, while in Tyro3 knockdown transgenic AD mice the number of amyloid plaques increased in the hippocampus [133]. Zhang and colleagues recently analysed the effects of Jujuboside A, a molecule with antioxidant, anti-inflammatory, and neuroprotective properties, in an APP/PS1 mouse model. They found that Jujuboside A exerted its activities through Axl-mediated pathways, and it was efficient in facilitating amyloid plaque clearance and ameliorating cognitive deficits, thus suggesting that Axl could stimulate microglial

phagocytic activity promoting amyloid clearance [134]. In line with these findings, a very recent study showed that melatonin administration was able to ameliorate cognitive functions both in healthy nontransgenic (NoTg) and AD transgenic (3xTg-AD) mice [135]. Authors detected not only a decrease of proinflammatory cytokine expression but also the modulation of Gas6 and its receptors and upregulation of proteasome activity, which is an important mechanism involved in both neurodegenerative and neuroinflammatory disorders [136, 137].

In human, few studies are aimed at investigating the relationship between TAM expression and amyloid pathology both in normal aging and in AD. Mattsson and colleagues analysed the baseline levels of CSF proteins involved in microglial activity and amyloid metabolism, assessing the longitudinal CSF levels of the peptide amyloid-β1-42 (Aβ42) decrease in cognitively healthy people [138]. Axl, chromogranin A, and angiotensin-converting enzyme were the most significant proteins associated with longitudinal Aβ42 decrease, suggesting that they might predict the development of amyloid pathology at the earliest stages of AD [138]. Axl plasma levels, along with other analytes involved in amyloid metabolism such as matrix metalloproteinase-9 and apolipoprotein E, were associated with amyloid burden measured by [^{11}C]-PiB PET imaging in AD subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort [139]. Sainaghi and colleagues reported the first evidence of a significantly increased CSF level of Gas6 in AD patients compared to controls [140]. The higher levels of Gas6, particularly in the early stage of disease, suggested a compensatory role of Gas6, with the aim of downregulating proinflammatory cytokine production and promoting amyloid clearance [140]. A very recent report investigated the genetic region-specific expression changes in AD and control brain homogenates, through a series of biochemical, molecular, and bioinformatics analyses [141]. The study shows an upregulation of genes related to the toll-like receptor signalling, usually involved in amplifying immune responses in the CNS, along with the upregulation of Pros1 in moderate stages of AD and an increase of Gas6 expression from normal cognition through AD-type pathology. Considering the role of TAMs in modulating toll-like receptor signalling, these findings suggest again that a dysregulation of TAMs may contribute to AD pathology [141].

PD is a progressive neurological disorder that affects both motor and nonmotor systems [142]. Widespread aggregation of the α-synuclein protein into inclusions called Lewy bodies, which can be detected in both the central and peripheral nervous systems, is the pathological hallmark of the disease [142, 143]. It is currently believed that a higher α-synuclein burden is associated to a more severe PD phenotype [144]. TAMs may play a role in PD pathogenesis, influencing microglial activation and phagocytosis and regulating alpha-synuclein deposition. Studies in animal models support this hypothesis. Indeed, in early-stage PD, deficiency of the transcriptional factor Nrf2, which regulates Axl and Mertk in microglial phagocytosis and inflammatory gene expression, exacerbated protein deposition, neuroinflammation, and neuronal loss [145]. Moreover, a work on a transgenic mouse model of hereditary PD, characterised by a deposition of alpha-synuclein predominantly in the spinal cord, showed an increased expression of Axl, mainly in the spinal cord but also in the brain, which was age correlated [31].

MS is a progressive autoimmune disease of the CNS, characterised by inflammation, demyelination, and neurodegeneration. Demyelination derives from cell infiltrates of proinflammatory T-helper lymphocytes [146]; oligodendrocyte loss and microglial activation strongly contribute to the pathological process, leading to axonal damage, which can also be present independently of lymphocyte infiltration and myelin damage [147]. Whether neurodegeneration is a primary or secondary event is not completely clear; nonetheless, it represents the major contributor to clinical disability [148]. TAMs have been extensively studied in animal models of MS and to a lesser extent in human [74].

Several studies underlined the protective role of TAMs in the "cuprizone model," which is particularly useful in studying factors which influence myelin damage and repair. The administration of cuprizone, a copper chelator, to adult mice, induces a toxic demyelination without affecting the blood-brain barrier; a spontaneous remyelination can be observed in the first week [149]. Gas6, Axl, and Mertk are upregulated in mice after cuprizone-induced demyelination, in parallel with microglial activation [117]. The absence of Axl in the mouse model delays recovery from cuprizone toxicity due to a deficit in phagocytosis of myelin debris and extends axonal damage [60]. Gas6 knockout mice show a more severe cuprizone-induced demyelination, a delayed remyelination, an increased microglial activation, and a greater oligodendrocyte loss [117]. Furthermore, the administration of Gas6 improves recovery from cuprizone-induced injury, favouring remyelination and cellular and myelin debris clearance [118].

EAE is a model of CNS severe inflammation, with demyelination and axonal damage, induced by immunization with myelin antigens or myelin-specific T lymphocyte transfer. In EAE, as showed in the cuprizone model, Gas6, Axl, and Mertk are upregulated. Gas6 knockout mice have more severe demyelination and axonal damage linked to the EAE, and the Gas6 delivery protects against demyelination and accelerates repair [113]. Axl deficiency increases inflammatory response and hinders cellular and myelin debris clearance in EAE [120].

Overall, these findings in MS and EAE models suggest a protective role of the TAM system, especially of Gas6 and Axl, stimulating recovery of myelin and axons, favouring remyelination, regulating microglial activation, and accelerating myelin debris clearance.

TAMs are involved in MS lesion formation in human and play a role in disease progression. Analysing brain homogenates from chronic active and chronic silent MS lesions, Weinger and colleagues found the elevated levels of membrane-bound Mertk and soluble Axl and Mertk, with an inverse correlation with the Gas6 levels in lesions. These findings indirectly confirmed the protective role of Gas6, whose reduction in chronic lesions, due to the bond with soluble receptors, contributed to sustain pathology [150].

Sainaghi and colleagues measured both the CSF and plasma levels of Gas6 in sixty-five MS patients comparing them with forty controls. CSF Gas6 concentration was significantly higher in patients than in controls, with an inverse correlation with the severity of the relapses [151]. This study confirmed again the primary role of Gas6 in favouring myelin repair and recovery from damage. Another study evaluated plasma concentration of total and free Pros1 in sixty-five MS patients and fifteen controls. Plasma levels of total Pros1 were decreased in MS patients compared with controls, with very low levels of plasma free Pros1 in patients with higher disease severity, suggesting ProS1 dosage as a potential marker of disease progression [152].

Finally, genome-wide association studies identified the Mertk as a novel risk gene for MS susceptibility, with several single-nucleotide polymorphisms within the gene suggestive for association with MS ([153]; Ma et al. 2011). Mertk is important in mediating myelin phagocytosis by myeloid cells, which in MS lesions are represented by microglia and also macrophages derived from circulating monocytes. A recent study confirmed, in MS-derived macrophages, an impaired phagocytosis relatively selective to myelin and linked to an abnormal reduction on expression of Mertk [154]. Treatment with TGFβ could restore phagocytosis and expression of the receptor and the ligand [154]. These results encourage the development of new molecular immunomodulation therapies which may have an impact on disease progression.

6. Conclusion

The research on the relationship between neuroinflammation and neurodegeneration is moving fast. New therapeutic strategies designed to downmodulate neurotoxic factors on the one hand and to shift the inflammatory response into a protective reaction on the other hand are ongoing with the aim of providing a potential clinical benefit. In this context, TAM pathways represent potential targets for therapeutic intervention, due to their wide range of activities in immune regulatory networks.

Therapeutic attempts to control neuroinflammatory responses and treat autoimmune diseases influencing TAM signalling have been conducted in vitro and in animal models. Mertk stimulation in macrophages blocked the production of a broad proinflammatory cytokine response induced by LPS [155]. In a mouse model of arthritis, treatment with Gas6 or Pros1 limited inflammatory responses, consequently reducing symptoms [156].

In the nervous system, to clarify the role of TAMs in myelin formation may help in developing therapeutic strategies to promote remyelination in MS or in CIDP. To better delineate the role of TAMs in microglial activation and phagocytosis of pathological protein aggregates may drive the advancement in therapeutic intervention in neurodegenerative diseases such as AD and PD. Further studies into the precise mechanisms of action of TAMs and the corresponding downstream signalling are the inevitable precondition to elaborate future disease-modifying interventions.

To selectively block or activate the precise mechanism underlining the different TAM activities may avoid possible off-target effects due to an unselective recruitment of the entire system. TAM inhibitors are available, but cross-reactivity is possible also with other RTKs [157]. Thus, potential side effects are linked to the use of the TAM modulator. Mertk-prolonged inhibition in rats can produce blindness [158]; a stable inhibition of the TAM system may produce autoimmunity responses [159]. In addition to the effects in tissue remodelling and repair, the TAM system has an important oncogenic potential, due to the ability to promote both proliferation and survival in several cells. However, precisely in cancer therapy, TAM signalling modulation has showed promising results. Mertk and Axl inhibition in astrocytoma cells increased apoptosis and autophagy, together with sensitivity to chemotherapy [91]. Mertk loss in glioblastoma cells inhibited invasive properties and increased chemosensitivity [160]. At the same time, Pros1 loss in glioblastoma cells decreased proliferation, cell migration, and invasion and increased apoptosis. These data suggest the need of a precise regulation of the single TAM/ligand signalling plus the downstream pathway to obtain a successful therapeutic strategy.

Finally, the fine regulation of TAM expression in both developmental and pathological processes makes this family a potential candidate as biomarkers in monitoring physiological development or disease progression and therapeutic effectiveness.

A deeper knowledge of the exact roles of TAMs in neuroinflammation and neurodegeneration will contribute in providing important basic resources to understand and counteract neurodegenerative and neuroinflammatory diseases.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was funded by the AGING Project, Department of Excellence, Università degli Studi del Piemonte Orientale.

References

[1] R. M. A. Linger, A. K. Keating, H. S. Earp, and D. K. Graham, "Tam receptor tyrosine kinases: biologic functions, signaling, and potential therapeutic targeting in human cancer," *Advances in Cancer Research*, vol. 100, pp. 35–83, 2008.

[2] A. M. Pierce and A. K. Keating, "TAM receptor tyrosine kinases: expression, disease and oncogenesis in the central nervous system," *Brain Research*, vol. 1542, pp. 206–220, 2014.

[3] B. Shafit-Zagardo, R. C. Gruber, and J. C. DuBois, "The role of TAM family receptors and ligands in the nervous system: from development to pathobiology," *Pharmacology & Therapeutics*, vol. 188, pp. 97–117, 2018.

[4] J. H. M. van der Meer, T. van der Poll, and C. van 't Veer, "TAM receptors, Gas6, and protein S: roles in inflammation and hemostasis," *Blood*, vol. 123, no. 16, pp. 2460–2469, 2014.

[5] M. Wium, J. Paccez, and L. Zerbini, "The dual role of TAM receptors in autoimmune diseases and cancer: an overview," *Cell*, vol. 7, no. 10, p. 166, 2018.

[6] J. Zhang and X. Qi, "The role of the TAM family of receptor tyrosine kinases in neural development and disorders," *Neuropsychiatry*, vol. 8, no. 2, pp. 428–437, 2018.

[7] M. R. Mark, D. T. Scadden, Z. Wang, Q. Gu, A. Goddard, and P. J. Godowski, "Rse, a novel receptor-type tyrosine kinase with homology to Axl/Ufo, is expressed at high levels in the brain," *The Journal of Biological Chemistry*, vol. 269, no. 14, pp. 10720–10728, 1994.

[8] A. L. Prieto, S. O'Dell, B. Varnum, and C. Lai, "Localization and signaling of the receptor protein tyrosine kinase tyro3 in cortical and hippocampal neurons," *Neuroscience*, vol. 150, no. 2, pp. 319–334, 2007.

[9] H. Funakoshi, T. Yonemasu, T. Nakano, K. Matumoto, and T. Nakamura, "Identification of Gas6, a putative ligand for Sky and Axl receptor tyrosine kinases, as a novel neurotrophic factor for hippocampal neurons," *Journal of Neuroscience Research*, vol. 68, no. 2, pp. 150–160, 2002.

[10] Q. M. Zhao, L. M. Li, C. Zhang, and R. Guo, "Expression and localization of receptor tyrosine kinase Tyro3 in rat brain," *Journal of Peking University (Health Science)*, vol. 44, no. 6, pp. 905–910, 2012.

[11] A. L. Prieto, J. L. Weber, and C. Lai, "Expression of the receptor protein-tyrosine kinases Tyro-3, Axl, and mer in the developing rat central nervous system," *The Journal of Comparative Neurology*, vol. 425, no. 2, pp. 295–314, 2000.

[12] A. L. Prieto, J. L. Weber, S. Tracy, M. J. Heeb, and C. Lai, "Gas6, a ligand for the receptor protein-tyrosine kinase Tyro-3, is widely expressed in the central nervous system," *Brain Research*, vol. 816, no. 2, pp. 646–661, 1999.

[13] A. Pierce, B. Bliesner, M. Xu et al., "Axl and Tyro3 modulate female reproduction by influencing gonadotropin-releasing hormone neuron survival and migration," *Molecular Endocrinology*, vol. 22, no. 11, pp. 2481–2495, 2008.

[14] R. Li, J. Chen, G. Hammonds et al., "Identification of Gas6 as a growth factor for human Schwann cells," *The Journal of Neuroscience*, vol. 16, no. 6, pp. 2012–2019, 1996.

[15] Y. Miyamoto, T. Torii, S. Takada et al., "Involvement of the Tyro3 receptor and its intracellular partner Fyn signaling in Schwann cell myelination," *Molecular Biology of the Cell*, vol. 26, no. 19, pp. 3489–3503, 2015.

[16] A. Angelillo-Scherrer, P. G. de Frutos, C. Aparicio et al., "Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis," *Nature Medicine*, vol. 7, no. 2, pp. 215–221, 2001.

[17] Y. Higuchi, T. Kubota, M. Koyanagi, T. Maeda, A. M. Feldman, and N. Makino, "Upregulation of anticoagulant proteins, protein S and tissue factor pathway inhibitor, in the mouse myocardium with cardio-specific TNF-α overexpression," *American Journal of Physiology. Heart and Circulatory Physiology*, vol. 302, no. 11, pp. H2352–H2362, 2012.

[18] M. Katagiri, Y. Hakeda, D. Chikazu et al., "Mechanism of stimulation of osteoclastic bone resorption through Gas6/-Tyro3, a receptor tyrosine kinase signaling, in mouse osteoclasts," *The Journal of Biological Chemistry*, vol. 276, no. 10, pp. 7376–7382, 2001.

[19] C. Lai, M. Gore, and G. Lemke, "Structure, expression, and activity of Tyro 3, a neural adhesion-related receptor tyrosine kinase," *Oncogene*, vol. 9, no. 9, pp. 2567–2578, 1994.

[20] Q. Lu, M. Gore, Q. Zhang et al., "Tyro-3 family receptors are essential regulators of mammalian spermatogenesis," *Nature*, vol. 398, no. 6729, pp. 723–728, 1999.

[21] Q. Lu and G. Lemke, "Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family," *Science*, vol. 293, no. 5528, pp. 306–311, 2001.

[22] N. Matsubara, Y. Takahashi, Y. Nishina et al., "A receptor tyrosine kinase, Sky, and its ligand Gas 6 are expressed in gonads and support primordial germ cell growth or survival in culture," *Developmental Biology*, vol. 180, no. 2, pp. 499–510, 1996.

[23] D. Prasad, C. V. Rothlin, P. Burrola et al., "TAM receptor function in the retinal pigment epithelium," *Molecular and Cellular Neurosciences*, vol. 33, no. 1, pp. 96–108, 2006.

[24] I. C. A. Taylor, S. Roy, P. Yaswen, M. R. Stampfer, and H. E. Varmus, "Mouse Mammary Tumors Express Elevated Levels of RNA Encoding the Murine Homolog of SKY, a Putative Receptor Tyrosine Kinase," *The Journal of Biological Chemistry*, vol. 270, no. 12, pp. 6872–6880, 1995.

[25] H. Wang, S. Cnhen, Y. Chen et al., "The role of Tyro 3 subfamily receptors in the regulation of hemostasis and megakaryocytopoiesis," *Haematologica*, vol. 92, no. 5, pp. 643–650, 2007.

[26] P. Bellosta, M. Costa, D. A. Lin, and C. Basilico, "The receptor tyrosine kinase ARK mediates cell aggregation by homophilic binding," *Molecular and Cellular Biology*, vol. 15, no. 2, pp. 614–625, 1995.

[27] R. Ji, L. Meng, X. Jiang et al., "TAM receptors support neural stem cell survival, proliferation and neuronal differentiation," *PLoS One*, vol. 9, no. 12, article e115140, 2014.

[28] S. L. Shankar, K. O'Guin, M. Cammer et al., "The growth arrest-specific gene product Gas6 promotes the survival of human oligodendrocytes via a phosphatidylinositol 3-kinase-dependent pathway," *The Journal of Neuroscience*, vol. 23, no. 10, pp. 4208–4218, 2003.

[29] J. D. Cahoy, B. Emery, A. Kaushal et al., "A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function," *The Journal of Neuroscience*, vol. 28, no. 1, pp. 264–278, 2008.

[30] R. Ji, S. Tian, H. J. Lu et al., "TAM receptors affect adult brain neurogenesis by negative regulation of microglial cell activation," *Journal of Immunology*, vol. 191, no. 12, pp. 6165–6177, 2013.

[31] L. Fourgeaud, P. G. Través, Y. Tufail et al., "TAM receptors regulate multiple features of microglial physiology," *Nature*, vol. 532, no. 7598, pp. 240–244, 2016.

[32] M. P. Allen, C. Zeng, K. Schneider et al., "Growth arrest-specific gene 6 (Gas6)/adhesion related kinase (Ark) signaling promotes gonadotropin-releasing hormone neuronal survival via extracellular signal-regulated kinase (ERK) and Akt," *Molecular Endocrinology*, vol. 13, no. 2, pp. 191–201, 1999.

[33] D. K. Graham, G. W. Bowman, T. L. Dawson, W. L. Stanford, H. S. Earp, and H. R. Snodgrass, "Cloning and developmental expression analysis of the murine c-mer tyrosine kinase," *Oncogene*, vol. 10, no. 12, pp. 2349–2359, 1995.

[34] F. Lafdil, M. N. Chobert, D. Couchie et al., "Induction of Gas6 protein in CCl4-induced rat liver injury and anti-apoptotic effect on hepatic stellate cells," *Hepatology*, vol. 44, no. 1, pp. 228–239, 2006.

[35] A. Neubauer, A. Fiebeler, D. K. Graham et al., "Expression of Axl, a transforming receptor tyrosine kinase, in normal and malignant hematopoiesis," *Blood*, vol. 84, no. 6, pp. 1931–1941, 1994.

[36] H. Wang, Y. Chen, Y. Ge et al., "Immunoexpression of Tyro 3 family receptors—Tyro 3, Axl, and Mer—and their ligand Gas6 in postnatal developing mouse testis," *The Journal of Histochemistry and Cytochemistry*, vol. 53, no. 11, pp. 1355–1364, 2005.

[37] M. Yanagita, H. Arai, K. Ishii et al., "Gas6 regulates mesangial cell proliferation through Axl in experimental glomerulonephritis," *The American Journal of Pathology*, vol. 158, no. 4, pp. 1423–1432, 2001.

[38] D. K. Graham, T. L. Dawson, D. L. Mullaney, H. R. Snodgrass, and H. S. Earp, "Cloning and mRNA expression analysis of a novel human protooncogene, c-mer," *Cell Growth & Differentiation*, vol. 5, pp. 647–657, 1994.

[39] W. H. Shao, Y. Zhen, J. Rosenbaum et al., "A protective role of Mer receptor tyrosine kinase in nephrotoxic serum-induced nephritis," *Clinical Immunology*, vol. 136, no. 2, pp. 236–244, 2010.

[40] Y. M. Wu, D. R. Robinson, and H. J. Kung, "Signal pathways in up-regulation of chemokines by tyrosine kinase MER/-NYK in prostate cancer cells," *Cancer Research*, vol. 64, no. 20, pp. 7311–7320, 2004.

[41] S. Xie, Y. Li, X. Li et al., "Mer receptor tyrosine kinase is frequently overexpressed in human non-small cell lung cancer, confirming resistance to erlotinib," *Oncotarget*, vol. 6, no. 11, 2015.

[42] E. . M. Behrens, P. Gadue, S.-y. Gong, S. Garrett, P. . L. Stein, and P. . L. Cohen, "The mer receptor tyrosine kinase: expression and function suggest a role in innate immunity," *European Journal of Immunology*, vol. 33, no. 8, pp. 2160–2167, 2003.

[43] C. Chen, Q. Li, A. L. Darrow et al., "Mer receptor tyrosine kinase signaling participates in platelet function," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 6, pp. 1118–1123, 2004.

[44] A. Zagórska, P. G. Través, E. D. Lew, I. Dransfield, and G. Lemke, "Diversification of TAM receptor tyrosine kinase function," *Nature Immunology*, vol. 15, no. 10, pp. 920–928, 2014.

[45] G. Zizzo, B. A. Hilliard, M. Monestier, and P. L. Cohen, "Efficient clearance of early apoptotic cells by human macrophages requires M2c polarization and MerTK induction," *Journal of Immunology*, vol. 189, no. 7, pp. 3508–3520, 2012.

[46] T. N. Stitt, G. Conn, M. Goret et al., "The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases," *Cell*, vol. 80, no. 4, pp. 661–670, 1995.

[47] C. Schneider, R. M. King, and L. Philipson, "Genes specifically expressed at growth arrest of mammalian cells," *Cell*, vol. 54, no. 6, pp. 787–793, 1988.

[48] G. C. Avanzi, M. Gallicchio, G. Cavalloni et al., "GAS6, the ligand of Axl and Rse receptors, is expressed in hematopoietic tissue but lacks mitogenic activity," *Experimental Hematology*, vol. 25, no. 12, pp. 1219–1226, 1997.

[49] Y. Ishimoto and T. Nakano, "Release of a product of growth arrest-specific gene 6 from rat platelets," *FEBS Letters*, vol. 466, no. 1, pp. 197–199, 2000.

[50] G. Manfioletti, C. Brancolini, G. Avanzi, and C. Schneider, "The protein encoded by a growth arrest specific gene (gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade," *Molecular and Cellular Biology*, vol. 13, no. 8, pp. 4976–4985, 1993.

[51] T. Nakano, K. I. Higashino, N. Kikuchi et al., "Vascular smooth muscle cell-derived, Gla-containing growth-potentiating factor for Ca^{2+}-mobilizing growth factors," *The Journal of Biological Chemistry*, vol. 270, no. 11, pp. 5702–5705, 1995.

[52] M. D. Binder and T. J. Kilpatrick, "TAM receptor signalling and demyelination," *Neuro-Signals*, vol. 17, no. 4, pp. 277–287, 2009.

[53] C. Maillard, M. Berruyer, C. M. Serre, M. Dechavanne, and P. D. Delmas, "Protein-S, a vitamin K-dependent protein, is a bone matrix component synthesized and secreted by osteoblasts," *Endocrinology*, vol. 130, no. 3, pp. 1599–1604, 1992.

[54] T. M. Hackeng, C. van 't Veer, J. C. Meijers, and B. N. Bouma, "Human protein S inhibits prothrombinase complex activity on endothelial cells and platelets via direct interactions with factors Va and Xa," *Journal of Biological Chemistry*, vol. 269, no. 33, pp. 21051–21058, 1994.

[55] M. J. Heeb, J. Rosing, H. M. Bakker, J. A. Fernandez, G. Tans, and J. H. Griffin, "Protein S binds to and inhibits factor Xa," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 7, pp. 2728–2732, 1994.

[56] C. V. Rothlin, E. A. Carrera-Silva, L. Bosurgi, and S. Ghosh, "TAM receptor signaling in immune homeostasis," *Annual Review of Immunology*, vol. 33, no. 1, pp. 355–391, 2015.

[57] T. Deng, Q. Chen, and D. Han, "The roles of TAM receptor tyrosine kinases in the mammalian testis and immunoprivileged sites," *Frontiers in Bioscience*, vol. 21, no. 2, pp. 316–327, 2016.

[58] T. Burstyn-Cohen, E. D. Lew, P. G. Través, P. G. Burrola, J. C. Hash, and G. Lemke, "Genetic dissection of tam receptor-ligand interaction in retinal pigment epithelial cell phagocytosis," *Neuron*, vol. 76, no. 6, pp. 1123–1132, 2012.

[59] J. Chen, K. Carey, and P. J. Godowski, "Identification of Gas6 as a ligand for Mer, a neural cell adhesion molecule related receptor tyrosine kinase implicated in cellular transformation," *Oncogene*, vol. 14, no. 17, pp. 2033–2039, 1997.

[60] H. J. Hoehn, Y. Kress, A. Sohn, C. F. Brosnan, S. Bourdon, and B. Shafit-Zagardo, "Axl-/- mice have delayed recovery and prolonged axonal damage following cuprizone toxicity," *Brain Research*, vol. 1240, pp. 1–11, 2008.

[61] K. Nagata, K. Ohashi, T. Nakano et al., "Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases," *The Journal of Biological Chemistry*, vol. 271, no. 47, pp. 30022–30027, 1996.

[62] B. C. Varnum, C. Young, G. Elliott et al., "Axl receptor tyrosine kinase stimulated by the vitamin K-dependent protein encoded by growth-arrest-specific gene 6," *Nature*, vol. 373, no. 6515, pp. 623–626, 1995.

[63] J. P. O'Bryan, Y. W. Fridell, R. Koski, B. Varnum, and E. T. Liu, "The transforming receptor tyrosine kinase, Axl, is post-translationally regulated by proteolytic cleavage," *The Journal of Biological Chemistry*, vol. 270, no. 2, pp. 551–557, 1995.

[64] S. Sather, K. D. Kenyon, J. B. Lefkowitz et al., "A soluble form of the Mer receptor tyrosine kinase inhibits macrophage clearance of apoptotic cells and platelet aggregation," *Blood*, vol. 109, no. 3, pp. 1026–1033, 2007.

[65] J. Stenhoff, B. Dahlback, and S. Hafizi, "Vitamin K-dependent Gas6 activates ERK kinase and stimulates growth of cardiac fibroblasts," *Biochemical and Biophysical Research Communications*, vol. 319, no. 3, pp. 871–878, 2004.

[66] E. A. Carrera Silva, P. Y. Chan, L. Joannas et al., "T cell-derived protein S engages TAM receptor signaling in dendritic cells to control the magnitude of the immune response," *Immunity*, vol. 39, no. 1, pp. 160–170, 2013.

[67] C. V. Rothlin, S. Ghosh, E. I. Zuniga, M. B. A. Oldstone, and G. Lemke, "TAM receptors are pleiotropic inhibitors of the innate immune response," *Cell*, vol. 131, no. 6, pp. 1124–1136, 2007.

[68] H. Guo, T. M. Barrett, Z. Zhong et al., "Protein S blocks the extrinsic apoptotic cascade in tissue plasminogen activator/N-methyl D-aspartate-treated neurons via Tyro3-Akt-FKHRL1 signaling pathway," *Molecular Neurodegeneration*, vol. 6, no. 1, p. 13, 2011.

[69] A. Anwar, A. K. Keating, D. Joung et al., "Mer tyrosine kinase (MerTK) promotes macrophage survival following exposure to oxidative stress," *Journal of Leukocyte Biology*, vol. 86, no. 1, pp. 73–79, 2009.

[70] K. L. Guttridge, J. C. Luft, T. L. Dawson et al., "Mer receptor tyrosine kinase signaling," *The Journal of Biological Chemistry*, vol. 277, no. 27, pp. 24057–24066, 2002.

[71] Y. Chen, H. Wang, N. Qi et al., "Functions of TAM RTKs in regulating spermatogenesis and male fertility in mice," *Reproduction*, vol. 138, no. 4, pp. 655–666, 2009.

[72] A. J. Carr, A. Vugler, J. Lawrence et al., "Molecular characterization and functional analysis of phagocytosis by human embryonic stem cell-derived RPE cells using a novel human retinal assay," *Molecular Vision*, vol. 15, pp. 283–295, 2009.

[73] P. Bellosta, Q. Zhang, S. P. Goff, and C. Basilico, "Signaling through the ark tyrosine kinase receptor protects from apoptosis in the absence of growth stimulation," *Oncogene*, vol. 15, no. 20, pp. 2387–2397, 1997.

[74] M. Bellan, M. Pirisi, and P. Sainaghi, "The Gas6/TAM system and multiple sclerosis," *International Journal of Molecular Sciences*, vol. 17, no. 11, p. 1807, 2016.

[75] J. W. Cave, M. Wang, and H. Baker, "Adult subventricular zone neural stem cells as a potential source of dopaminergic replacement neurons," *Frontiers in Neuroscience*, vol. 8, p. 16, 2014.

[76] A. Gely-Pernot, V. Coronas, T. Harnois et al., "An endogenous vitamin K-dependent mechanism regulates cell proliferation in the brain subventricular stem cell niche," *Stem Cells*, vol. 30, no. 4, pp. 719–731, 2012.

[77] K. Zelentsova-Levytskyi, Z. Talmi, G. Abboud-Jarrous, T. Capucha, T. Sapir, and T. Burstyn-Cohen, "Protein S negatively regulates neural stem cell self-renewal through Bmi-1 signaling," *Frontiers in Molecular Neuroscience*, vol. 10, p. 124, 2017.

[78] K. Zelentsova, Z. Talmi, G. Abboud-Jarrous et al., "Protein S regulates neural stem cell quiescence and neurogenesis," *Stem Cells*, vol. 35, no. 3, pp. 679–693, 2017.

[79] T. V. P. Bliss and G. L. Collingridge, "A synaptic model of memory: long-term potentiation in the hippocampus," *Nature*, vol. 361, no. 6407, pp. 31–39, 1993.

[80] T. F. Roberts, K. A. Tschida, M. E. Klein, and R. Mooney, "Rapid spine stabilization and synaptic enhancement at the onset of behavioural learning," *Nature*, vol. 463, no. 7283, pp. 948–952, 2010.

[81] W. S. Chung, N. J. Allen, and C. Eroglu, "Astrocytes control synapse formation, function, and elimination," *Cold Spring Harbor Perspectives in Biology*, vol. 7, no. 9, 2015.

[82] W. S. Chung, L. E. Clarke, G. X. Wang et al., "Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways," *Nature*, vol. 504, no. 7480, pp. 394–400, 2013.

[83] M. Schwanzel-Fukuda and D. W. Pfaff, "Origin of luteinizing hormone-releasing hormone neurons," *Nature*, vol. 338, no. 6211, pp. 161–164, 1989.

[84] M. P. Allen, M. Xu, D. A. Linseman et al., "Adhesion-related kinase repression of gonadotropin-releasing hormone gene expression requires Rac activation of the extracellular signal-regulated kinase pathway," *Journal of Biological Chemistry*, vol. 277, no. 41, pp. 38133–38140, 2002.

[85] S. Salian-Mehta, M. Xu, A. J. Knox et al., "Functional consequences of AXL sequence variants in hypogonadotropic hypogonadism," *The Journal of Clinical Endocrinology and Metabolism*, vol. 99, no. 4, pp. 1452–1460, 2014.

[86] Z. Zhong, Y. Wang, H. Guo et al., "Protein S protects neurons from excitotoxic injury by activating the TAM receptor Tyro3-phosphatidylinositol 3-kinase-Akt pathway through its sex hormone-binding globulin-like region," *The Journal of Neuroscience*, vol. 30, no. 46, pp. 15521–15534, 2010.

[87] J. Onken, R. Torka, S. Korsing et al., "Inhibiting receptor tyrosine kinase AXL with small molecule inhibitor BMS-777607 reduces glioblastoma growth, migration, and invasion in vitro and in vivo," *Oncotarget*, vol. 7, no. 9, pp. 9876–9889, 2016.

[88] S. N. Popova, M. Bergqvist, A. Dimberg et al., "Subtyping of gliomas of various WHO grades by the application of immunohistochemistry," *Histopathology*, vol. 64, no. 3, pp. 365–379, 2014.

[89] K. Staflin, T. Zuchner, G. Honeth, A. Darabi, and C. Lundberg, "Identification of proteins involved in neural progenitor cell targeting of gliomas," *BMC Cancer*, vol. 9, no. 1, 2009.

[90] M. Hutterer, P. Knyazev, A. Abate et al., "Axl and growth arrest-specific gene 6 are frequently overexpressed in human gliomas and predict poor prognosis in patients with glioblastoma multiforme," *Clinical Cancer Research*, vol. 14, no. 1, pp. 130–138, 2008.

[91] A. K. Keating, G. K. Kim, A. E. Jones et al., "Inhibition of Mer and Axl receptor tyrosine kinases in astrocytoma cells leads to increased apoptosis and improved chemosensitivity," *Molecular Cancer Therapeutics*, vol. 9, no. 5, pp. 1298–1307, 2010.

[92] S. Krause, C. Pfeiffer, S. Strube et al., "Mer tyrosine kinase promotes the survival of t(1;19)-positive acute lymphoblastic leukemia (ALL) in the central nervous system (CNS)," *Blood*, vol. 125, no. 5, pp. 820–830, 2015.

[93] H. Huang, S. Colella, M. Kurrer, Y. Yonekawa, P. Kleihues, and H. Ohgaki, "Gene expression profiling of low-grade diffuse astrocytomas by cDNA arrays," *Cancer Research*, vol. 60, no. 24, pp. 6868–6874, 2000.

[94] S. Ammoun, L. Provenzano, L. Zhou et al., "Axl/Gas6/NFκB signalling in schwannoma pathological proliferation,

adhesion and survival," *Oncogene*, vol. 33, no. 3, pp. 336–346, 2014.

[95] G. Johansson, P. C. Peng, P. Y. Huang et al., "Soluble AXL: a possible circulating biomarker for neurofibromatosis type 1 related tumor burden," *PLoS One*, vol. 9, no. 12, article e115916, 2014.

[96] M. R. Crittenden, J. Baird, D. Friedman et al., "Mertk on tumor macrophages is a therapeutic target to prevent tumor recurrence following radiation therapy," *Oncotarget*, vol. 7, no. 48, pp. 78653–78666, 2016.

[97] J. A. Stratton, A. Holmes, N. L. Rosin et al., "Macrophages regulate Schwann cell maturation after nerve injury," *Cell Reports*, vol. 24, no. 10, pp. 2561–2572.e6, 2018.

[98] G. Lemke and Q. Lu, "Macrophage regulation by Tyro 3 family receptors," *Current Opinion in Immunology*, vol. 15, no. 1, pp. 31–36, 2003.

[99] S. Scutera, T. Fraone, T. Musso et al., "Survival and migration of human dendritic cells are regulated by an IFN-α-inducible Axl/Gas6 pathway," *Journal of Immunology*, vol. 183, no. 5, pp. 3004–3013, 2009.

[100] C. A. Bonjardim, P. C. P. Ferreira, and E. G. Kroon, "Interferons: signaling, antiviral and viral evasion," *Immunology Letters*, vol. 122, no. 1, pp. 1–11, 2009.

[101] M. N. Sharif, D. Sosic, C. V. Rothlin et al., "Twist mediates suppression of inflammation by type I IFNs and Axl," *The Journal of Experimental Medicine*, vol. 203, no. 8, pp. 1891–1901, 2006.

[102] Q. Li, Q. Lu, H. Lu, S. Tian, and Q. Lu, "Systemic autoimmunity in TAM triple knockout mice causes inflammatory brain damage and cell death," *PLoS One*, vol. 8, no. 6, article e64812, 2013.

[103] R. S. Scott, E. J. McMahon, S. M. Pop et al., "Phagocytosis and clearance of apoptotic cells is mediated by MER," *Nature*, vol. 411, no. 6834, pp. 207–211, 2001.

[104] H. M. Seitz, T. D. Camenisch, G. Lemke, H. S. Earp, and G. K. Matsushima, "Macrophages and dendritic cells use different Axl/MerTK/Tyro3 receptors in clearance of apoptotic cells," *Journal of Immunology*, vol. 178, no. 9, pp. 5635–5642, 2007.

[105] M. O. Hall, A. L. Prieto, M. S. Obin et al., "Outer segment phagocytosis by cultured retinal pigment epithelial cells requires Gas6," *Experimental Eye Research*, vol. 73, no. 4, pp. 509–520, 2001.

[106] T. Nakano, Y. Ishimoto, J. Kishino et al., "Cell adhesion to phosphatidylserine mediated by a product of growth arrest-specific gene 6," *The Journal of Biological Chemistry*, vol. 272, no. 47, pp. 29411–29414, 1997.

[107] D. Vollrath, D. Yasumura, G. Benchorin et al., "*Tyro3* modulates *Mertk*-associated retinal degeneration," *PLoS Genetics*, vol. 11, no. 12, article e1005723, 2015.

[108] J. J. Neher, J. V. Emmrich, M. Fricker, P. K. Mander, C. Thery, and G. C. Brown, "Phagocytosis executes delayed neuronal death after focal brain ischemia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 43, pp. E4098–E4107, 2013.

[109] C. Comi and G. Tondo, "Insights into the protective role of immunity in neurodegenerative disease," *Neural Regeneration Research*, vol. 12, no. 1, pp. 64-65, 2017.

[110] L. Castelli, C. Comi, A. Chiocchetti et al., "ICOS gene haplotypes correlate with IL10 secretion and multiple sclerosis evolution," *Journal of Neuroimmunology*, vol. 186, no. 1-2, pp. 193–198, 2007.

[111] C. Comi, G. Cappellano, A. Chiocchetti et al., "The impact of osteopontin gene variations on multiple sclerosis development and progression," *Clinical and Developmental Immunology*, vol. 2012, Article ID 212893, 6 pages, 2012.

[112] F. Su, F. Bai, and Z. Zhang, "Inflammatory cytokines and Alzheimer's disease: a review from the perspective of genetic polymorphisms," *Neuroscience Bulletin*, vol. 32, no. 5, pp. 469–480, 2016.

[113] R. C. Gruber, A. K. Ray, C. T. Johndrow et al., "Targeted GAS6 delivery to the CNS protects axons from damage during experimental autoimmune encephalomyelitis," *The Journal of Neuroscience*, vol. 34, no. 49, pp. 16320–16335, 2014.

[114] R. Akkermann, A. Aprico, A. A. Perera et al., "The TAM receptor Tyro3 regulates myelination in the central nervous system," *Glia*, vol. 65, no. 4, pp. 581–591, 2017.

[115] F. Blades, A. Aprico, R. Akkermann, S. Ellis, M. D. Binder, and T. J. Kilpatrick, "The TAM receptor TYRO3 is a critical regulator of myelin thickness in the central nervous system," *Glia*, vol. 66, no. 10, pp. 2209–2220, 2018.

[116] M. D. Binder, J. Xiao, D. Kemper, G. Z. M. Ma, S. S. Murray, and T. J. Kilpatrick, "Gas6 increases myelination by oligodendrocytes and its deficiency delays recovery following cuprizone-induced demyelination," *PLoS One*, vol. 6, no. 3, article e17727, 2011.

[117] M. D. Binder, H. S. Cate, A. L. Prieto et al., "Gas6 deficiency increases oligodendrocyte loss and microglial activation in response to cuprizone-induced demyelination," *The Journal of Neuroscience*, vol. 28, no. 20, pp. 5195–5206, 2008.

[118] V. Tsiperson, X. Li, G. J. Schwartz, C. S. Raine, and B. Shafit-Zagardo, "Gas6 enhances repair following cuprizone-induced demyelination," *PLoS One*, vol. 5, no. 12, article e15748, 2010.

[119] A. Brosius Lutz, W. S. Chung, S. A. Sloan et al., "Schwann cells use tam receptor-mediated phagocytosis in addition to autophagy to clear myelin in a mouse model of nerve injury," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 114, no. 38, pp. E8072–E8080, 2017.

[120] J. G. Weinger, C. F. Brosnan, O. Loudig et al., "Loss of the receptor tyrosine kinase Axl leads to enhanced inflammation in the CNS and delayed removal of myelin debris during experimental autoimmune encephalomyelitis," *Journal of Neuroinflammation*, vol. 8, no. 1, p. 49, 2011.

[121] A. K. Ray, J. C. DuBois, R. C. Gruber et al., "Loss of Gas6 and Axl signaling results in extensive axonal damage, motor deficits, prolonged neuroinflammation, and less remyelination following cuprizone exposure," *Glia*, vol. 65, no. 12, pp. 2051–2069, 2017.

[122] R. M. Ransohoff, "How neuroinflammation contributes to neurodegeneration," *Science*, vol. 353, no. 6301, pp. 777–783, 2016.

[123] G. Cappellano, M. Carecchio, T. Fleetwood et al., "Immunity and inflammation in neurodegenerative diseases," *American Journal of Neurodegenerative Disease*, vol. 2, no. 2, pp. 89–107, 2013.

[124] D. C. Lee, J. Rizer, J. B. Hunt, M. L. B. Selenica, M. N. Gordon, and D. Morgan, "Review: experimental manipulations of microglia in mouse models of Alzheimer's pathology: activation reduces amyloid but hastens tau pathology," *Neuropathology and Applied Neurobiology*, vol. 39, no. 1, pp. 69–85, 2013.

[125] F. Zhang, R. Zhong, S. Li et al., "Acute Hypoxia Induced an Imbalanced M1/M2 Activation of Microglia through NF-κB

Signaling in Alzheimer's Disease Mice and Wild-Type Littermates," *Frontiers in Aging Neuroscience*, vol. 9, p. 282, 2017.

[126] L. Zhong, X. F. Chen, Z. L. Zhang et al., "DAP12 stabilizes the C-terminal fragment of the triggering receptor expressed on myeloid cells-2 (TREM2) and protects against LPS-induced pro-inflammatory response," *The Journal of Biological Chemistry*, vol. 290, no. 25, pp. 15866–15877, 2015.

[127] G. T. Liberatore, V. Jackson-Lewis, S. Vukosavic et al., "Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease," *Nature Medicine*, vol. 5, no. 12, pp. 1403–1409, 1999.

[128] Z. Gao and S. E. Tsirka, "Animal models of MS reveal multiple roles of microglia in disease pathogenesis," *Neurology Research International*, vol. 2011, Article ID 383087, 9 pages, 2011.

[129] J. Brelstaff, A. M. Tolkovsky, B. Ghetti, M. Goedert, and M. G. Spillantini, "Living neurons with tau filaments aberrantly expose phosphatidylserine and are phagocytosed by microglia," *Cell Reports*, vol. 24, no. 8, pp. 1939–1948.e4, 2018.

[130] C. Cerami, L. Iaccarino, and D. Perani, "Molecular imaging of neuroinflammation in neurodegenerative dementias: the role of in vivo PET imaging," *International Journal of Molecular Sciences*, vol. 18, no. 5, p. 993, 2017.

[131] H. Gnahn, F. Hefti, R. Heumann, M. E. Schwab, and H. Thoenen, "NGF-mediated increase of choline acetyltransferase (ChAT) in the neonatal rat forebrain: evidence for a physiological role of NGF in the brain?," *Brain Research*, vol. 9, no. 1, pp. 45–52, 1983.

[132] Q. Wang, Q. J. Lu, B. Xiao, Y. Zheng, and X. M. Wang, "Expressions of Axl and Tyro-3 receptors are under regulation of nerve growth factor and are involved in differentiation of PC12 cells," *Neuroscience Bulletin*, vol. 27, no. 1, pp. 15–22, 2011.

[133] Y. Zheng, Q. Wang, B. Xiao, Q. Lu, Y. Wang, and X. Wang, "Involvement of Receptor Tyrosine Kinase Tyro3 in Amyloidogenic APP Processing and β-Amyloid Deposition in Alzheimer's Disease Models," *PLoS One*, vol. 7, no. 6, article e39035, 2012.

[134] M. Zhang, C. Qian, Z. G. Zheng et al., "Jujuboside A promotes Aβ clearance and ameliorates cognitive deficiency in Alzheimer's disease through activating Axl/HSP90/PPARγ pathway," *Theranostics*, vol. 8, no. 15, pp. 4262–4278, 2018.

[135] R. Corpas, C. Griñán-Ferré, V. Palomera-Ávalos et al., "Melatonin induces mechanisms of brain resilience against neurodegeneration," *Journal of Pineal Research*, vol. 65, no. 4, article e12515, 2018.

[136] E. Bellavista, A. Santoro, D. Galimberti, C. Comi, F. Luciani, and M. Mishto, "Current understanding on the role of standard and immunoproteasomes in inflammatory/immunological pathways of multiple sclerosis," *Autoimmune Diseases*, vol. 2014, Article ID 739705, 12 pages, 2014.

[137] Y. Zhang, X. Chen, Y. Zhao, M. Ponnusamy, and Y. Liu, "The role of ubiquitin proteasomal system and autophagy-lysosome pathway in Alzheimer's disease," *Reviews in the Neurosciences*, vol. 28, no. 8, pp. 861–868, 2017.

[138] N. Mattsson, P. Insel, R. Nosheny et al., "CSF protein biomarkers predicting longitudinal reduction of CSF β-amyloid42 in cognitively healthy elders," *Translational Psychiatry*, vol. 3, no. 8, article e293, 2013.

[139] S. J. Kiddle, M. Thambisetty, A. Simmons et al., "Plasma based markers of [^{11}C] PiB-PET brain amyloid burden," *PLoS One*, vol. 7, no. 9, article e44260, 2012.

[140] P. P. Sainaghi, M. Bellan, F. Lombino et al., "Growth arrest specific 6 concentration is increased in the cerebrospinal fluid of patients with Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 55, no. 1, pp. 59–65, 2017.

[141] M. Herrera-Rivero, F. Santarelli, F. Brosseron, M. P. Kummer, and M. T. Heneka, "Dysregulation of TLR5 and TAM ligands in the Alzheimer's brain as contributors to disease progression," *Molecular Neurobiology*, vol. 56, no. 9, pp. 6539–6550, 2019.

[142] L. V. Kalia, S. K. Kalia, P. J. McLean, A. M. Lozano, and A. E. Lang, "α-Synuclein oligomers and clinical implications for Parkinson disease," *Annals of Neurology*, vol. 73, no. 2, pp. 155–169, 2013.

[143] C. Comi, L. Magistrelli, G. D. Oggioni et al., "Peripheral nervous system involvement in Parkinson's disease: evidence and controversies," *Parkinsonism & Related Disorders*, vol. 20, no. 12, pp. 1329–1334, 2014.

[144] L. Corrado, F. de Marchi, S. Tunesi et al., "The length of *SNCA* Rep1 microsatellite may influence cognitive evolution in Parkinson's disease," *Frontiers in Neurology*, vol. 9, p. 213, 2018.

[145] I. Lastres-Becker, A. Ulusoy, N. G. Innamorato et al., "α-Synuclein expression and Nrf2 deficiency cooperate to aggravate protein aggregation, neuronal death and inflammation in early-stage Parkinson's disease," *Human Molecular Genetics*, vol. 21, no. 14, pp. 3173–3192, 2012.

[146] S. Dhib-Jalbut, "Pathogenesis of myelin/oligodendrocyte damage in multiple sclerosis," *Neurology*, vol. 68, no. 22, Supplement 3, pp. S13–S21, 2007.

[147] P. M. Matthews, E. Pioro, S. Narayanan et al., "Assessment of lesion pathology in multiple sclerosis using quantitative MRI morphometry and magnetic resonance spectroscopy," *Brain*, vol. 119, no. 3, pp. 715–722, 1996.

[148] J. M. Frischer, S. Bramow, A. Dal-Bianco et al., "The relation between inflammation and neurodegeneration in multiple sclerosis brains," *Brain*, vol. 132, no. 5, pp. 1175–1189, 2009.

[149] O. Torkildsen, L. A. Brunborg, K. M. Myhr, and L. Bø, "The cuprizone model for demyelination," *Acta Neurologica Scandinavica*, vol. 117, no. s188, pp. 72–76, 2008.

[150] J. G. Weinger, K. M. Omari, K. Marsden, C. S. Raine, and B. Shafit-Zagardo, "Up-regulation of soluble Axl and Mer receptor tyrosine kinases negatively correlates with Gas6 in established multiple sclerosis lesions," *The American Journal of Pathology*, vol. 175, no. 1, pp. 283–293, 2009.

[151] P. P. Sainaghi, L. Collimedaglia, F. Alciato et al., "Growth arrest specific gene 6 protein concentration in cerebrospinal fluid correlates with relapse severity in multiple sclerosis," *Mediators of Inflammation*, vol. 2013, Article ID 406483, 7 pages, 2013.

[152] G. Z. M. Ma, L. L. Giuffrida, M. M. Gresle et al., "Association of plasma levels of protein S with disease severity in multiple sclerosis," *Multiple Sclerosis Journal - Experimental, Translational and Clinical*, vol. 1, 2015.

[153] M. D. Binder, A. D. Fox, D. Merlo et al., "Common and low frequency variants in MERTK are independently associated with multiple sclerosis susceptibility with discordant association dependent upon HLA-DRB1*15:01 status," *PLoS Genetics*, vol. 12, no. 3, article e1005853, 2016.

[154] L. M. Healy, J. H. Jang, S. Y. Won et al., "MerTK-mediated regulation of myelin phagocytosis by macrophages generated from patients with MS," *Neurology Neuroimmunology and neuroinflammation*, vol. 4, no. 6, article e402, 2017.

[155] T. D. Camenisch, B. H. Koller, H. S. Earp, and G. K. Matsushima, "A novel receptor tyrosine kinase, Mer, inhibits TNF-α production and lipopolysaccharide-induced endotoxic shock," *Journal of Immunology*, vol. 162, no. 6, pp. 3498–3503, 1999.

[156] B. T. van den Brand, S. Abdollahi-Roodsaz, E. A. Vermeij et al., "Therapeutic efficacy of Tyro3, Axl, and Mer tyrosine kinase agonists in collagen-induced arthritis," *Arthritis and Rheumatism*, vol. 65, no. 3, pp. 671–680, 2013.

[157] C. T. Cummings, W. Zhang, K. D. Davies et al., "Small molecule inhibition of MERTK is efficacious in non-small cell lung cancer models independent of driver oncogene status," *Molecular Cancer Therapeutics*, vol. 14, no. 9, pp. 2014–2022, 2015.

[158] X. Liu, Y. Zhang, Y. He, J. Zhao, and G. Su, "Progress in histopathologic and pathogenetic research in a retinitis pigmentosa model," *Histology and Histopathology*, vol. 30, no. 7, pp. 771–779, 2015.

[159] L. Xu, F. Hu, H. Zhu et al., "Soluble tam receptor tyrosine kinases in rheumatoid arthritis: correlation with disease activity and bone destruction," *Clinical and Experimental Immunology*, vol. 192, no. 1, pp. 95–103, 2018.

[160] Y. Wang, G. Moncayo, P. Morin et al., "Mer receptor tyrosine kinase promotes invasion and survival in glioblastoma multiforme," *Oncogene*, vol. 32, no. 7, pp. 872–882, 2013.

Hindawi
Disease Markers
Volume 2019, Article ID 2304931, 15 pages
https://doi.org/10.1155/2019/2304931

Review Article

Gas6/TAM Signaling Components as Novel Biomarkers of Liver Fibrosis

Carlo Smirne[1], **Cristina Rigamonti**[1], **Carla De Benedittis**[1], **Pier Paolo Sainaghi**[1,2,3], **Mattia Bellan**[1,2,3], **Michela Emma Burlone**[1], **Luigi Mario Castello**[1] and **Gian Carlo Avanzi**[1,3]

[1]*Internal Medicine Division, Maggiore della Carità Hospital, Department of Translational Medicine, Università del Piemonte Orientale (UPO), Novara, Italy*
[2]*Immunorheumatology Unit, CAAD (Center for Autoimmune and Allergic Diseases), "Maggiore della Carità" Hospital, Novara, Italy*
[3]*IRCAD (Interdisciplinary Research Center of Autoimmune Diseases), Novara, Italy*

Correspondence should be addressed to Carlo Smirne; carlo.smirne@med.uniupo.it

Received 5 April 2019; Revised 20 June 2019; Accepted 12 August 2019; Published 8 September 2019

Academic Editor: Michele Malaguarnera

Liver fibrosis consists in the accumulation of extracellular matrix components mainly derived from activated hepatic stellate cells. This is commonly the result of chronic liver injury repair and represents an important health concern. As liver biopsy is burdened with many drawbacks, not surprisingly there is great interest to find new reliable noninvasive methods. Among the many are new potential fibrosis biomarkers under study, some of the most promising represented by the growth arrest-specific gene 6 (Gas6) serum protein and its family of tyrosine kinase receptors, namely, Tyro3, Axl, and MERTK (TAM). Gas6/TAM system (mainly, Axl and MERTK) has in fact recently emerged as an important player in the progression of liver fibrosis. This review is aimed at giving an overall perspective of the roles played by these molecules in major chronic liver diseases. The most promising findings up to date acknowledge that both Gas6 and its receptor serum levels (such as sAxl and, probably, sMERTK) have been shown to potentially allow for easy and accurate measurement of hepatic fibrosis progression, also providing indicative parameters of hepatic dysfunction. Although most of the current scientific evidence is still preliminary and there are no in vivo validation studies on large patient series, it still looks very promising to imagine a possible future prognostic role for these biomarkers in the multidimensional assessment of a liver patient. One may also speculate on a potential role for this system targeting (e.g., with small molecule inhibitors against Axl) as a therapeutic strategy for liver fibrosis management, always bearing in mind that any such therapeutic approach might face toxicity.

1. Introduction

1.1. Hepatic Fibrosis: Pathophysiology and Clinical Importance. All hepatologists wish they had a crystal ball in their clinic to enable them to determine whether or not their immediate patient has liver fibrosis or not. This is because liver fibrosis is a predominate key component of essentially all chronic liver diseases. It is the formation of scar tissue in response to parenchymal injuries such as chronic hepatitis B (CHB) and C (CHC), nonalcoholic fatty liver disease (NAFLD), or alcoholism (ALD). The continuous and progressive replacement of hepatocytes by the extracellular matrix and fibrous tissue eventually leads to liver cirrhosis, which in turn may lead to liver failure or promote a conducive microenvironment for cancer development, in particular hepatocellular carcinoma (HCC) [1, 2]. Whatever the etiology of liver injury, it is the activation of hepatic stellate cells (HSCs) that is responsible for liver fibrosis, being HSCs the main collagen-producing cells in the damaged liver [3, 4]. HSCs transform during chronic liver injuries from a quiescent state into a myofibroblast-like phenotype (HSCs/MFBs), which proliferate and migrate towards areas of necrosis and regeneration [5, 6]. The main action of HSCs/MFBs consists in a profound alteration of the extracellular matrix (ECM) composition due to the upregulation of proteins such as α-smooth muscle actin (α-SMA), interstitial collagens such as

collagen 1A1, and matrix metalloproteinases (MMPs) such as MMP9 as well as tissue inhibitors of metalloproteinases (TIMPs), and proteoglycans. Activated HSCs also generate hepatic cytokines such as transforming growth factor-β, platelet-derived growth factor, connective tissue growth factor, fibroblast growth factor, hepatocyte growth factor, and vascular endothelial growth factor and recruit inflammatory mono- and polymorphonuclear leukocytes that produce chemokines, including monocyte chemotactic protein-(MCP-) 1, regulated on activation normal T cell expressed and secreted (RANTES), chemokine (C-C motif) ligand 21 (CCL21), and C-C chemokine receptor type 5 (CCR5). Although HSCs' critical role in liver fibrosis was proposed nearly two decades ago [7], more recent data demonstrate that, regardless of the underlying etiology of liver disease, the majority of myofibroblasts comes from the liver-resident HSC population [8]. While liver fibrosis was once broadly thought of as an irreversible process, there is now substantial evidence that, at least from a speculative point of view, a near-normal hepatic architecture can be restored upon cessation of injury [9]. However, these promising findings must be offset by the fact that, after cessation of the fibrotic triggering insult, around half of the activated HSCs survive in an apparently quiescent state, as they are primed to quickly reactivate into myofibroblasts in response to fibrogenic stimuli [10, 11]. This leaves room for doubt that antifibrotic therapies meant to inhibit activated HSCs, although beneficial to prevent ECM deposition, may be sufficient to revert fibrosis permanently.

In any case, accurately defining the current fibrosis stage reached by a patient along the course of his/her disease is, as previously mentioned, of quintessential clinical importance, since crucial decisions, such as starting monitoring for complications (e.g., esophageal varices or HCC), depend on it. Moreover, the presence and extent of liver fibrosis help to predict prognosis and to prompt treatment decisions in various chronic liver diseases. For instance, different international treatment guidelines mention that the severity of liver fibrosis should be considered, regardless of serum alanine aminotransferase level, for starting antiviral treatment for CHB [12, 13]. In conclusion, there are a multiplicity of reasons for which it is crucial to diagnose and assess the extent of liver fibrosis.

1.2. Liver Biopsy for Staging of Fibrosis. Liver biopsy is still considered as the gold standard method to assess liver fibrosis; moreover, it provides useful information about diagnosis as well as other damaging processes such as necrosis, inflammation, and steatosis [14]. All most widely used methods to assess histological fibrosis are based on the description of the elements that mark the progression of the disease, such as periportal fibrosis, septal fibrosis, and/or nodule formation [15].

One obvious and insurmountable limitation of liver biopsy is that it is perceived as unduly invasive.

Furthermore, an insufficient sample size and divergence based on differing experience among pathologists can lead to significant interobserver disagreement. Risks associated with liver biopsy include pain (84%), bleeding (0.5%), damage to the biliary system (0.2%), and infections (0.1%), with a mortality rate of approximately 0.01% [16]. Finally, the cost of liver biopsy can be significant, leading to a questionable cost-effectiveness ratio [17].

1.3. Current Clinical Noninvasive Techniques to Assess Hepatic Fibrosis. These limitations of liver biopsy have given urgency for the development of alternative diagnostic procedures for liver fibrosis. As a result, noninvasive techniques have gained popularity in current clinical settings, leading to a reduction of liver biopsies to stage the degree of liver fibrosis; however, they also have several pitfalls.

The most traditional alternatives to invasive procedures are represented by medical imaging. Ultrasonography, for example, can suggest the presence of fibrosis and cirrhosis but it is neither sensitive nor specific in its implementation, performing positively only in late stages of liver cirrhosis, when the signs of portal hypertension develop [18]. Computed tomography and magnetic resonance are more sensitive and specific but are burdened by the association of high costs and inadequate interrater reliability among different radiologists; moreover, the extensive use of computed tomography scan is limited by radiological risks [19].

As a result, more innovative noninvasive approaches have been (and are being) designed. The two principal approaches that have been validated in large patient cohorts with various etiologies are elastographic techniques measuring liver stiffness and the detection and quantification of serum markers.

Elastographic methods, which test liver stiffness, are represented mainly by transient elastography (FibroScan®). Alternative techniques include point or multidimensional shear wave elastography and magnetic resonance elastography. All of these procedures, in addition to requiring expensive equipment, may be inaccurate in obese or ascitic patients and may lead to overestimation of fibrosis in patients with high necroinflammatory activity [20]. Moreover, being the result of the sum of inflammation and fibrosis in the liver parenchyma, liver stiffness *per se* may not be the ideal candidate to monitor for fibrosis regression.

The advantages of biomarkers over liver biopsy, besides being minimally invasive, are, at least from a speculative point of view, their cost, ease of application, interlaboratory reproducibility, and broad availability [21]. The rationale of their use derives from the notorious ability of the liver to either produce or modify a multiplicity of chemicals, a property that has long been explored to estimate, from the changes in their blood concentration, the degree to which liver function is impaired and/or to which extent organ damage is in addition to monitoring therapies. Indeed, liver biochemistry panels (e.g., aminotransferases, alkaline phosphatase, γ-glutamyl transferase, bilirubin, albumin, and prothrombin time) are included in almost all laboratory routines, being informative, relatively cheap, and prone to repeat testing [22]. Conceptually, fibrosis is of no exception. By-products spilling in the blood as a result of the deposition and removal of ECM produced by HSCs and other hepatic cells can be taken as a proxy measure of what is occurring in the liver parenchyma and are generally referred to as direct markers of fibrosis (as opposed to the aforementioned markers for liver injury which are considered indirect

markers of the same). Typically, serum levels of the former markers are elevated with progressing fibrosis and have a tendency to decrease with response to treatment [23]. As a result, their assessment may be useful for bringing about effective treatment, but they are neither organ specific nor readily available, unlike what would be required for an ideal biomarker [21]. An exhaustive classification according to their molecular structure can be found in a recent review from Nallagangula et al. [24].

As a general rule, although a single direct marker may serve as an indicator of disease severity, there is growing consensus that a combination of multiple markers as an integrated panel will enhance the performance characteristics in terms of specificity and sensitivity. This is why patients can now be profiled based on artificial intelligence algorithms that produce scores by combining different biochemical parameters (e.g., direct and/or indirect fibrosis markers and/or blood platelet count), including, in some cases, demographics such as age or gender. Some of the main scoring systems for liver fibrosis which have been implemented in clinical practice include the aspartate aminotransferase-to-platelet ratio (APRI) [25, 26], fibrosis- (FIB-) 4 [27], Fibro index [28], Bonacini index [29], Forns test [30], and NAFLD fibrosis score [31]. Unfortunately, none of these approaches have produced highly accurate results for liver fibrosis assessment to date [32] and their use in clinical practice is not comparable to those of prognostic scores, such as the Child-Pugh-Turcotte classification system [33] and the Model for End-stage Liver Disease score [34]. In this context, we also need to account for some derived scores, such as FibroTest [35], Fibrometer [36], Hepascore [37], and enhanced liver fibrosis [38], which include more specific blood tests such as direct fibrosis markers (e.g., hyaluronic acid [36–38], procollagen III amino terminal peptide [38], and tissue inhibitor of metalloproteinase 1 [38]), that are not routinely available. Again, the lines of evidence about their reliability and cost-effectiveness are not sufficient to support their use in clinical practice.

1.4. Evolving Biomarker Candidates for Liver Fibrosis. The aforementioned limitations of most current surrogate markers of liver fibrosis to provide stepwise follow-up (meaning a sensitive and specific manner for the detection and differentiation between the various stages of liver fibrosis and the possibility to detect modest progression or regression of fibrosis) explain why, on the one hand, liver biopsy has not yet been abandoned and why, on the other, there is great cultural eagerness to find new reliable noninvasive indicators, also due to the putative treatments for liver fibrosis appearing on the horizon. All newly discovered candidate markers may therefore play a vital role in the assessment of chronic liver injury which needs further evaluation. However, statistical comparison should always be made with established biomarkers and panels in large-scale multietiology validation studies [24, 39].

Among the many new potential markers which are under study, one of the most promising is represented by growth arrest-specific gene 6 (Gas6) serum protein and its family of receptors, namely, Tyro3, Axl, and MERTK (TAM). This system has long been demonstrated to have a pivotal role in fibrogenesis and in the progression of chronic liver diseases, yet it is believed that we are currently verging on a breakthrough in research due to the increasing knowledge of the fine interplay of these factors with the various mechanisms involved in liver damage. There is in fact a growing consent on its potential use also as a useful novel biomarker for the detection of liver fibrosis in vivo. However, many controversies remain due to the complexity of the biological systems involved.

The purpose of this work is to precisely review the literature data, highlighting the areas where the current lines of evidence are more concrete and those that still need further confirmation or validation.

2. Gas6/TAM Receptors

2.1. Biology of Gas6/TAM Receptors. TAM is one of the twenty subfamilies of receptor tyrosine kinases [40]. Members of the TAM receptor family are Tyro3, Axl, and myeloid-epithelial-reproductive tyrosine kinase (MERTK). All comprise two immunoglobulin-like and two fibronectin type III repeats in their extracellular domains in tandem. These are connected to a single-pass transmembrane domain and a cytoplasmic protein tyrosine kinase. Upon ligand binding, the receptor dimerizes and the tyrosine kinase becomes activated [41, 42]. TAM receptors differ in the physiological tissue expression. Axl is expressed in a wide variety of tissues and organs including the hippocampus, cerebellum, heart, skeletal muscle, liver, kidney, testis, brain, monocytes, macrophages, platelets, endothelial cells, and dendritic cell.

MERTK expression is found in the ovary, prostate, testis, lung, retina, and kidney and macrophages, dendritic cells, natural killer cells, megakaryocytes, and platelets. Tyro3 is most prominent in the nervous system, but it is expressed also in the ovaries, testis, breast, lung, kidney, osteoclasts, retina, monocytes/macrophages, and platelets [43]. Noteworthily, each receptor can be produced also as a soluble form (sAxl/sMERTK/sTyro3) [44].

First cloned in 1991, TAM receptors were all considered orphan receptors until 1995 [45]. In that year, their vitamin K-dependent ligands, protein S and Gas6, were identified [46–48]. While both Gas6 and protein S share common features of domain organization and both require dimerization and γ-carboxylation for their activity as TAM ligands, they have differential specificities and affinities to TAM receptors following their markedly different affinities. It is now generally accepted that Gas6 activates Axl, Tyro3, and MERTK and that protein S activates MERTK and Tyro3. More in detail, Axl is preferentially activated by Gas6 with 100–1,000x higher binding affinity over Tyro3 and MERTK, suggesting that it may be the most relevant of the three receptors for Gas6 in many tissues, whereas affinity between protein S and Axl has never been shown. MERTK displays lower sensitivity to both ligands, and it is observed to have the greatest phosphatidylserine (PS) dependence on ligand-induced activation. Tyro3 is preferentially activated by protein C. Moreover, Tyro3 and MERTK biological activation is enhanced

in the presence of PS, implicating mainly both these receptors in the clearance of apoptotic cells.

Whatever the receptor, in many cells, the activation of TAMs is coupled with the downstream activation of the phosphoinositide 3 kinase (PI3K)/AKT pathway. Most of this downstream PI3K signaling is nucleated through a TAM-autophosphorylated Grb2-binding site, which is located 18 residues carboxy terminal to the kinase domain and is conserved in all three TAMs. Coupling to phospholipase C, ERK1/2, Ras, and MAP kinase activation have also been described in many different cells [43, 49–51].

Coming back to the two TAM ligands, it is important to note that they differ in tissue expression patterns. More in detail, while natural anticoagulant protein S is mainly synthesized in the liver, Gas6 is produced predominantly in the heart, kidneys, and lungs and, to a lesser extent, in the liver. Other important tissues where Gas6 is expressed are endothelial cells [52], vascular smooth muscle cells [53], and bone marrow [54]. Gas6 has also been shown to be present in murine platelets, but this presence in humans has been debated [55]. From a morphological point of view, the two proteins share a high structural homology and sequence identity. However, they have clearly different biological roles [56, 57]. Protein S has mainly a TAM-independent inhibitory effect on hemostasis [58–60]. The Gas6/TAM system has instead clearly emerged from basic and clinical studies to have rather pleiotropic effects with many biological functions, sometimes playing more than one role at a time, as frequently seen in human biology [61]. Specifically regarding the area of coagulation, Gas6 seems to stimulate hemostasis playing a complementary role in platelet function [62], and it has been proposed as a biomarker for the diagnosis of pulmonary embolism [63]. But, in recent years, several other signaling functions of TAM receptors have been described, such as stimulation of cell growth and proliferation, inhibition of apoptosis [53, 64], mediation of efferocytosis (e.g., the process by which dying cells are removed by phagocytes) [65], and modulation of inflammation [66]. These effects probably explain why the overexpression of TAMs (mostly Axl and MERTK) can drive conventional oncogenic signaling and survival pathways in different types of cancers, while also playing an important role in epithelial to mesenchymal transition and metastasis [42]. As a consequence, the overexpression of TAM receptors has been associated with chemoresistance and poor survival outcomes [67].

A current research field that deserves a separate discussion is the activity of Gas6/TAM on the immune system [68]. Gas6 activation of the TAM receptors (specifically, MERTK and Axl isolated from circulating monocytes and tissue macrophages) was initially found to inhibit Toll-like receptor (TLR) signaling, which in turn is a known trigger of rapid inflammatory cytokine production in various cell types [69]. Conversely, TLR signaling was demonstrated to markedly decrease Gas6 expression in mouse macrophages through the activation of the nuclear factor-κB, further facilitating—in a self-regulatory mechanism—the TLR-mediated inflammation [70]. Furthermore, the Gas6/TAM system was shown to be directly involved in the clearance of apoptotic bodies [71]. As a matter of fact, Gas6 recognizes phosphatidylserine, a lipid normally expressed on the inner face of the plasma membrane and exposed on the external membrane during apoptosis, and bridges it with the TAM receptors, driving macrophages to the recognition of apoptotic cells and to their subsequent phagocytosis, stimulating natural killer cell development [72]. Other recent studies revealed that Gas6/TAM signaling is involved in inflammation by enhancing interactions between endothelial cells and leukocytes [73]. Moreover, the induction of Axl limits cytokine synthesis in activated monocytes or dendritic cells [74]. Based on these premises, it is not surprising that there has been speculation on its possible role for the system in preventing autoimmunity [75]. On the contrary, defects in TAM signaling have been associated with numerous autoimmune diseases and degenerative diseases, since an impaired clearance of apoptotic bodies and an inappropriate inflammatory response are considered critical for the deranged immune response observed in these conditions. The role of TAM receptors has been for instance studied in diseases such as rheumatoid arthritis [76], multiple sclerosis [77–79], systemic lupus erythematosus [80], Sjögren syndrome [81], and Alzheimer's disease [82].

The complexity of the crosstalk between Gas6 and its receptors has increased to a further extent by the fact that in many of the aforementioned diseases, such as rheumatoid arthritis and lupus erythematosus, an impairment of the physiological balance between the transmembrane and the inactive soluble form of the receptors has been observed, suggesting that an increased cleavage of the receptors could have biological relevance in the pathogenesis of these conditions [76, 83]. The most studied is probably sAxl. Physiologically, Axl is cleaved by a disintegrin and metalloproteinase (ADAM) 10 and 17 in a protein kinase C-dependent fashion causing the release of sAxl which maintains the ability to interact with Gas6 [84, 85]. Thus, the release of sAxl and its involvement in a negative feedback loop by Gas6 binding together with the γ-secretase-mediated release of a sAxl intracellular domain (ICD) suggest bidirectional signaling.

2.2. Role of Gas6/TAM under Healthy and Pathological Conditions in the Liver. In recent years, the Gas6/TAM interaction has been described to be relevant in inflammatory and healing processes of the liver; in fact, Gas6 globally seems to play a protective role in response to liver injury.

In the liver, Gas6 is mainly expressed in Kupffer cells with levels below those observed in other tissues such as those found in the lung, kidney, or heart [56]. However, after specific liver injury, other hepatic cell types may participate in its production. For instance, Gas6 produced by HSCs together with its receptor Axl participate in the signaling involved in the injury repair mechanisms. Moreover, it has been shown in animal models that Gas6 expression is also significantly upregulated in injured areas by the other key cellular actors involved after acute or chronic liver damage, such as macrophages, HSCs/MFBs, and liver progenitor cells (LPCs). In this context, Gas6 exerts an antiapoptotic effect on both HSCs and HSCs/MFBs, acting as a survival factor, probably supporting transient HSC/MFB accumulation during liver healing [86]. For instance, Gas6 produced by HSCs and

infiltrating macrophages together with its receptor Axl participate in the signaling (which includes, among others, the aforementioned Axl/PI3K/AKT pathway) involved in the wound healing response to liver injury by carbon tetrachloride (CCl_4), and LPCs induce Gas6 production after hepatectomy [86–88]. Moreover, an early increase in serum Gas6 levels has been demonstrated following liver ischemia/reperfusion (I/R) exposure [89].

Consistent with these findings, in Gas6−/− knockout (KO) mice, abnormal wound healing after CCl_4-induced liver damage compared with wild-type animals has been reported, with decreased expression of activation markers for Kupffer cells (such as CD14, TNF-alpha, IL6, and MCP-1) and HSCs (such as α-SMA and collagen type 1); as a consequence, decreased macrophage and HSC/MFB recruitment has also been shown in damaged areas. So Gas6 deficiency, by limiting cytokine/chemokine release, prevents hepatocyte proliferation, macrophage infiltration in liver necrotic areas (which, in turn, is mediated by a direct chemotactic effect of Gas6), and accumulation of myofibroblasts in healing areas. Interestingly, in Gas6 KO mice, a positive feedback on Axl expression was observed, with the concomitant induction after CCl_4 treatment of the suppressor of cytokine signaling (SOCS) 1, suggesting that the delayed liver repair in deficient mice may be a consequence of an inhibitory signal arising from Axl receptor overexpression [88].

A similar mechanism probably explains what has been described in hepatic I/R models. As already mentioned, in mice following I/R exposure, an early increase in serum Gas6 levels was reported. Unlike wild-type mice, Gas6-/- mice were highly sensitive to partial hepatic I/R, with 90% of mice dying within 12 hours of reperfusion due to massive hepatocellular injury. I/R induced early hepatic AKT phosphorylation in wild-type but not in Gas6-/- mice, whereas hepatic IL-1β and TNF mRNA levels (e.g., lipopolysaccharide- (LPS-) induced cytokines) were higher in Gas6-/- mice compared to wild-type mice. In line with the in vivo data, in vitro studies indicated that Gas6 induced AKT phosphorylation in primary mouse hepatocytes protecting them from hypoxia-induced cell death, while Gas6 diminished IL-1β and TNF in murine macrophages. Finally, the protective role of Gas6 on cell growth and survival during tissue repair was confirmed by the fact that in vivo recombinant Gas6 treatment not only rescued Gas6 knockout mice from I/R-induced severe liver damage but also attenuated hepatic damage in wild-type mice following I/R. Thus, it may be speculated that Gas6 could emerge as a potential therapeutic target to reduce postischemic hepatic damage [89].

Synthesizing to the fullest extent, the protective role of Gas6/TAM on the liver is mediated by its strong profibrogenic potential. However, as in all biological processes, even an initially favorable action—like a physiological reparative process—can become, if out of control and especially if protracted in time, a factor of damage itself, since an excessive fibrotic apposition in the liver can in turn become a pathophysiological mechanism of hepatic injury. In this sense, Gas6/TAM has a role like that of a "two-faced Janus," depending on clinical contexts. These concepts will be further clarified in the following paragraphs.

2.3. Role of Gas6/TAM in Liver Fibrosis. The Gas6/TAM system has recently emerged as an important player in progression to liver fibrosis through the aforementioned control of inflammation and liver repair. Not surprisingly, the focus of the few pathophysiological studies currently available is the modulation of HSC activation, because of its recognized role in the liver fibrosis associated to chronic liver injury of any etiology, being HSCs the main collagen-producing cells in any damaged liver [3, 4]. The most convincing study comes from a murine model of Barcena et al. The authors used both a genetic model of Axl deficiency (Axl KO) and a pharmacologic approach, the Axl inhibitor BGB324. HSCs were obtained from wild-type and Axl−/− mice, treated with recombinant Gas6 protein (rGas6), Axl siRNAs, or BGB324, and analyzed by western blot and real-time PCR. Experimental fibrosis was then studied in CCl_4-treated wild-type and Axl−/− mice and in combination with Axl inhibitor. After five weeks of CCl_4 treatment, wild-type mice exhibited a marked increase in Gas6 and sAxl serum levels compared to controls, indicating that this pathway is upregulated during CCl_4-induced liver fibrosis. In primary mouse HSCs, Gas6 and Axl levels paralleled HSC activation. rGas6 phosphorylated Axl and AKT prior to HSC phenotypic changes, while Axl siRNA silencing reduced HSC activation. Moreover, BGB324 blocked Axl/AKT phosphorylation and diminished HSC activation. In addition, Axl KO mice displayed decreased HSC activation in vitro and liver fibrogenesis after chronic damage by CCl_4 administration. Similarly, BGB324 reduced collagen deposition and CCl_4-induced liver fibrosis in mice [90].

Based on these premises, it is not hazardous to hypothesize that the Gas6/TAM system may have a prominent role in the pathogenesis of major chronic liver diseases, with particular reference to fibrosis development. However, it must be said that up to now the amount of evidence is still rather scarce, and further clinical studies of adequate potency are needed.

2.3.1. Gas6/TAM System and Nonalcoholic Fatty Liver Disease. Taking into account the aforementioned limitations, one of the most important liver disease models which has been studied is NAFLD, which includes simple nonalcoholic fatty liver and the more serious nonalcoholic steatohepatitis (NASH). This nosological entity is one of the leading causes of liver-related morbidity and mortality, at least in developed Western countries. Whatever its etiology, it is characterized by fat storage in hepatocytes, lobular inflammation, elevated local and systemic cytokines, activation of HSCs, and expansion of LPCs in periportal areas, both in animal and human models [91, 92]. NAFLD is a risk factor associated with toxic and metabolic fatty liver disease and can progress to end-stage cirrhosis [93]. According to the two-hit model of NAFLD, steatosis is the first hit that increases hepatocyte vulnerability to any secondary insult eliciting an inflammatory response, but most probably, both events are tightly interconnected since fat accumulation per se induces oxidative injury and inflammatory cytokine synthesis [94]. The persistent low-grade inflammation due to chronic hepatocyte damage

also plays a critical role in LPC expansion, which may play a part in fibrosis [91, 92, 95, 96].

To address the role of Gas6 in NAFLD and in the progression to liver fibrosis, an animal model was studied, e.g., Gas6 KO mice fed with a choline-deficient ethionine-supplemented diet (CDE) or receiving a CCl_4 treatment [97]. Gas6 deficiency attenuated hepatic steatosis by limiting CDE-induced downregulation of genes involved in β-oxidation observed in wild-type animals. Moreover, Gas6-deficient mice displayed a reduction of hepatic inflammation, revealed by limited F4/80-positive macrophage infiltration, decreased expression of IL-1β, TNF-α, MCP-1, and lymphotoxin-β, and attenuated LPC response to CDE diet. Gas6 deficiency moreover reduced CDE-induced fibrogenesis and hepatic myofibroblast activation, decreased expression of TGF-β and collagen type 1 mRNAs, and increased Axl protein levels. After chronic CCl_4 injury, Gas6-deficient mice also exhibited reduced liver fibrosis as a consequence of defective macrophage recruitment compared with wild-type animals. The authors concluded that the improvement of steatohepatitis and fibrosis in Gas6−/− mice was linked to an inhibition of the liver inflammatory response (similar to other previously mentioned models) which in turn regulates lipid metabolism and macrophage recruitment. Thus, this study highlights the possible deleterious effect of Gas6 in the progression of steatosis to steatohepatitis and fibrosis. However, it has to be mentioned that in this CDE model no induction of SOCS1 and 3 could be observed, as previously observed in the CCl_4 acute model of liver injury [98], thus making the functional relevance of Axl overexpression in Gas6-deficient mice still uncertain. Another possible weakness of the work is that the other components of the TAM family (e.g., Tyro3 and MERTK) were not tested, though they may contribute to the Gas6 effects described in this NAFLD animal model.

While, to the best of our knowledge, no current data are available on Tyro3 role in NAFLD-related fibrogenesis in vivo or in vitro, there are some pieces of evidence about the pathophysiological role of Axl and MERTK.

For what concerns the former one, its distinctive subcellular signaling during NASH development and the efficacy of its intervention to prevent diet-induced liver fibrosis remain to be explained. However, there are several preliminary pieces of evidence that indicate that it may play an important role in NAFLD progression. In particular, in a letter from Mari et al., it is commented that increased Axl levels have been detected in mouse models of NASH, anticipating a significant role for Gas6/Axl in human NASH pathology [98]. These data were recently confirmed in a research from Tutusaus et al. in which it was described how Axl expression was elevated in NAFLD patients and in mouse models of NASH. Among individuals with different degrees of NAFLD (steatosis/fibrosis/cirrhosis), only cirrhotic patients displayed increased Gas6 and MERTK serum levels. However, Axl values were significantly elevated in all NASH groups in parallel to disease progression. Consistent with Axl influence in HSC transdifferentiation, in human activated HSC cells (LX2), the expression of profibrogenic genes after Axl activation was blocked by the selective Axl inhibitor BGB324. Axl control of inflammatory response was then analyzed in activated human THP-1 macrophages. While Gas6 reduced LPS-induced gene expression, Axl inhibition did not affect it. Finally, mice fed with a high-fat diet choline-deficient with methionine restriction (HFCD) developed significant hepatic steatosis and fibrosis and exhibited increased sAxl levels, recapitulating human NASH observations. Besides inhibiting Axl, BGB324 administration increased circulating Gas6, favoring Gas6 liver protection. This protective effect was confirmed also in HFCD-fed mice which showed reduced liver fibrosis and hepatic inflammation. Taken together, these data seem to suggest that sAxl levels are an early marker of NASH that correlates with disease development and, at least in experimental NASH models, that therapeutic inhibition of Axl can diminish liver fibrosis by blocking HSC activation and reducing hepatic inflammation, possibly due to Gas6 hepatoprotective action [99].

The other TAM receptor which has been studied in this disease is MERTK. The latter one is a well-known key component for the initiation of efferocytosis [42, 100] and is overexpressed in mouse HSCs activated by culture in plastic and in experimental models of liver fibrosis (e.g., after induction of chronic liver damage in response to CCl_4 administration or bile duct ligation) [90, 101]. Moreover, agonists of LXR, a nuclear receptor favoring lipogenesis, increase MERTK expression in monocytes [102]. Therefore, MERTK and its variants could act as central players in the control of apoptosis, immune response, HSC activation, and steatosis modulation, e.g., all factors involved in the pathogenesis of NAFLD and its fibrotic progression to steatohepatitis and cirrhosis. Based on a genome-wide study in patients with CHC which, amongst several susceptibility loci for severity and progression of liver fibrosis, identified as the strongest one the homozygosity for rs4374383 G>A single nucleotide polymorphism, a non-coding variant in the MERTK gene [103], an in vivo and in vitro study was conducted on NAFLD. In a large cohort of patients with histological diagnosis of NAFLD, the protective AA genotype was associated with lower MERTK hepatic expression (fibrosis F2-F4 in 19% of patients with MERTK AA compared to 30% of those with MERTK GG/GA); the AA genotype remained associated with clinically significant fibrosis also at multiple logistic regression analysis. Similar results were observed also when considering severe fibrosis (F3-F4) as histological outcome. The prevalence of NAFLD was not affected by MERTK genotype (39.7% in MERTK AA vs. 44.1% in MERTK GG/GA), but severe steatosis was observed in 8% of patients with MERTK AA compared with 21% with MERTK GG/GA genotype. Again, MERTK AA genotype remained associated with severe steatosis at multiple logistic regression. MERTK was overexpressed in the liver of NAFLD patients with F2-F4 fibrosis, mainly in HSCs and macrophages (but not in hepatocytes). Similarly, the receptor was more represented in an animal model of fibrogenesis (e.g., mice fed with a methionine- and choline-deficient diet). Furthermore, the exposure of cultured human HSCs to the MERTK ligand Gas6 increased cell activation and migration and induced the expression of the profibrogenic procollagen I. These effects were counteracted by the inhibition (both with specific small molecule inhibitor UNC569

and siRNA) of MERTK activity, which also resulted in apoptotic death of HSCs. The results of this research seem to provide sufficient evidence for considering MERTK AA genotype as an appealing new genetic biomarker in natural history, pathophysiological, and interventional studies in NAFLD [98, 104, 105].

2.3.2. Gas6/TAM System and Other Liver Diseases. Taking into account other liver disease models, some preliminary in vivo data are available for ALD and CHC infection. In the previously mentioned Barcena et al. paper, the authors recruited a small sample of patients (30 ALD, 51 CHC) who all had hepatic fibrosis staged by liver biopsy. Additionally, in both groups, patients were evenly distributed in regard to the different degrees of liver disease, although in the ALD group no moderate fibrosis cases (METAVIR F2-F3) were included. Gas6 and sAxl serum levels were measured before initiation of treatments. In ALD patients, both an increase of Gas6 and sAxl were found in serum levels of cirrhotic patients, showing close correlation to the severity of the disease, although behaving differently. Specifically, sAxl concentration had already augmented in individuals with compensated cirrhosis compared to initial fibrosis, while Gas6 levels had increased markedly in the decompensated cirrhosis group. Moreover, a remarkable correlation was found between the MELD score and both proteins. In CHC patients, Gas6 levels were significantly different among individuals with established fibrosis (F2) and patients with initial fibrosis (F0 and F1 groups). In addition, F2 fibrosis patients' sAxl levels displayed significant changes in comparison to individuals with advanced fibrosis or cirrhosis (F3/F4 group). The authors therefore could provide groundbreaking evidence emphasizing for the first time the relevance of the Gas6/Axl pathway also during the development of ALD- and CHC-induced liver damage, supporting Gas6 and sAxl serum levels as indicative parameters of hepatic dysfunction and fibrosis development in liver disease and suggesting their possible future prognostic role within a patient multidimensional evaluation [90].

The report that sAxl levels are increased in advanced fibrosis/cirrhosis has been confirmed in a much wider population including 75 healthy controls, 400 chronic liver disease patients of various etiologies (chronic viral hepatitis, autoimmune hepatitis, cholestatic liver disease, and NAFLD) and fibrosis stages (including cirrhosis), and 347 HCC patients [106]. For cirrhosis, sAxl showed a sensitivity of 80.8% and a specificity of 92.0% at a cutoff of 54.0 ng/ml.

In any case, Axl is not the only component of the TAM family which has demonstrated a putative role in hepatic fibrosis progression. For instance, there are growing pieces of evidence of MERTK involvement, at least for what concerns CHC. More in detail, a genetic predisposition with regard to an accelerated fibrosis (demonstrated by liver histology and/or transient elastography) has been reported for what concerns the aforementioned rs4374383 G>A single nucleotide polymorphism. As shown in other diseases, it is likely that patients carrying the GG/GA genotypes have a significantly higher hepatic MERTK expression, although the underlying mechanism is unknown [104]. This in turn will lead to a dysregulation of the phagocytosis of apoptotic cells by macrophages and, more in general, of various inflammatory responses [103, 107–109]. It has to be noted that, although the rs4374383 SNP is not located in a regulatory MERTK region, a high number of SNPs are in high linkage disequilibrium (LD) with it. Thus, another SNP or SNPs in high LD could be causally responsible. This issue was investigated by Cavalli et al., who suggested that rs6726639A allele, in high LD with rs4374383 ($r^2 = 0.94$), could promote the binding of interferon regulatory factor 1 (IRF1) to this region [110] and serve to activate or repress the expression of a high number of genes involved in the immune response [111]. The preferential binding of IRF1 to the A allele compared to the C allele would downregulate MERTK in patients carrying the A allele, protecting against CHC liver fibrosis and HCC. So, in genetic association studies, the two SNPs (rs4374383 and rs6726639) may be interchangeable for predicting liver fibrosis progression.

The results of the aforementioned studies could leave room to a possible future role for the targeting of TAM receptors (e.g., with small molecule inhibitors against Axl or MERTK) as a therapeutic strategy for liver fibrosis management, with the caveat that any such therapeutic approach might face toxicity. The measurement of the soluble levels of Gas6 and its receptors (e.g., sAxl and sMERTK [102]) could furthermore be the basis of providing an easy and accurate measurement of hepatic fibrosis progression, since numerous other targets for antifibrotic agents are difficult to be analyzed or to enter early-phase clinical studies due to the lack of sensitive markers to follow the effects [90].

Returning to clinical studies whose purpose is to test the role of plasma Gas6 as a novel putative biomarker of hepatic fibrosis in different disease models, a paper from Bellan et al. deserves a mention [112]. A fair number (113) of patients were studied, the vast majority (81%) being affected by CHC infection. Fibrosis was staged by transient elastography and/or, whenever feasible and accepted, by liver biopsy; again, all stages of hepatic disease were represented, from initial fibrosis to decompensated cirrhosis. Authors confirmed Barcena's finding that patients with histological demonstration of severe fibrosis had significantly higher Gas6 plasma concentrations; they were also able to demonstrate for the first time that Gas6 plasma concentration was directly correlated to liver stiffness measured by transient elastography. Even more relevant, the diagnostic accuracy of Gas6 was comparable to that of liver elastography both in ruling out and in detecting severe liver fibrosis. A proposed threshold of 30 ng/ml for Gas6 plasma concentration was able to rule out a clinically relevant degree of fibrosis with an 84% sensitivity and 56% specificity, while values >42 ng/ml identified severe fibrosis with a sensitivity of 64% and a specificity of 95%; however, taking into account that the majority of patients was affected by chronic viral hepatitis, some caution should be exercised before automatically generalizing these conclusions when other conditions can be factors.

2.3.3. Gas6/TAM System: Does It Have a Role in Fibrosis Complications? Of noteworthy importance, in the previously reported paper from Bellan et al. [112], the authors noted a

nonstatistically significant trend toward higher Gas6 concentrations in patients affected by cirrhosis complications (e.g., esophageal varices and HCC). These reports are to some extent the expected consequence of the association with the severity of fibrosis, since both conditions complicate the natural history of cirrhosis. The former ones are the direct consequence of a major hepatic fibrosis complication, e.g., portal hypertension.

The linkage with the latter is biologically more complex to explain and also remains plausible for several relevant reasons. The Gas6/Axl (both in transmembrane and soluble forms) system has been, for instance, claimed to be connected to the promotion of tumor invasion in various solid malignancies, as recently confirmed in a meta-analysis conducted on 3,344 total patients (379 with HCC) from 25 studies. Axl overexpression was significantly correlated with poor prognosis (2.03-fold increase in mortality in all solid tumor patients); in a subgroup analysis of different cancer types, Axl overexpression was correlated with shorter overall survival in a few tumors, including HCC (combined HR of 1.89 (95% CI 1.37–2.60, $p < 0.001$)) [113]. The pathophysiological rationale of Gas6/Axl deleterious role probably consists in its capacity to activate HSCs and modulate hepatocyte differentiation, as suggested by a preliminary study which demonstrated that in HCC cancer cell lines Gas6/Axl can enhance cell invasiveness through transcriptional activation of Slug which induces epithelial to mesenchymal transition (EMT) [114]. Notably, under physiological conditions, Gas6 and its receptor Axl are not expressed in hepatocytes. However, Axl is strongly expressed in malignant hepatocytes of about 40% of HCC patients showing progression towards metastasis [115]. Moreover, as previously described in immune stem and cancer cells, EMT-transformed hepatocytes upregulate the expression of Axl and secrete Gas6 revealing a possible autocrine/paracrine regulation loop in the Gas6/Axl pathway [116]. In the background of fibrosis, sinusoidal endothelial cells, activated HSCs, and Axl-positive myofibroblasts as well as Kupffer cells release Gas6 into the tumor microenvironment of HCC, causing a Gas6-enriched HCC stroma. These data suggest that Axl signaling drives HCC progression in the presence of large amounts of bioactive Gas6 and is of even more particular interest as tyrosine kinase inhibition is one of the most exploited antitumoral approaches of targeted therapies (e.g., bosutinib) [114, 117, 118]. However, since very complex mechanisms are involved that go extensively beyond the simple induction of hepatic fibrosis, the precise analysis of the possible oncogenic roles of the Gas6/TAM system in HCC signaling (in many cases still lacking solid evidence) remains outside the purpose of the present review.

A further analysis of the possible association of Gas6 plasma concentrations with the presence of esophageal varices comes from the same research group which extended the abovementioned preliminary finding in a large cohort of CHC-infected cirrhotic patients [119]. The clinical rationale for such a research is that early detection of patients with varices at high risk of bleeding (e.g., large varices) is crucial in cirrhotic patients, but sparing endoscopy to low-risk patients would be worthy of consideration. With this in mind, noninvasive methods, such as Baveno VI criteria, have been proposed to stratify the risk of esophageal varices (suggested cutoffs for which screening for esophageal varices can be safely omitted: liver stiffness at transient elastography < 20 kPa and a platelet count $> 150 \times 10^9$/l) but unfortunately have some limitations [120]. In the studied cohort, a total of 74/160 (46%) patients had esophageal varices that were large in 17/160 (11%) cases. 34/160 patients (21%) satisfied Baveno VI criteria to avoid variceal screening, but one of them had large varices at upper gastrointestinal endoscopy (sensitivity 94%). Serum Gas6 values increased from 63 ng/ml among patients without varices to 75 ng/ml among patients with small varices and to 98 ng/ml among those with large varices. A plasma Gas6 value < 45 ng/ml, detected in 34/160 (21%) patients, was 94% sensitive (but only 23% specific) in identifying patients without large varices; one of these patients (different from the subject missed by Baveno VI criteria) had large varices at upper gastrointestinal endoscopy. The authors could then conclude that plasma Gas6 concentration is a highly sensitive test to identify patients with large varices, outperforming the platelet count as a single biomarker of large varices and proving to be comparable to the diagnostic performance of Baveno VI criteria. This could provide the initial rationale for a future role for Gas6 in clinical settings in which liver elastography is still not available or in those patients for whom a reliable liver stiffness cannot be obtained (e.g., for ascites or morbid obesity).

For what concerns other severe complications of cirrhosis, e.g., portal hypertension-induced ascites, the role of other TAM system members has been demonstrated, with particular regard to MERTK. This prorestorative marker shows a two-faced activity: while for instance it is abundantly expressed in liver macrophages during the resolution phases of several diseases (e.g., acetaminophen-induced liver injury) [121], it has also been identified as a potent suppressor of T cell responses [122]. Regarding the latter activity, there are for instance some pieces of evidence about the development of immunoparesis in patients with acute on-chronic liver failure (ACLF) involving the unbalanced activation and overexpression of MERTK on monocytes/macrophages in the circulation and tissue sites of inflammation [123–125]. The great influence of MERTK-positive monocytes was confirmed in a late Antoniades work that studied ACLF patients with ascites. Immunometabolic profiling of their ascites revealed profound disturbances in myeloid cells with upregulated MERTK expression, impaired proinflammatory responses to LPS, preferential lipid metabolism, and evidence of epithelial cell death. The impact of these perturbations on bacterial clearance could predispose to an increased susceptibility to infections such as spontaneous bacterial peritonitis (another severe complication of cirrhosis), but this still requires further exploration [126].

Notably, coming back to HCC, some preliminary data exist on a similar pattern of severe myeloid impairment. As a matter of fact, in this tumor, it has been reported the expansion of a MERTK-expressing immunosuppressive tumor-associated macrophage population that suppresses host innate and adaptive immune responses. In the same study,

neoplastic patients, compared with controls, had also a significant increase in MERTK-expressing circulating monocytes (and in Gas6, as previously mentioned). Inhibition of MERTK signaling restored their proinflammatory capabilities, thereby identifying a possible novel immunotherapeutic target in HCC [127].

2.3.4. Possible Implications of Soluble Axl in Liver Fibrosis. As previously mentioned, Axl can be cleaved and released in serum as sAxl. Since the latter is still able to bind Gas6 and is therefore capable of depleting the ligand, it is considered to be a critical determinant in affecting autocrine or paracrine Axl signaling [128, 129]. Consequently, hepatic fibrosis progression should be subsequently attenuated by diminished Gas6/Axl signaling, resulting in a phenotype comparable to the one of chemically challenged Gas6 KO mice [97]. However, serum Gas6 levels have been shown to be elevated in patients with advanced fibrosis and cirrhosis as well as in HCC patients [85, 101]. Moreover, high Axl expression as well as high sAxl levels independently correlate with fibrosis/cirrhosis [90, 106, 130]. These findings are contradictory to the hypothesis that ectodomain shedding of Axl only leads to signal dampening. There is in fact evidence that the ICD of Axl could remain active supporting the belief of both a Gas6-independent signaling, in parallel with a Gas6-dependent one, which has been revealed by the previously mentioned Gas6 KO and Axl KO studies showing reduced fibrogenesis [90, 97]. This hypothesis is supported by the Holstein et al. group that proposed there might be a switch predisposing liver fibrosis, cirrhosis, or even HCC development, where even in the event of a cleavage of Axl, the inhibitory Axl shedding mechanism is circumvented due to the presence of abundant nonshedded Axl receptors that will overcome the loss of proteolytically cleaved Axl. Available free Gas6 is then able to bind increasingly expressed Axl receptor and stimulate Gas6/Axl signaling driving fibrosis in the liver [117]. This Gas6-independent signaling hypothesis implicates that proteases are recruited to cleave the Axl ectodomain after Gas6-mediated Axl activation. In this scenario, the ICD could remain active and could still be able to phosphorylate effector molecules [117]. However, it is an open question as to whether ectodomain shedding occurs after Axl homodimerization and ICD activation. Interestingly, a mechanism of shedding prior to receptor activation with ligand-independent signaling has been reported for ErbB2 [131].

3. Conclusions

In the present paper, we have reviewed current evidence regarding the use of Gas6 and its TAM receptors as potential biomarkers of liver fibrosis.

The rationale for interest in Gas6 system derives from the proven role of the Gas6 pathway in the HSC transdifferentiation process from a normal vitamin A-storing to an ECM-remodeling phenotype. This indeed is what initializes fibrosis. Despite recent progress in understanding the biology of HSCs, the mechanisms are not yet fully understood. In fact, in addition to the treatment/withdrawal of the underlying cause, fibrosis regression in chronic liver diseases is not accomplished by any antifibrotic drug despite the experimental description of an array of pharmacological targets [3, 6]. Based on these premises, the exact biological roles of Gas6 pathways, though undoubtedly relevant in human liver pathology, are still under investigation at least in regard to the fibrogenesis process. At any rate, what has clearly emerged from preclinical and clinical studies is that Gas6/TAM is a profibrogenic route. This means that it is beneficial/reparative in the event of acute damage but profibrogenic/harmful if the insult chronicizes. In this context, not only does the evidence available so far make it interesting to test the potential use of these system-related proteins as serological markers of disease progression/fibrosis but we may also speculate that this pathway may provide a new therapeutic target not only for liver fibrosis but also for different chronic liver diseases. Moreover, the existence of specific inhibitors [132, 133], already in clinical trials, may facilitate the biomedical translation of preclinical studies.

In the current state of the art, in essence, a sufficient amount of data has now accumulated showing that Gas6 and its receptors, such as Axl and MERTK, play a relevant role in the major models of chronic liver diseases. However, reference has been limited to approximately a few hundred patients tested in vivo. While results of the studies conducted to date are promising, some major drawbacks remain. First, to prove accurate staging of liver fibrosis by these novel serological markers, liver biopsies will still be needed to identify the specific stage of fibrosis in each patient. The lack of such material results in the usual incapacity of most studies to report other facets of biomarkers beyond the ability to identify late stage fibrosis or cirrhosis, as compared to transient elastography or validated serological scoring algorithms. Second, large-scale multietiology validation of such novel serum markers is still needed. The efficiency of the biomarkers should be tested prospectively on large patient cohorts with differences in age, gender, etiology of liver disease, etc. Moreover, it is reasonable that these novel biomarkers might find, as many other noninvasive analytes, their best use within more complex algorithms rather than in the simple measurement of their plasma concentrations. For example, in the paper from Barcena et al., an algorithm containing sAxl and Gas6 could achieve an even stronger correlation ($r^2 = 0.86$) with the MELD score than the two analytes taken individually, suggesting that the measurement of both proteins provides a better evaluation of liver functionality [90]. Most likely, however, the best diagnostic solution will be achieved combining these markers with more variables, not necessarily directly related to the fibrosis itself. A first preliminary confirmation comes from the work of Staufer et al. in which sAxl performed better in predicting advanced liver fibrosis (≥F2) when combined with serum albumin (in a sAxl/albumin ratio) in various chronic liver diseases [130].

Finally, the fine mechanisms of this like pleiotropic system have still to be fully clarified. First of all, an important issue about published data is that most have involved the assay of single ligand against single TAM receptor. However, some analysis has demonstrated that invalidation of one TAM receptor might induce a compensatory enhancement

of one or two other TAM receptors and also vice versa in the case of upregulation of a single receptor [134, 135]. However, the functional consequences of this reciprocal regulation remain unclear. The complexity of Gas6/TAM system is also revealed by what happens when it is the ligand (e.g., Gas6) to be deficient. In these cases, a high and constitutive expression of Axl is found, which reveals a negative control exerted by Gas6 on its high-affinity Axl receptor expression. However, the functional relevance of Axl overexpression, at least in Gas6-deficient mice, is still uncertain and deserves further studies [88, 97]. Another Axl negative feedback regulation, which needs to be better clarified in liver pathology, involves microRNA (miRNA). There are some preliminary data in tumoral cells showing that miR-34a may target the $3'$ UTR of Axl mRNA to posttranscriptionally inhibit Axl expression, modulating apoptosis in cancer cells, and revealing functional implication of miRNA in the carcinogenic process. On its turn, Axl overexpression may induce miR-34a expression [136]. Obviously, also many positive feedbacks regulate Gas6/TAM system. There is for instance an interesting recent report that again needs to be validated in hepatic diseases about a novel oncogenic long noncoding antisense RNA (Gas6-AS1) that can control the expression of its cognate sense gene Gas6 at the transcriptional or translational levels. Its net effects consist in supporting tumor progression via inducing an increase of Axl levels and driveling Axl signaling pathway activation [137]. Another partially unresolved issue concerning Gas6/TAMs is, as previously mentioned, whether the shedding of activated Axl receptors could lead to Gas6-independent signaling, with potential consequences not only on fibrogenesis but also on hepatic oncogenesis [117]. Other issues which need further investigation concern the other relevant TAM receptor in liver pathology, e.g., MERTK. As a matter of fact, it still has to be resolved the particular contribution of sMERTK to hepatic inflammation and fibrogenesis. It is believed, like other soluble TAM receptors, to compete with the bound receptors and thus inhibit their function. In other chronic disease models, significantly lower expression of MERTK in monocytes has been described; conversely, sMERTK expression was increased compared to controls and related to disease severity. Moreover, the metalloproteinase ADAM 17, responsible for cleavage of MERTK to its soluble form, has been shown to be increased in patient monocytes [138]. These observations suggest that functional deficiency of TAM receptor-mediated regulation of inflammation may contribute to chronic inflammation and, translating this to liver physiopathology, be a potential driver of fibrosis progression. It would then be interesting to evaluate if sMERTK levels are altered in patients with steatohepatitis or viral infection. However, additional aspects of MERTK liver biology deserve, in our opinion, to be further analyzed. For example, future research should verify if MERTK inhibition or MERTK KO mice display reduced fibrosis, as already observed in Axl KO mice or after pharmacological Axl inhibition [90].

In conclusion, in the foreseeable future, Gas6/TAM receptors have a strong pathophysiological rationale and a potential use as serological markers of disease progression in chronic liver diseases; moreover, the system may be targeted in future liver therapies (e.g., in clinical trials testing small molecule inhibitors). If these tools were to be further optimized by improving their accuracy, while at the same time handling other possible confounding factors, their presence in a liver clinic may provide a means for making the correct diagnosis, analogous to having a much longed for crystal ball.

Disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors are grateful to Steven Louis Singer, B.S.B.A., for his attentive English revision. This study was funded by the AGING Project—Department of Excellence—DIMET, Università degli Studi del Piemonte Orientale.

References

[1] M. Pinter, M. Trauner, M. Peck-Radosavljevic, and W. Sieghart, "Cancer and liver cirrhosis: implications on prognosis and management," *ESMO Open*, vol. 1, no. 2, article e000042, 2016.

[2] C. Coulouarn, A. Corlu, D. Glaise, I. Guenon, S. S. Thorgeirsson, and B. Clement, "Hepatocyte-stellate cell cross-talk in the liver engenders a permissive inflammatory microenvironment that drives progression in hepatocellular carcinoma," *Cancer Research*, vol. 72, no. 10, pp. 2533–2542, 2012.

[3] A. Mallat and S. Lotersztajn, "Cellular mechanisms of tissue fibrosis. 5. Novel insights into liver fibrosis," *American Journal of Physiology-Cell Physiology*, vol. 305, no. 8, pp. C789–C799, 2013.

[4] R. Bataller and D. A. Brenner, "Liver fibrosis," *Journal of Clinical Investigation*, vol. 115, no. 2, pp. 209–218, 2005.

[5] S. L. Friedman, "Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver," *Physiological Reviews*, vol. 88, no. 1, pp. 125–172, 2008.

[6] S. L. Friedman, D. Sheppard, J. S. Duffield, and S. Violette, "Therapy for fibrotic diseases: nearing the starting line," *Science Translational Medicine*, vol. 5, no. 167, article 167sr1, 2013.

[7] R. Bataller and D. A. Brenner, "Hepatic stellate cells as a target for the treatment of liver fibrosis," *Seminars in Liver Disease*, vol. 21, no. 3, pp. 437–452, 2001.

[8] I. Mederacke, C. C. Hsu, J. S. Troeger et al., "Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology," *Nature Communications*, vol. 4, no. 1, 2013.

[9] E. L. Ellis and D. A. Mann, "Clinical evidence for the regression of liver fibrosis," *Journal of Hepatology*, vol. 56, no. 5, pp. 1171–1180, 2012.

[10] J. S. Troeger, I. Mederacke, G.–. Y. Gwak et al., "Deactivation of hepatic stellate cells during liver fibrosis resolution in mice," *Gastroenterology*, vol. 143, no. 4, pp. 1073–1083.e22, 2012.

[11] T. Kisseleva, M. Cong, Y. Paik et al., "Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 24, pp. 9448–9453, 2012.

[12] P. Lampertico, K. Agarwal, T. Berg et al., "EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection," *Journal of Hepatology*, vol. 67, no. 2, pp. 370–398, 2017.

[13] S. K. Sarin, M. Kumar, G. K. Lau et al., "Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update," *Hepatology International*, vol. 10, no. 1, pp. 1–98, 2016.

[14] P. Bedossa, D. Dargère, and V. Paradis, "Sampling variability of liver fibrosis in chronic hepatitis C," *Hepatology*, vol. 38, no. 6, pp. 1449–1457, 2003.

[15] Z. D. Goodman, "Grading and staging systems for inflammation and fibrosis in chronic liver diseases," *Journal of Hepatology*, vol. 47, no. 4, pp. 598–607, 2007.

[16] S. Lee and D. Y. Kim, "Non-invasive diagnosis of hepatitis B virus-related cirrhosis," *World Journal of Gastroenterology*, vol. 20, no. 2, pp. 445–459, 2014.

[17] T. Pasha, S. Gabriel, T. Therneau, E. R. Dickson, and K. D. Lindor, "Cost-effectiveness of ultrasound-guided liver biopsy," *Hepatology*, vol. 27, no. 5, pp. 1220–1226, 1998.

[18] R. Allan, K. Thoirs, and M. Phillips, "Accuracy of ultrasound to identify chronic liver disease," *World Journal of Gastroenterology*, vol. 16, no. 28, pp. 3510–3520, 2010.

[19] S. K. Venkatesh, M. Yin, N. Takahashi, J. F. Glockner, J. A. Talwalkar, and R. L. Ehman, "Non-invasive detection of liver fibrosis: MR imaging features vs. MR elastography," *Abdominal Imaging*, vol. 40, no. 4, pp. 766–775, 2015.

[20] U. Arena, F. Vizzutti, G. Corti et al., "Acute viral hepatitis increases liver stiffness values measured by transient elastography," *Hepatology*, vol. 47, no. 2, pp. 380–384, 2008.

[21] M. Pinzani, K. Rombouts, and S. Colagrande, "Fibrosis in chronic liver diseases: diagnosis and management," *Journal of Hepatology*, vol. 42, no. 1, pp. S22–S36, 2005.

[22] D. R. Dufour, J. A. Lott, F. S. Nolte, D. R. Gretch, R. S. Koff, and L. B. Seeff, "Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring," *Clinical Chemistry*, vol. 46, no. 12, pp. 2050–2068, 2000.

[23] M. Grigorescu, "Noninvasive biochemical markers of liver fibrosis," *Journal of Gastrointestinal and Liver Diseases*, vol. 15, no. 2, pp. 149–159, 2006.

[24] K. S. Nallagangula, S. K. Nagaraj, L. Venkataswamy, and M. Chandrappa, "Liver fibrosis: a compilation on the biomarkers status and their significance during disease progression," *Future Science*, vol. 4, no. 1, article FSO250, 2017.

[25] C.-T. Wai, J. K. Greenson, R. J. Fontana et al., "A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C," *Hepatology*, vol. 38, no. 2, pp. 518–526, 2003.

[26] C. Fabris, C. Smirne, P. Toniutto et al., "Assessment of liver fibrosis progression in patients with chronic hepatitis C and normal alanine aminotransferase values: the role of AST to the platelet ratio index," *Clinical Biochemistry*, vol. 39, no. 4, pp. 339–343, 2006.

[27] R. K. Sterling, E. Lissen, N. Clumeck et al., "Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection," *Hepatology*, vol. 43, no. 6, pp. 1317–1325, 2006.

[28] M. Koda, Y. Matunaga, M. Kawakami, Y. Kishimoto, T. Suou, and Y. Murawaki, "FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C," *Hepatology*, vol. 45, no. 2, pp. 297–306, 2007.

[29] M. Bonacini, G. Hadi, S. Govindarajan, and K. L. Lindsay, "Utility of a discriminant score for diagnosing advanced fibrosis or cirrhosis in patients with chronic hepatitis C virus infection," *American Journal of Gastroenterology*, vol. 92, no. 8, pp. 1302–1304, 1997.

[30] X. Forns, S. Ampurdanès, J. M. Llovet et al., "Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model," *Hepatology*, vol. 36, no. 4, pp. 986–992, 2002.

[31] P. Angulo, J. M. Hui, G. Marchesini et al., "The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD," *Hepatology*, vol. 45, no. 4, pp. 846–854, 2007.

[32] G. L.-H. Wong, "Non-invasive assessments for liver fibrosis: the crystal ball we long for," *Journal of Gastroenterology and Hepatology*, vol. 33, no. 5, pp. 1009–1015, 2018.

[33] C. G. Child and J. G. Turcotte, "Surgery and portal hypertension," *Major Problems in Clinical Surgery*, vol. 1, pp. 1–85, 1964.

[34] P. S. Kamath, R. H. Wiesner, M. Malinchoc et al., "A model to predict survival in patients with end-stage liver disease," *Hepatology*, vol. 33, no. 2, pp. 464–470, 2001.

[35] E. Rossi, L. Adams, A. Prins et al., "Validation of the FibroTest biochemical markers score in assessing liver fibrosis in hepatitis C patients," *Clinical Chemistry*, vol. 49, no. 3, pp. 450–454, 2003.

[36] P. Calès, J. Boursier, F. Oberti et al., "FibroMeters: a family of blood tests for liver fibrosis," *Gastroentérologie Clinique et Biologique*, vol. 32, no. 6, pp. 40–51, 2008.

[37] L. A. Adams, M. Bulsara, E. Rossi et al., "Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection," *Clinical Chemistry*, vol. 51, no. 10, pp. 1867–1873, 2005.

[38] W. M. Rosenberg, M. Voelker, R. Thiel et al., "Serum markers detect the presence of liver fibrosis: a cohort study," *Gastroenterology*, vol. 127, no. 6, pp. 1704–1713, 2004.

[39] J. Lambrecht, S. Verhulst, I. Mannaerts, H. Reynaert, and L. A. van Grunsven, "Prospects in non-invasive assessment of liver fibrosis: liquid biopsy as the future gold standard," *Biochimica et Biophysica Acta - Molecular Basis of Disease*, vol. 1864, no. 4, pp. 1024–1036, 2018.

[40] D. R. Robinson, Y. M. Wu, and S. F. Lin, "The protein tyrosine kinase family of the human genome," *Oncogene*, vol. 19, no. 49, pp. 5548–5557, 2000.

[41] T. Sasaki, P. G. Knyazev, N. J. Clout et al., "Structural basis for Gas6-Axl signalling," *The EMBO Journal*, vol. 25, no. 1, pp. 80–87, 2006.

[42] R. M. A. Linger, A. K. Keating, H. S. Earp, and D. K. Graham, "TAM receptor tyrosine kinases: biologic functions, signaling, and potential therapeutic targeting in human cancer," *Advances in Cancer Research*, vol. 100, pp. 35–83, 2008.

[43] A. M. Pierce and A. K. Keating, "TAM receptor tyrosine kinases: expression, disease and oncogenesis in the central nervous system," *Brain Research*, vol. 1542, pp. 206–220, 2014.

[44] M. Wium, J. D. Paccez, and L. F. Zerbini, "The dual role of TAM receptors in autoimmune diseases and cancer: an overview," *Cells*, vol. 7, no. 10, p. 166, 2018.

[45] C. Lai and G. Lemke, "An extended family of protein-tyrosine kinase genes differentially expressed in the vertebrate nervous system," *Neuron*, vol. 6, no. 5, pp. 691–704, 1991.

[46] T. N. Stitt, G. Conn, M. Goret et al., "The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases," *Cell*, vol. 80, no. 4, pp. 661–670, 1995.

[47] B. C. Varnum, C. Young, G. Elliott et al., "Axl receptor tyrosine kinase stimulated by the vitamin K-dependent protein encoded by growth-arrest-specific gene 6," *Nature*, vol. 373, no. 6515, pp. 623–626, 1995.

[48] K. Nagata, K. Ohashi, T. Nakano et al., "Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases," *Journal of Biological Chemistry*, vol. 271, no. 47, pp. 30022–30027, 1996.

[49] G. Lemke, "Biology of the TAM receptors," *Cold Spring Harbor Perspectives in Biology*, vol. 5, no. 11, article a009076, 2013.

[50] J. Zhang and X. Qi, "The role of the TAM family of receptor tyrosine kinases in neural development and disorders," *Neuropsychiatry*, vol. 8, no. 2, pp. 428–437, 2018.

[51] V. Davra, S. G. Kimani, D. Calianese, and R. B. Birge, "Ligand activation of TAM family receptors-implications for tumor biology and therapeutic response," *Cancers*, vol. 8, no. 12, p. 107, 2016.

[52] G. Manfioletti, C. Brancolini, G. Avanzi, and C. Schneider, "The protein encoded by a growth arrest-specific gene (gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade," *Molecular and Cellular Biology*, vol. 13, no. 8, pp. 4976–4985, 1993.

[53] T. Nakano, K. Higashino, N. Kikuchi et al., "Vascular smooth muscle cell-derived, Gla-containing growth-potentiating factor for Ca^{2+}-mobilizing growth factors," *Journal of Biological Chemistry*, vol. 270, no. 11, pp. 5702–5705, 1995.

[54] G. C. Avanzi, M. Gallicchio, G. Cavalloni et al., "GAS6, the ligand of Axl and Rse receptors, is expressed in hematopoietic tissue but lacks mitogenic activity," *Experimental Hematology*, vol. 25, no. 12, pp. 1219–1226, 1997.

[55] J. M. E. M. Cosemans, R. van Kruchten, S. Olieslagers et al., "Potentiating role of Gas6 and Tyro3, Axl and Mer (TAM) receptors in human and murine platelet activation and thrombus stabilization," *Journal of Thrombosis and Haemostasis*, vol. 8, no. 8, pp. 1797–1808, 2010.

[56] L. Bellido-Martin and P. G. de Frutos, "Vitamin K-dependent actions of Gas6," *Vitamins and Hormones*, vol. 78, pp. 185–209, 2008.

[57] L. Fernández-Fernández, L. Bellido-Martin, and P. Garcia de Frutos, "Growth arrest-specific gene 6 (GAS6). An outline of its role in haemostasis and inflammation," *Thrombosis and Haemostasis*, vol. 100, no. 10, pp. 604–610, 2008.

[58] F. J. Walker, "Regulation of activated protein C by a new protein. A possible function for bovine protein S," *Journal of Biological Chemistry*, vol. 255, no. 12, pp. 5521–5524, 1980.

[59] M. J. Heeb, R. M. Mesters, G. Tans, J. Rosing, and J. H. Griffin, "Binding of protein S to factor Va associated with inhibition of prothrombinase that is independent of activated protein C," *Journal of Biological Chemistry*, vol. 268, no. 4, pp. 2872–2877, 1993.

[60] M. J. Heeb, J. Rosing, H. M. Bakker, J. A. Fernandez, G. Tans, and J. H. Griffin, "Protein S binds to and inhibits factor Xa," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 7, pp. 2728–2732, 1994.

[61] M. Bellan, L. M. Castello, and M. Pirisi, "Candidate biomarkers of liver fibrosis: a concise, pathophysiology-oriented review," *Journal of Clinical and Translational Hepatology*, vol. 6, no. 3, pp. 1–9, 2018.

[62] J. H. M. van der Meer, T. van der Poll, and C. van 't Veer, "TAM receptors, Gas6, and protein S: roles in inflammation and hemostasis," *Blood*, vol. 123, no. 16, pp. 2460–2469, 2014.

[63] P. P. Sainaghi, F. Alciato, S. Carnieletto et al., "Gas6 evaluation in patients with acute dyspnea due to suspected pulmonary embolism," *Respiratory Medicine*, vol. 103, no. 4, pp. 589–594, 2009.

[64] S. Goruppi, E. Ruaro, and C. Schneider, "Gas6, the ligand of Axl tyrosine kinase receptor, has mitogenic and survival activities for serum starved NIH3T3 fibroblasts," *Oncogene*, vol. 12, no. 3, pp. 471–480, 1996.

[65] H. A. Anderson, C. A. Maylock, J. A. Williams, C. P. Paweletz, H. Shu, and E. Shacter, "Serum-derived protein S binds to phosphatidylserine and stimulates the phagocytosis of apoptotic cells," *Nature Immunology*, vol. 4, no. 1, pp. 87–91, 2003.

[66] T. D. Camenisch, B. H. Koller, H. S. Earp, and G. K. Matsushima, "A novel receptor tyrosine kinase, Mer, inhibits TNF-α production and lipopolysaccharide-induced endotoxic shock," *The Journal of Immunology*, vol. 162, no. 6, pp. 3498–3503, 1999.

[67] C. Gjerdrum, C. Tiron, T. Hoiby et al., "Axl is an essential epithelial-to-mesenchymal transition-induced regulator of breast cancer metastasis and patient survival," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 3, pp. 1124–1129, 2010.

[68] Q. Lu and G. Lemke, "Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family," *Science*, vol. 293, no. 5528, pp. 306–311, 2001.

[69] C. V. Rothlin, E. A. Carrera-Silva, L. Bosurgi, and S. Ghosh, "TAM receptor signaling in immune homeostasis," *Annual Review of Immunology*, vol. 33, no. 1, pp. 355–391, 2015.

[70] T. Deng, Y. Zhang, Q. Chen, K. Yan, and D. Han, "Toll-like receptor-mediated inhibition of Gas6 and ProS expression facilitates inflammatory cytokine production in mouse macrophages," *Immunology*, vol. 135, no. 1, pp. 40–50, 2012.

[71] R. S. Scott, E. J. McMahon, S. M. Pop et al., "Phagocytosis and clearance of apoptotic cells is mediated by MER," *Nature*, vol. 411, no. 6834, pp. 207–211, 2001.

[72] M. Kurowska-Stolarska, S. Alivernini, E. G. Melchor et al., "MicroRNA-34a dependent regulation of AXL controls the activation of dendritic cells in inflammatory arthritis," *Nature Communications*, vol. 8, no. 1, article 15877, 2017.

[73] M. Tjwa, L. Bellido-Martin, Y. Lin et al., "Gas6 promotes inflammation by enhancing interactions between endothelial cells, platelets, and leukocytes," *Blood*, vol. 111, no. 8, pp. 4096–4105, 2008.

[74] C. V. Rothlin, S. Ghosh, E. I. Zuniga, M. B. A. Oldstone, and G. Lemke, "TAM receptors are pleiotropic inhibitors of the innate immune response," *Cell*, vol. 131, no. 6, pp. 1124–1136, 2007.

[75] N. Qi, P. Liu, Y. Zhang, H. Wu, Y. Chen, and D. Han, "Development of a spontaneous liver disease resembling autoimmune hepatitis in mice lacking tyro3, axl and mer receptor tyrosine kinases," *PLoS One*, vol. 8, no. 6, article e66604, 2013.

[76] L. Xu, F. Hu, H. Zhu et al., "Soluble TAM receptor tyrosine kinases in rheumatoid arthritis: correlation with disease activity and bone destruction," *Clinical and Experimental Immunology*, vol. 192, no. 1, pp. 95–103, 2018.

[77] J. G. Weinger, K. M. Omari, K. Marsden, C. S. Raine, and B. Shafit-Zagardo, "Up-regulation of soluble Axl and Mer receptor tyrosine kinases negatively correlates with Gas6 in established multiple sclerosis lesions," *American Journal of Pathology*, vol. 175, no. 1, pp. 283–293, 2009.

[78] P. P. Sainaghi, L. Collimedaglia, F. Alciato et al., "Growth arrest specific gene 6 protein concentration in cerebrospinal fluid correlates with relapse severity in multiple sclerosis," *Mediators of Inflammation*, vol. 2013, Article ID 406483, 7 pages, 2013.

[79] M. Bellan, M. Pirisi, and P. P. Sainaghi, "The Gas6/TAM system and multiple sclerosis," *International Journal of Molecular Sciences*, vol. 17, no. 11, article 1807, 2016.

[80] H. Zhu, X. Sun, L. Zhu et al., "The expression and clinical significance of different forms of Mer receptor tyrosine kinase in systemic lupus erythematosus," *Journal of Immunology Research*, vol. 2014, Article ID 431896, 12 pages, 2014.

[81] C. H. Chen, H. C. Chen, C. C. Chang et al., "Growth arrest-specific 6 protein in patients with Sjögren syndrome: determination of the plasma level and expression in the labial salivary gland," *PLoS One*, vol. 10, no. 10, article e0139955, 2015.

[82] P. P. Sainaghi, M. Bellan, F. Lombino et al., "Growth arrest specific 6 concentration is increased in the cerebrospinal fluid of patients with Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 55, no. 1, pp. 59–65, 2017.

[83] L. Ballantine, A. Midgley, D. Harris, E. Richards, S. Burgess, and M. W. Beresford, "Increased soluble phagocytic receptors sMer, sTyro3 and sAxl and reduced phagocytosis in juvenile-onset systemic lupus erythematosus," *Pediatric Rheumatology*, vol. 13, no. 1, 2015.

[84] J. P. O'Bryan, Y. W. Fridell, R. Koski, B. Varnum, and E. T. Liu, "The transforming receptor tyrosine kinase, Axl, is post-translationally regulated by proteolytic cleavage," *Journal of Biological Chemistry*, vol. 270, no. 2, pp. 551–557, 1995.

[85] J. J. Orme, Y. Du, K. Vanarsa et al., "Heightened cleavage of Axl receptor tyrosine kinase by ADAM metalloproteases may contribute to disease pathogenesis in SLE," *Clinical Immunology*, vol. 169, pp. 58–68, 2016.

[86] F. Lafdil, M. N. Chobert, D. Couchie et al., "Induction of Gas6 protein in CCl4-induced rat liver injury and anti-apoptotic effect on hepatic stellate cells," *Hepatology*, vol. 44, no. 1, pp. 228–239, 2006.

[87] D. Couchie, F. Lafdil, N. Martin–Garcia, Y. Laperche, E. S. Zafrani, and P. Mavier, "Expression and role of Gas6 protein and of its receptor Axl in hepatic regeneration from oval cells in the rat," *Gastroenterology*, vol. 129, no. 5, pp. 1633–1642, 2005.

[88] F. Lafdil, M. N. Chobert, V. Deveaux et al., "Growth arrest-specific protein 6 deficiency impairs liver tissue repair after acute toxic hepatitis in mice," *Journal of Hepatology*, vol. 51, no. 1, pp. 55–66, 2009.

[89] L. Llacuna, C. Bárcena, L. Bellido-Martín et al., "Growth arrest-specific protein 6 is hepatoprotective against murine ischemia/reperfusion injury," *Hepatology*, vol. 52, no. 4, pp. 1371–1379, 2010.

[90] C. Bárcena, M. Stefanovic, A. Tutusaus et al., "Gas6/Axl pathway is activated in chronic liver disease and its targeting reduces fibrosis via hepatic stellate cell inactivation," *Journal of Hepatology*, vol. 63, no. 3, pp. 670–678, 2015.

[91] M. M. Richardson, J. R. Jonsson, E. E. Powell et al., "Progressive fibrosis in nonalcoholic steatohepatitis: association with altered regeneration and a ductular reaction," *Gastroenterology*, vol. 133, no. 1, pp. 80–90, 2007.

[92] T. Roskams, S. Q. Yang, A. Koteish et al., "Oxidative stress and oval cell accumulation in mice and humans with alcoholic and nonalcoholic fatty liver disease," *The American Journal of Pathology*, vol. 163, no. 4, pp. 1301–1311, 2003.

[93] E. Fassio, E. Álvarez, N. Domínguez, G. Landeira, and C. Longo, "Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies," *Hepatology*, vol. 40, no. 4, pp. 820–826, 2004.

[94] C. P. Day, "From fat to inflammation," *Gastroenterology*, vol. 130, no. 1, pp. 207–210, 2006.

[95] A. D. Clouston, E. E. Powell, M. J. Walsh, M. M. Richardson, A. J. Demetris, and J. R. Jonsson, "Fibrosis correlates with a ductular reaction in hepatitis C: roles of impaired replication, progenitor cells and steatosis," *Hepatology*, vol. 41, no. 4, pp. 809–818, 2005.

[96] K. N. Lowes, B. A. Brennan, G. C. Yeoh, and J. K. Olynyk, "Oval cell numbers in human chronic liver diseases are directly related to disease severity," *The American Journal of Pathology*, vol. 154, no. 2, pp. 537–541, 1999.

[97] A. Fourcot, D. Couchie, M. N. Chobert et al., "Gas6 deficiency prevents liver inflammation, steatohepatitis, and fibrosis in mice," *American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 300, no. 6, pp. G1043–G1053, 2011.

[98] M. Marí, A. Tutusaus, P. García de Frutos, and A. Morales, "Genetic and clinical data reinforce the role of GAS6 and TAM receptors in liver fibrosis," *Journal of Hepatology*, vol. 64, no. 4, pp. 983-984, 2016.

[99] A. Tutusaus, E. D. Gregorio, B. Cucarull et al., "AXL increase in NASH patients and anti-fibrotic efficacy of AXL inhibition in experimental NASH," *Journal of Hepatology*, vol. 68, article S394, Supplement 1, 2018.

[100] G. Zizzo, B. A. Hilliard, M. Monestier, and P. L. Cohen, "Efficient clearance of early apoptotic cells by human macrophages requires M2c polarization and MerTK induction," *The Journal of Immunology*, vol. 189, no. 7, pp. 3508–3520, 2012.

[101] S. De Minicis, E. Seki, H. Uchinami et al., "Gene expression profiles during hepatic stellate cell activation in culture and in vivo," *Gastroenterology*, vol. 132, no. 5, pp. 1937–1946, 2007.

[102] G. Zizzo and P. L. Cohen, "The PPAR-γ antagonist GW9662 elicits differentiation of M2c-like cells and upregulation of the MerTK/Gas6 axis: a key role for PPAR-γ in human

macrophage polarization," *Journal of Inflammation*, vol. 12, no. 1, 2015.

[103] E. Patin, Z. Kutalik, J. Guergnon et al., "Genome-wide association study identifies variants associated with progression of liver fibrosis from HCV infection," *Gastroenterology*, vol. 143, no. 5, pp. 1244–1252.e12, 2012.

[104] S. Petta, L. Valenti, F. Marra et al., "MERTK rs4374383 polymorphism affects the severity of fibrosis in non-alcoholic fatty liver disease," *Journal of Hepatology*, vol. 64, no. 3, pp. 682–690, 2016.

[105] S. Petta and F. Marra, "Reply to 'Genetic and clinical data reinforce the role of GAS6 and TAM receptors in liver fibrosis'," *Journal of Hepatology*, vol. 64, no. 4, pp. 984-985, 2016.

[106] M. Dengler, K. Staufer, H. Huber et al., "Soluble Axl is an accurate biomarker of cirrhosis and hepatocellular carcinoma development: results from a large scale multicenter analysis," *Oncotarget*, vol. 8, no. 28, pp. 46234–46248, 2017.

[107] K. Matsuura and Y. Tanaka, "Host genetic variants influencing the clinical course of hepatitis C virus infection," *Journal of Medical Virology*, vol. 88, no. 2, pp. 185–195, 2016.

[108] S. Rüeger, P. Y. Bochud, J. F. Dufour et al., "Impact of common risk factors of fibrosis progression in chronic hepatitis C," *Gut*, vol. 64, no. 10, pp. 1605–1615, 2015.

[109] M. A. Jiménez-Sousa, A. Z. Gómez-Moreno, D. Pineda-Tenor et al., "The myeloid-epithelial-reproductive tyrosine kinase (MERTK) rs4374383 polymorphism predicts progression of liver fibrosis in hepatitis C virus-infected patients: a longitudinal study," *Journal of Clinical Medicine*, vol. 7, no. 12, p. 473, 2018.

[110] M. Cavalli, G. Pan, H. Nord, E. Wallén Arzt, O. Wallerman, and C. Wadelius, "Genetic prevention of hepatitis C virus-induced liver fibrosis by allele-specific downregulation of MERTK," *Hepatology Research*, vol. 47, no. 8, pp. 826–830, 2017.

[111] T. Tamura, M. Ishihara, M. S. Lamphier et al., "An IRF-1-dependent pathway of DNA damage-induced apoptosis in mitogen-activated T lymphocytes," *Nature*, vol. 376, no. 6541, pp. 596–599, 1995.

[112] M. Bellan, G. Pogliani, C. Marconi et al., "Gas6 as a putative noninvasive biomarker of hepatic fibrosis," *Biomarkers in Medicine*, vol. 10, no. 12, pp. 1241–1249, 2016.

[113] S. Zhang, X. S. Xu, J. X. Yang, J. H. Guo, T. F. Chao, and Y. Tong, "The prognostic role of Gas6/Axl axis in solid malignancies: a meta-analysis and literature review," *OncoTargets and Therapy*, vol. 11, pp. 509–519, 2018.

[114] H. J. Lee, Y. M. Jeng, Y. L. Chen, L. Chung, and R. H. Yuan, "Gas6/Axl pathway promotes tumor invasion through the transcriptional activation of Slug in hepatocellular carcinoma," *Carcinogenesis*, vol. 35, no. 4, pp. 769–775, 2014.

[115] E. B. Rankin and A. J. Giaccia, "The receptor tyrosine kinase AXL in cancer progression," *Cancers*, vol. 8, no. 11, p. 103, 2016.

[116] P. Reichl, M. Dengler, F. van Zijl et al., "Axl activates autocrine transforming growth factor-β signaling in hepatocellular carcinoma," *Hepatology*, vol. 61, no. 3, pp. 930–941, 2015.

[117] E. Holstein, M. Binder, and W. Mikulits, "Dynamics of Axl receptor shedding in hepatocellular carcinoma and its implication for theranostics," *International Journal of Molecular Sciences*, vol. 19, no. 12, article 4111, 2018.

[118] D. K. Graham, D. DeRyckere, K. D. Davies, and H. S. Earp, "The TAM family: phosphatidylserine sensing receptor tyrosine kinases gone awry in cancer," *Nature Reviews Cancer*, vol. 14, no. 12, pp. 769–785, 2014.

[119] M. Bellan, P. P. Sainaghi, M. T. Minh et al., "Gas6 as a predictor of esophageal varices in patients affected by hepatitis C virus related-chronic liver disease," *Biomarkers in Medicine*, vol. 12, no. 1, pp. 27–34, 2018.

[120] R. de Franchis and on behalf of the Baveno VI Faculty, "Expanding consensus in portal hypertension: report of the Baveno VI consensus workshop: stratifying risk and individualizing care for portal hypertension," *Journal of Hepatology*, vol. 63, no. 3, pp. 743–752, 2015.

[121] Z. Li and S. A. Weinman, "Regulation of hepatic inflammation via macrophage cell death," *Seminars in Liver Disease*, vol. 38, no. 4, pp. 340–350, 2018.

[122] R. Cabezón, E. A. Carrera-Silva, G. Flórez-Grau et al., "MERTK as negative regulator of human T cell activation," *Journal of Leukocyte Biology*, vol. 97, no. 4, pp. 751–760, 2015.

[123] C. Bernsmeier, A. Singanayagam, V. C. Patel, J. Wendon, and C. G. Antoniades, "Immunotherapy in the treatment and prevention of infection in acute-on-chronic liver failure," *Immunotherapy*, vol. 7, no. 6, pp. 641–654, 2015.

[124] C. Bernsmeier, O. T. Pop, A. Singanayagam et al., "Patients with acute-on-chronic liver failure have increased numbers of regulatory immune cells expressing the receptor tyrosine kinase MERTK," *Gastroenterology*, vol. 148, no. 3, pp. 603–615.e14, 2015.

[125] S. K. Mukherjee, A. Wilhelm, and C. G. Antoniades, "TAM receptor tyrosine kinase function and the immunopathology of liver disease," *American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 310, no. 11, pp. G899–G905, 2016.

[126] A. Singanayagam, D. Erminelli, E. Triantafyllou et al., "THU-052-Immunometabolic profiling of ascites from patients with acute-on-chronic liver failure reveals increased MerTK+ immunosuppressive myeloid cells and cell death markers with preferential lipid metabolism compared to cirrhosis without organ failure," *Journal of Hepatology*, vol. 70, no. 1, article e183, 2019.

[127] S. K. Mukherjee, O. T. Pop, E. Triantafyllou et al., "MER tyrosine kinase positive tumour associated macrophages are a novel therapeutic target in hepatocellular carcinoma," *Journal of Hepatology*, vol. 64, no. 2, pp. S574–S575, 2016.

[128] C. Ekman, J. Stenhoff, and B. Dahlbäck, "Gas6 is complexed to the soluble tyrosine kinase receptor Axl in human blood," *Journal of Thrombosis and Haemostasis*, vol. 8, no. 4, pp. 838–844, 2010.

[129] M. A. Miller, R. J. Sullivan, and D. A. Lauffenburger, "Molecular pathways: receptor ectodomain shedding in treatment, resistance, and monitoring of cancer," *Clinical Cancer Research*, vol. 23, no. 3, pp. 623–629, 2017.

[130] K. Staufer, M. Dengler, H. Huber et al., "The non-invasive serum biomarker soluble Axl accurately detects advanced liver fibrosis and cirrhosis," *Cell Death & Disease*, vol. 8, no. 10, article e3135, 2017.

[131] J. Wang, N. Willumsen, Q. Zheng, Y. Xue, M. A. Karsdal, and A. C. Bay-Jensen, "Bringing cancer serological diagnosis to a new level: focusing on HER2, protein ectodomain shedding and neoepitope technology," *Future Oncology*, vol. 9, no. 1, pp. 35–44, 2013.

[132] S. J. Holland, A. Pan, C. Franci et al., "R428, a selective small molecule inhibitor of Axl kinase, blocks tumor spread and prolongs survival in models of metastatic breast cancer," *Cancer Research*, vol. 70, no. 4, pp. 1544–1554, 2010.

[133] K. F. Ludwig, W. du, N. B. Sorrelle et al., "Small-molecule inhibition of Axl targets tumor immune suppression and enhances chemotherapy in pancreatic cancer," *Cancer Research*, vol. 78, no. 1, pp. 246–255, 2018.

[134] M. D. Binder and T. J. Kilpatrick, "TAM receptor signalling and demyelination," *Neurosignals*, vol. 17, no. 4, pp. 277–287, 2009.

[135] A. M. Grabiec, A. Goenka, M. E. Fife, T. Fujimori, and T. Hussell, "Axl and MerTK receptor tyrosine kinases maintain human macrophage efferocytic capacity in the presence of viral triggers," *European Journal of Immunology*, vol. 48, no. 5, pp. 855–860, 2018.

[136] C. Y. Cho, J. S. Huang, S. G. Shiah et al., "Negative feedback regulation of AXL by miR-34a modulates apoptosis in lung cancer cells," *RNA*, vol. 22, no. 2, pp. 303–315, 2016.

[137] P. Zhang, Q. Dong, H. Zhu, S. Li, L. Shi, and X. Chen, "Long non-coding antisense RNA GAS6-AS1 supports gastric cancer progression via increasing GAS6 expression," *Gene*, vol. 696, pp. 1–9, 2019.

[138] I. J. Lee, B. A. Hilliard, M. Ulas et al., "Monocyte and plasma expression of TAM ligand and receptor in renal failure: Links to unregulated immunity and chronic inflammation," *Clinical Immunology*, vol. 158, no. 2, pp. 231–241, 2015.

Hindawi
Disease Markers
Volume 2019, Article ID 6156493, 12 pages
https://doi.org/10.1155/2019/6156493

Review Article

Gas6/TAM Axis in Sepsis: Time to Consider Its Potential Role as a Therapeutic Target

Livia Salmi,[1] **Francesco Gavelli**,[1] **Filippo Patrucco**,[1] **Marina Caputo**,[1] **Gian Carlo Avanzi**,[2] **and Luigi Mario Castello**[1]

[1]*Department of Translational Medicine, University of Piemonte Orientale, Via Solaroli 17, 28100 Novara, Italy*
[2]*University of Piemonte Orientale, Via Duomo 6, 13100 Vercelli, Italy*

Correspondence should be addressed to Livia Salmi; livia.salmi@uniupo.it

Received 29 March 2019; Accepted 30 July 2019; Published 14 August 2019

Academic Editor: Lance A. Liotta

Tyrosine kinase receptors are transmembrane proteins involved in cell signaling and interaction. Among them, the TAM family (composed by Tyro 3, Axl, and Mer) represents a peculiar subgroup with an important role in many physiological and pathological conditions. Despite different mechanisms of activation (e.g., protein S and Galactin-3), TAM action is tightly related to their common ligand, a protein named growth arrest-specific 6 (Gas6). Since the expression of both TAM and Gas6 is widely distributed among tissues, any alteration of one of these components can lead to different pathological conditions. Moreover, as they are indispensable for homeostasis maintenance, in recent years a growing interest has emerged regarding their role in the regulation of the inflammatory process. Due to this involvement, many authors have demonstrated the pivotal role of the Gas6/TAM axis in both sepsis and the sepsis-related inflammatory responses. In this narrative review, we highlight the current knowledge as well as the last discoveries on TAM and Gas6 implication in different clinical conditions, notably in sepsis and septic shock. Lastly, we underline not only the feasible use of Gas6 as a diagnostic and prognostic biomarker in certain systemic acute conditions but also its potential therapeutic role in these life-threatening diseases.

1. Brief "TAM" Story

Tyrosine kinase receptors (RTKs) are transmembrane proteins often implicated in cell-to-cell communication. Until now, 58 RTKs have been identified [1]; these receptors pilot, through phosphorylation, an enormous amount of essential signaling pathways, regulating proliferation, survival, and apoptosis.

Among RTKs, Tyro3, Axl, and Mer (gene name *Mertk*) share structural similarity (notably two Ig-like domains, two fibronectin type III domains, a hydrophobic transmembrane domain, and a tyrosine kinase domain) and they are grouped in the so-called "TAM family" (Figure 1). Despite their deep resemblance, TAM receptors are expressed by different cell types and tissues (Table 1): Tyro3 is generally localized in the nervous system, whereas Mer and Axl have been found in different tissues and they are frequently coexpressed by the same cells [2]. This coexpression can be either equivalent in some cells, such as Kupffer cells in the liver and red pulp macrophages in the spleen, or unbalanced in others, such as for $CD68^+$ tingible macrophages, which are primarily Mer^+, and $CD11c^+$ white pulp dendritic cells (DCs), which are mostly Axl^+ [3].

TAM were discovered and cloned by several groups in the 90s [2]. In the first years from their discovery, their role in the maintenance of homeostatic balance through the regulation of the phagocytosis of apoptotic bodies (efferocytosis) was demonstrated [21]. Gradually, their role in the innate inflammatory response and in the regulation of cell proliferation and apoptosis was elucidated, leading to growing interest. In fact, a deficiency in TAM expression is related to autoimmunity diseases [2] and, oppositely, their

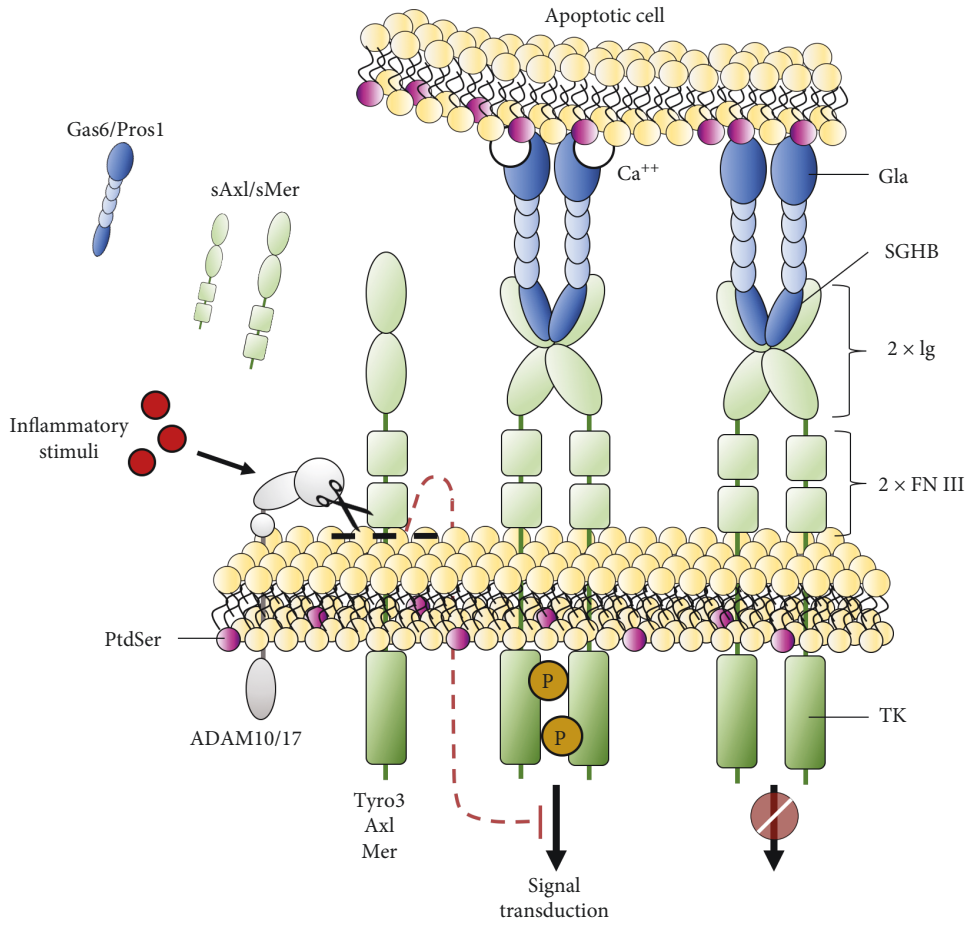


Figure 1: TAM structures and posttranslational regulation. Schematic representation of TAM receptors and their ligands. All TAM receptors share structural domains, i.e., the tyrosine kinase (TK) domain, the transmembrane domain, two fibronectin type III domains (FN III), and two Ig-like domains (Ig) from the C-terminal to the N-terminal (right). The TAM ligands Gas6 and Pros1 share a sex hormone-binding globulin (SHBG) domain and a gamma-carboxyglutamic acid-rich (Gla) domain (right). The Gla domain binds phosphatidylserine (PtdSer) exposed in the outer/external side of the apoptotic cell plasma-membrane, while the SHBG domain interacts with TAM receptor Ig-like domains on the surface of TAM-expressing cells, thus acting as "bridge" proteins (right). The binding itself does not result in receptor activation that occurs through receptor transphosphorylation and in a Ca^{++}-dependent fashion (center). For Mer and Axl, the signal transduction is shut down by proteolytic cleavage of the receptor ectodomain (shedding), which is mediated by the transmembrane disintegrin and metalloproteinase (ADAM) 17 and/or ADAM10. Shedding can be induced by inflammatory stimuli (e.g., lipopolysaccharide) leading to the extracellular domain release of the receptor and generating a soluble Axl (sAxl) and soluble Mer (sMer) form able to interact with and sequester the ligands Gas6 and Pros1 (left).

overexpression or aberrant activation (i.e., gain-of-function mutations) is associated with the development and progression of cancer [22].

In this context, the complex network of TAM functions has been clarified in recent years, as it seems more linked to the environmental context, or "*milieu*," rather than to the expressing cell/tissue, such as neurodegenerative diseases [23], autoimmune diseases, and cancer [24]. TAM activation, which occurs through tyrosine cross-phosphorylation, is normally mediated by the binding with their ligands, growth arrest-specific 6 (Gas6) and protein S (Pros1). Gas6 and Pros1 share in the C-terminal portion the "sex hormone-binding globulin (SHBG) domain," which binds the TAM Ig-like domains. The N-terminal portion includes the γ-carboxylate "gamma-carboxyglutamic acid-rich (Gla) domain," responsible for binding the phospholipid phosphatidylserine (PtdSer) in a Ca^{++}-dependent reaction (Figure 1).

Gas6 is able to bind and activate all TAM receptors, while Pros1 can only bind Mer and Tyro3, without interacting with Axl. In 2014, Lew et al. published a detailed paper showing that Gas6 is capable of binding and activating all TAM, but the most powerful effect was observed following Axl activation. Moreover, both murine and human recombinant Pros1 can bind and activate murine Tyro3 and Mer (but not Axl) in vitro. Lastly, they showed that the PtdSer-binding Gla domain present on Gas6, PtdSer itself, and Ca^{++} are all essential to achieve a full receptor activation, but none of them is involved in receptor binding [25]. Interestingly, Gas6/Pros1-TAM receptor binding is not able to determine the receptor activation per se [25]; so all the conditions described above need to be fulfilled in order to trigger the numerous signal transduction pathways, such as phosphoinositide 3-kinase (PI3K)/Akt, mitogen-activated protein kinase (MAP kinase), nuclear factor-light-

Table 1: The widespread expression of the TAM receptor.

	Tyro3	Axl	Mer
Brain	(i) Microglial cells [4] (ii) Astrocytes [4]	(i) Microglial cells [4] (ii) Astrocytes [4]	(i) Microglial cells [4] (ii) Astrocytes [4]
Heart			(i) Cardiomyocytes [5]
Breast			(i) Mammary epithelial cells [6]
Lung		(i) Macrophages $CD11bl^{ow}CD11c^{high}$ [7]	(i) *Alveolar macrophages* [8]
Liver	(i) *Kupffer cells* [9]	(i) *Kupffer cells* [9] (ii) *HSCs (q/a)* [9] (iii) *LSECs* [9] (iv) *Hepatocytes* [9]	(i) *Kupffer cells* [9] (ii) *HSC (a)* [9] (iii) *LSEC* [9]
Spleen		(i) DCs $CD11c^{high}$ [10]	(i) Macrophages $F4/80^{high}$, $B220^{-}$, $CD11c^{+}$ and $MHCII^{+}$ red pulp [11] (ii) Macrophages $F4/80^{+}CD68^{+}$ (tingible body) [11]
Kidney	(i) *Podocytes* [12]		(i) *Podocytes* [12]
Testis	(i) Sertoli cells [13]	(i) Sertoli cells [13]	(i) Sertoli $cells^{low}$ [13] (ii) Leydig cells [13]
Peritoneum		(i) Macrophages [14]	(i) Macrophages [14]
Blood/BM derived	(i) *Platelets* [15] (ii) $Monocytes^{low}$ (iii) Monocyte-derived $macrophages^{low}$ [16] (iv) NK cells [17] (v) DC $CD11c^{+}$ [18]	(i) *Platelets* [15] (ii) $Monocytes^{high}$ (iii) Monocyte-derived $macrophages^{low}$ [16] (iv) NK cells [17] (v) DC $CD11c^{+}$ [18]	(i) *Platelets* [15] (ii) $Monocytes^{low}$ (iii) Monocyte-derived $macrophages^{high}$ [16] (iv) NK cells [17] (v) DC $CD11c^{+}$ [18] (vi) DCs $CD11b^{+}$ and $B220^{+}$ [19] (vii) NKT cells [20]

Italic shows TAM expression located in human cells; all the others were found in murine cells. BM derived: bone marrow derived; HSCs: hepatic stellate cells; LSECs: liver sinusoidal endothelial cells; DCs: dendritic cells; NK: natural killer; NKT: natural killer T.

chain-enhancer of activated B cells (NF-κB), signal transducer and activator of transcription protein (STAT), phospholipase C (PLC), growth factor receptor-bound protein 2 (Grb2), Raf-1, extracellular-signal-regulated kinase (ERK), and others [26–28]. Rothlin et al. demonstrated that TAM signaling triggers the expression of the suppressor of cytokine signaling proteins, SOCS1 and SOCS3. In fact, in dendritic cells from mice knockout for all three TAM receptors (TAM triple knockout; TAM TKO), the induction of SOCS1 was substantially impaired [29, 30].

Until now, different mutations on TAM receptors have been linked to defined genetic diseases: primarily many MerTK mutations were associated with retinal degenerations [31]. In particular, TAM receptors differ from other RTKs since we know from mouse models that TAM genes can be ablated without any major effect on embryonic development [32]. As a consequence, TAM TKO mice are indistinguishable from their wild-type (WT) counterparts and this aspect appears peculiar because usually the absence of expression of other RTKs leads to severe embryonic development impairment, with intrauterine death [33]. Although during the first three life-weeks no macroscopic difference can be observed between TAM TKO and TAM WT mice, after this period TAM TKO mice develop several degenerative phenotypes. Male TAM TKO mice are infertile in adult life, a condition that is related to impaired sexual development and spermatogenesis. Indeed, Sertoli cells express all three TAM receptors as well as both ligands, Gas6 and Pros1, which allow them to manage, in an autocrine fashion, the phagocytosis of apoptotic germ cells (around 10^8/day in human male) [34]. The absence of TAM receptors results in incorrect efferocytosis and accumulation of apoptotic cells, damaging sexual organs. Still, both in adult TAM TKO and single $Mer^{-/-}$ mice, the impairment of phagocytosis causes the accumulation of apoptotic debris in the retina, causing a nearly complete absence of photoreceptors [35, 36] and blindness [32].

Since one of the main functions of TAM receptors is to modulate the immune homeostasis [2, 37], it is reasonable to consider their implication in autoimmune phenotypes. Qi et al. have demonstrated that TAM TKO mice develop a spontaneous liver disease which resembles autoimmune hepatitis. These mice exhibited chronic hepatitis, with progressive inflammatory cell infiltration and elevated cytokine levels in the liver [38]. Moreover, TAM TKO mice displayed splenomegaly, lymphadenopathy, and lymphocyte infiltration in nearly all tissues around 4-6 weeks after birth [37].

Also coagulation was impaired with both thrombosis and hemorrhages, especially in the brain, as well as skin lesions and hemophilic-like phenotypes with swollen joints [37].

Additionally, these mice generate high levels of circulating autoantibodies directed against dsDNA, collagens, and phospholipids, such as cardiolipin, PtdSer, phosphatidylethanolamine, and phosphatidylinositol [37].

Thus, we can summarize that TAM TKO mice have an autoimmune phenotype with features comparable to systemic lupus erythematosus (SLE), psoriasis, and rheumatoid arthritis [2, 37]. Antigen-presenting cells (APCs) from TAM TKO mice have a dysregulated activity in response to inflammatory stimuli, demonstrating a reduced tolerogenic behavior with the hyperproduction of type 1 interferons, interleukin (IL) 12, and overexpression of MHC class II and CD86 [29, 37]. This expression pattern is consistent with the splenomegaly and lymphadenopathy observed in adult TAM TKO mice.

Despite their structural homology, following activation TAM receptor signaling is shut down in different ways: the signal desensitization that occurs through the shedding of the ectodomain by proteolytic cleavage was reported for Mer and Axl [39, 40]. In spite of soluble Tyro3 increasing levels in the bloodstream in different chronic diseases [41, 42], this signal desensitization mechanism has not been described for Tyro3 yet (Figure 1). Between the TAM-common-fibronectin type III domains and the transmembrane domain, the proline residue Pro^{485} present in the Mer sequence makes it susceptible to cleavage by the metalloproteinase ADAM17, a disintegrin and metalloproteinase domain 17 [39], also known as tumor necrosis factor-alpha converting enzyme (TACE). Although the examination of the cleavage site sequence of several substrates shed by ADAM17 indicates that the distance between ADAM17 and its target is more important than the specific sequence in ectodomain shedding, the site direct mutagenesis of the Pro^{485} cleavage site results in Mer resistance to proteolysis [39].

The activation of pattern-recognition receptors (PRRs) with lipopolysaccharide (LPS) or polycytidylic acid (Poly:C) in macrophages results in the induction of cleavage of the Mer extracellular domain. Furthermore, LPS- and polyinosinic:polycytidylic acid- (PolyI:C-) induced Mer shedding is dependent on ADAM17, as it is abrogated in ADAM17 gene knockdown macrophages. Sather et al. have shown that the shedding of the Mer ectodomain results in the inactivation of the receptor and in additional neutralization of TAM ligands, which are sequestered by the released soluble form of the receptor ectodomain [43]. This autoregulatory mechanism is not exclusive to Mer but it has been described also for Axl. The cleavage, which generates the soluble and circulating Axl (sAxl), is induced by ADAM17 and another metalloproteinase, ADAM10 [44] (Figure 1). In 2010, Ekman et al. demonstrated that Gas6 is trapped by sAxl. In their elegant study, they hypothesized the absence of free-Gas6 circulating in the bloodstream in healthy subjects, since the molar concentration of sAxl is higher than the one of Gas6, thus suggesting that Gas6 released from cells is quickly bound by sAxl [45]. This seems related to the higher affinity of Gas6 for Axl in comparison to Mer. Indeed, Gas6 binds Axl with a dissociation constant in the subnanomolar range, whereas its affinity for Mer is at least 10-fold lower [46]. So, according to the interpretation suggested by Ekman et al., in the presence of Axl the interaction between Gas6 and Mer or soluble Mer (sMer) might be prevented. Conversely, a previous study published by Sather et al. demonstrated that both sAxl and sMer are able to inhibit the Gas6 activity. The authors focused on sMer, showing that the inactive sMer/Gas6-complex leads to a defective macrophage-mediated engulfment of apoptotic cells. Furthermore, they showed that the release of sMer is associated with a decrease of platelet aggregation in vitro and it could prevent the fatal collagen/epinephrine-induced thromboembolism in mice [43].

2. TAM Ligands: Mediators in Cell-to-Cell Interactions

To date, Gas6 and Pros1 are the most known TAM ligands, but other new potential ones have been described: tubby, tubby-like protein 1 [47], and galactin-3 (Gal-3) [48] seem to preferentially activate Mer during phagocytosis. However, little is still known regarding these new TAM ligands and this issue is beyond the scope of this review.

Both Gas6 and Pros1 are members of the vitamin K-dependent protein family: in fact, they contain a Gla domain in which the glutamate residues are posttranslationally modified to form gamma-carboxyglutamate through a vitamin K-dependent carboxylation. This latter reaction is required to confer to these proteins their activities. Moreover, Gas6 and Pros1 contain the SHBG-like domain that makes them unique compared to other vitamin K-dependent proteins: this domain shares 30% sequence identity with SHBG, it replaces the serine-protease domain found in other vitamin K-dependent plasma proteases [49], and it is devoid of enzymatic activity [50].

Pros1 circulates in plasma at a concentration of 346 nmol/L [51], and its expression can be found in several organs, such as the liver, kidney, lungs, and gonads [51], where it is produced by different cell types, like hepatocytes, endothelial cells, megakaryocytes, and osteoblasts [52]. Pros1 heterozygous deficiency is associated with an elevated risk of thrombosis development, whereas homozygous deficiency is lethal during embryonic development [51]. As stated above, Pros1, together with Gas6, is the most studied TAM ligand; it presents ~42% homology sequence with Gas6, and it specifically binds/activates Mer and Tyro3. Although Gas6 and Pros1 share structural homology, their functions are dissimilar, since the functions of Gas6 are limited to binding TAM. Instead, it is important to specify that Pros1 circulates in the bloodstream in two different forms: 60% of Pros1 is bound to the C4b-binding protein, while the remaining 40% of Pros1 is freely circulating [53]. Thus, only the "free Pros1" can bind and activate Mer and Tyro3. In addition, Pros1 contributes to the downregulation of thrombin formation by stimulating the activity as a nonenzymatic cofactor of both activated protein C and tissue factor pathway inhibitor [54, 55]. This latter essential function is TAM independent.

Gas6 interacts with TAM through its SHBG-like domain, positioned at the C-terminus of its sequence, activating downstream signaling pathways, such as PLCγ, PI3K, ERK, and NF-κB, and regulating cell survival, proliferation, migration, differentiation, adhesion, and apoptosis [56, 57].

Gas6 expression has been described in $CD11b^{+}F4/80^{+}$ bone marrow macrophages [58], in microglia [59], in peritoneal macrophages [14, 60], in apoptotic thymocytes [19], in Sertoli cells [61], and in $CD11c^{+}$ dendritic cells of colon carcinoma [60]. Moreover, Gas6 is particularly expressed by endothelial cells, platelets, and leukocytes [62, 63].

Despite this, the biological role of Gas6 is not completely understood yet. Goruppi et al. showed that Gas6 is able to induce proliferation in vitro and to promote survival in the murine fibroblast cell line NIH-3T3 [64].

During the last years, different groups studying Gas6-TAM interaction focused on inflammation and tissue homeostasis, since in the presence of the Gla domain binding a PtdSer and the SHBG-like domain binding the Ig-like domain of TAM, Gas6 works as a bridge between apoptotic cells and the effector cells (Figure 1).

3. Gas6 and TAM Involvement in the Pathophysiology of Different Acute and Chronic Diseases

Gas6 and Pros1 are secreted in the bloodstream and, interestingly, Gas6 plasma levels in humans (~18 ng/mL) are two logarithms lower than Pros1 plasmatic ones [65]. Gas6 expression and its concentration in the bloodstream and in different compartments were found to change in several pathological conditions, both chronic and acute. These data allowed hypothesizing a role for Gas6 in the physiopathology of different diseases and using it as a tool for prognostic stratification in several specific contexts. For example, Bellan et al. demonstrated a correlation between plasmatic Gas6 levels and liver stiffness due to hepatic fibrosis from several etiologies [66]. In this context, they have introduced thresholds of plasmatic Gas6 for liver fibrosis (30 ng/mL) and severe fibrosis (42 ng/mL). Furthermore, the role of Gas6 as a predictor of esophageal varices was esteemed in patients affected by hepatitis C virus-related chronic liver disease [67]. In 2017, Staufer et al. strongly demonstrated the utility of sAxl and Gas6 serum levels as a diagnostic tool for advanced fibrosis, cirrhosis, and hepatocellular carcinoma on 392 patients, 361 of whom were affected by chronic liver disease from different etiologies. Moreover, they suggested the sAxl/albumin ratio as a better biomarker, since this ratio increases the accuracy to detect the degrees of these chronic liver diseases [68]. The use of Gas6 as a noninvasive biomarker has been proposed also by Li et al. in the early detection of diabetic nephropathy [69]. On the contrary, they observed decreased levels of Gas6 in diabetic patients suffering from the underestimated nephropathy and have proposed Gas6 (cutoff ~9 ng/mL) as a better biomarker than cystatin C and creatinine. Concerning the renal pathophysiology, it has been shown that not only Gas6 but also sMer and sAxl have a potential role as biomarkers in patients affected by chronic kidney disease (CKD). Monocytes derived from CKD and hemodialysis patients showed a downregulation of Mer and Axl expression, both at RNA and plasma-membrane protein levels. However, plasmatic sMer and sAxl levels were remarkably higher in comparison to healthy subjects and they resulted to be positively associated with Gas6 levels in plasma of CKD patients [70].

Moreover, Sainaghi et al. found high Gas6 levels in the cerebrospinal fluid (CSF) of patients with a diagnosis of Alzheimer's disease (AD), with values that were doubled compared to the control group. The authors justified these findings as a compensatory mechanism: they hypothesized a Gas6 attempt to downregulate the proinflammatory cytokines, which are partially responsible for neuronal death [71]. Additionally, Gas6 has been found poorly expressed in the plasma of patients affected by multiple sclerosis, unlike sMer and sAxl [72]. However, Gas6 levels were found higher in CSF of these patients compared with control group, correlating with the relapse severity of the disease [73, 74]. Gas6 protein concentration in CSF was also found elevated in patients with chronic inflammatory demyelinating polyneuropathy (CIDP) [75].

The Gas6 role as biomarker in SLE patients, particularly for those developing lupus nephritis and cutaneous vasculitis, suggested by Wu et al. in 2014 [76], has been recently confirmed by Gong et al. [77]. In addition, they showed an increase in the levels of soluble forms of Mer and Axl in these patients and they correlated the high levels of soluble receptors to proliferative glomerulonephritis.

However, the association between autoimmune diseases, SLE, and (s)TAM level/role is well established and reviewed elsewhere [24, 78].

Since TAM and their ligands have a wide range of functions and are expressed all over the body, it is reasonable to think of their possible involvement in acute diseases as well. It is reported that plasma Gas6 concentration is increased in patients with acute dyspnea due to heart failure and even more in patients with systemic or pulmonary infection [79]. Llacuna et al., for example, assumed a feasible therapeutic role of Gas6 after ischemia/reperfusion- (I/R-) induced hepatic injury in mice. They demonstrated that Gas6 homeostasis is regulated during I/R with its local release aimed at plugging the injury during the first phase; then, they observed a drastic decrease of Gas6 RNA during the reperfusion phase. Using mice knockout for Gas6 ($Gas6^{-/-}$), the authors highlighted an increased susceptibility to hepatic I/R injury associated to enhanced expression of proinflammatory cytokines, such as IL-1β and tumor necrosis factor α (TNF-α), and increased levels of hepatic transaminases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)). Moreover, they intravenously injected recombinant Gas6 (rGas6) in mice after hepatic I/R, in both Gas6 WT and $Gas6^{-/-}$ mice, observing that rGas6 injection not only rescued null mice from I/R-mediated liver injury but it also proved to be useful in protecting WT mice against hepatic I/R damage [80].

The therapeutic role of Gas6 has been suggested also by two other research groups using mouse models of sepsis-induced kidney injury [81] and sepsis-induced lung injury

[82]. Chen et al. reported that intravenous injection of rGas6 immediately after sepsis induction exerts protective effects by reducing serum urea nitrogen, creatinine, and renal tissue apoptosis, thus attenuating the pathological damage and increasing the survival rate in a mouse model of sepsis-induced acute kidney injury following cecal ligation puncture (CLP) [81]. On the other hand, Giangola et al. reported that rGas6 administration behaves as an anti-inflammatory agent capable of abrogating sepsis-induced organ dysfunction and neutrophil-induced acute lung injury (ALI), resulting in the amelioration of the overall survival in a mouse model of CLP-induced sepsis [82].

4. An Open Window on Sepsis

Sepsis is one of the most common life-threatening diseases widespread in the world [83]. A crucial point concerning sepsis is to reach a fast diagnosis because of the multiple comorbidities and underlying diseases presented by septic patients [84].

The sepsis definition, in use until 2016, was based on the host's inflammatory responses. Recently, physicians and researchers have begun to break up the pathophysiology of sepsis discovering that the host reaction to sepsis involves not only the inflammatory milieu but also a modification in nonimmunological pathways [85]. This latest understanding led to a review of the sepsis definition and, in 2016, the Sepsis-3 conference defined sepsis as a "life-threatening organ dysfunction caused by a deregulated host response to infection" and septic shock as a "subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality" [86]. In this context, despite the presence of international recommendations [87], many points regarding the appropriate treatment still remain debatable [88–90]. As for the definition, diagnostic criteria have also changed and currently diagnosis is based on the detection of organ dysfunctions evaluated with the Sequential (Sepsis-Related) Organ Failure Assessment (SOFA) score.

In the past, the SOFA score was created with the aim of calculating the number and severity of the dysfunction in six organ systems (notably pulmonary, coagulation, hepatobiliary, cardiovascular, renal, and neurologic) [91]. The Sepsis-3 definitions also introduced a new diagnostic tool useful in the early identification of patients at risk of sepsis in the emergency department (ED): the quik-SOFA (qSOFA) [92].

Over the last decade, there has been great interest in finding out biomarkers that could improve both sepsis diagnosis and sepsis prognosis [93–95]. In 2017, Kim et al. demonstrated a possible prognostic utility of procalcitonin (PCT), presepsin (sCD14-subtypes), soluble suppression of tumorigenicity 2 (sST2), and Gal-3 in sepsis.

They suggested that a multimarker approach could be beneficial for an optimized management of patients with sepsis [93]. The idea of a multimarker approach has been recently reclaimed by Mearelli et al. in a multicenter prospective study comprising a large cohort of patients. They developed and validated a high-performing, reproducible, and cost-effective algorithm to assist physicians of the emergency department in distinguishing sepsis/septic shock from noninfectious systemic inflammatory response syndrome (SIRS) [96]. Nowadays, it is becoming evident that the use of biomarkers in clinical procedures can be helpful and essential for a correct diagnosis, to discriminate non-infectious SIRS, sepsis, and septic shock patients, and to estimate the prognosis.

The abovementioned Gal-3 is one of the novel Mer ligands identified by Caberoy et al. They showed that Gal-3 stimulates the phagocytosis of apoptotic cells and cellular debris through Mer activation [48]. Since Gal-3 is involved in efferocytosis and it was found significantly higher in patients with sepsis and septic shock, Ferreira et al. induced sepsis in both WT and Gal-3 knockout mice showing that the absence of Gal-3 was protective against sepsis. This phenomenon seems to be associated with the ability of Gal-3 to limit neutrophil migration to primary sites of infection, consequently favoring bacterial spreading and death [97].

The employment of TAM and their ligands as biomarkers in septic patients has already been described more than ten years ago. Borgel et al.'s and Gibot et al.'s groups were among the first to depict the correlation between Gas6 and sepsis condition in 2006 and 2007, respectively [98, 99]. Few years later, Ekman et al. confirmed that Gas6 levels are increased during sepsis [100], finding a correlation between Gas6 and the degree of organ damage. In addition, they showed an increase of sAxl as well, although without the same magnitude of Gas6. Indeed, Gas6 levels strongly correlated with IL-6 and PCT levels and the number of failing organs. Thus, Gas6 levels were associated with disease severity and organ dysfunction. New studies have been conducted on a cohort of septic patients diagnosed following the Sepsis-3 criteria [101, 102]. In a cohort of 129 patients, it was reported that Gas6 plasmatic levels at admission in an intensive care unit (ICU) were higher in nonsurvivors than survivors [101]. However, neither Gas6 nor sAxl levels investigated in this study were able to discriminate bacteremic from nonbacteremic patients or Gram-negative versus Gram-positive infections. Moreover, Gas6 was compared with well-known inflammatory/severity biomarkers and evidence was found for a correlation between Gas6 levels and IL-6, IL-8, IL-10, sAxl, and PCT levels. Gas6 and IL-8 were the only two biomarkers found to be differently expressed between survivors and nonsurvivors. Therefore, these two biomarkers seem to be able to predict mortality in septic/shock patients at the time of ICU admission. In the same study, Gas6 performed better than procalcitonin and C-reactive protein, which are broadly used to diagnose infection, even though Gas6 levels between survivors and nonsurvivors remained constant over time. According to these findings, Gas6 cannot predict sepsis evolution, unlike other inflammatory mediators, such as TNF-α and IL-1β [101]. The role of Gas6 in septic patients was recently highlighted also in sepsis-related acute lung injury (ALI) by Yeh et al. [102]. Indeed, ALI is one of the complications of sepsis, and it is known for its contribution to sudden deaths and morbidity [103]. In this study published in 2017, the authors enrolled 129 patients with a

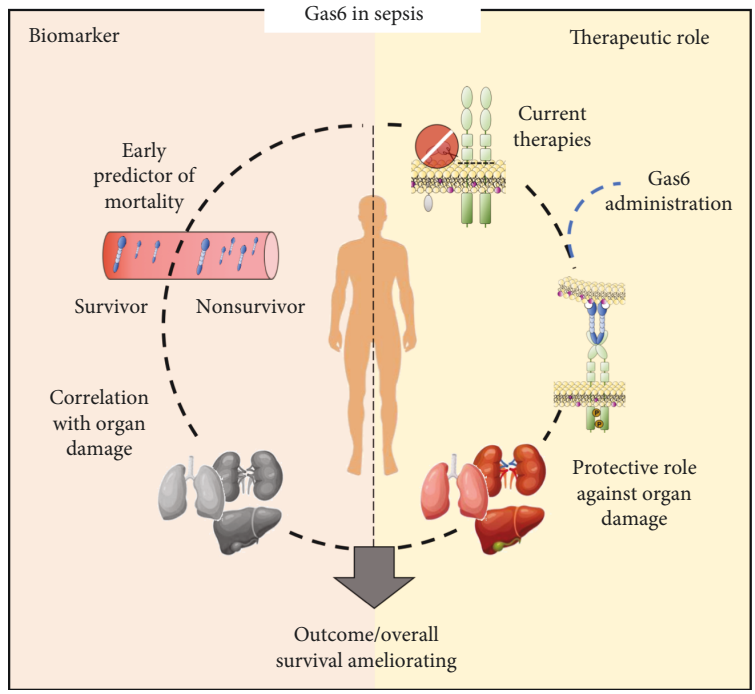


Figure 2: Gas6: the paradoxical role in sepsis. During sepsis, Gas6 could be used as an early biomarker in the routine management of septic patients since Gas6 plasma levels, measured at the time of ICU admission, can predict mortality and multiorgan failure. The high levels of Gas6 released in the bloodstream during sepsis seem to be aimed at counterbalancing sepsis dysfunctions; however, because inflammatory stimuli downregulate TAM receptors, the Gas6 overrelease is ineffective. Current therapy for sepsis is aimed at decreasing inflammatory stimuli. Gas6 administration after current therapy could operate on activated TAM receptors and protect the organs from sepsis-induced damage. The combination of a correct early diagnosis and the protective effects mediated by Gas6 could ameliorate the outcome/overall survival of patients.

diagnosis of sepsis and they compared the patients with and without ALI, observing that Gas6 levels, together with IL-6 and IL-8 levels, were significantly elevated among patients who developed ALI. Since nowadays a prompt and correct ALI diagnosis is mandatory in order to develop an effective treatment, the authors suggested Gas6 as an early predictor of ALI. Moreover, they suggested that Gas6 could also improve the parameters of the lung injury prediction score, such as its discrimination and its positive and negative predictive values [102].

The role of Gas6 in inflammatory contexts seems to be mainly related to its interaction with Mer [104, 105]. Mer has a pivotal role in counterbalancing the proinflammatory effects of toll-like receptor 4 (TRL4) activation induced by LPS, as demonstrated by Lee et al. using an anti-Mer neutralizing antibody [104].

Natural occurring regulatory T cells (Tregs) play a central role in maintaining immunologic homeostasis and tolerance. Different studies reported an expansion in both percentage and number of Tregs along with an increase in their suppressive function during sepsis [106]. Heuer et al. showed that adoptive transfer of in vitro-stimulated Tregs was able to increase the survival and the bacterial clearance in a mouse model of CLP-induced polymicrobial sepsis [107]. Zhao et al. demonstrated that Tregs express both Mer and Axl and that Gas6 administration in vivo increases forkhead box P3 (Foxp3) expression and suppressive activity by $CD4^+CD25^+$ Tregs. In vitro stimulation of Tregs by Gas6 had no effects on IL-10 and transforming growth factor $\beta 1$ (TGF-$\beta 1$) production, but it increased Foxp3 and cytotoxic T-lymphocyte antigen 4 (CTLA-4) expression as well as the suppressive activity in a dose-dependent manner [108]. Hence, these studies suggest a possible role of Gas6 in tuning the immune response during sepsis by linking the innate and adaptive immune system.

However, the issue of comparing the response of the murine model of sepsis with human pathology is still open [109]. Regarding the focus of this review, we still know little about the response of TAM receptors and Gas6 in a murine sepsis model. Moreover, the levels of Gas6 and sAxl in both healthy and septic mice are not clear. Thus, the possibility that sAxl sequesters the endogenous circulating Gas6 is present in mice as well as in humans [45]. However, the administration of a large amount of exogenous Gas6 could overcome this problem by ameliorating the sepsis-induced multiorgan failure in septic mice, as recently demonstrated by Ni et al. [110]. Therefore, also in sepsis, where Gas6 levels are high, the injection of exogenous Gas6 seems to improve the outcome.

Summarizing, on the basis of previous studies, it is possible to hypothesize the use of Gas6 as a biomarker in the complex pathophysiology of sepsis, since several data seem to suggest a role of Gas6 as a useful biomarker for discriminating between noninfectious SIRS, sepsis, and septic shock. Furthermore, Gas6 came out as an early predictor of mortality and was able to identify some life-threatening

sepsis complications. Moreover, Gas6 administration could be envisaged as a therapeutic reinforcement to the current treatment, since it showed to be able to ameliorate the overall survival and to partially protect from the organ dysfunction in a mouse model of sepsis. In conclusion, the Gas6/TAM axis activation possibly ameliorates the tissue hypoperfusion, thus restoring the physiological tissue homeostasis and preserving organ function, with a positive impact on sepsis prognosis (Figure 2).

Abbreviations

Gas6: Growth arrest-specific 6
RTKs: Tyrosine kinase receptors
DCs: Dendritic cells
BM: Bone marrow
HSCs: Hepatic stellate cells
LSECs: Liver sinusoidal endothelial cells
NK: Natural killer
NKT: Natural killer T
Pros1: Protein S
SHGB: Sex hormone-binding globulin
Gla: Gamma-carboxyglutamic acid rich
PtdSer: Phosphatidylserine
PI3K: Phosphoinositide 3 kinase
MAP: Mitogen-activated protein
NF-κB: Nuclear factor-light-chain-enhancer of activated B cells
STAT: Signal transducer and activator of transcription protein
PLC: Phospholipase C
Grb2: Growth factor receptor-bound protein 2
ERK: Extracellular-signal-regulated kinase
SOCS: Suppressor of cytokine signaling proteins
TKO: Triple knockout
WT: Wild type
SLE: Systemic lupus erythematosus
APCs: Antigen-presenting cells
IL: Interleukin
ADAM17: A disintegrin and metalloproteinase domain 17
TACE: Tumor necrosis factor-alpha converting enzyme
PRRs: Pattern-recognition receptors
LPS: Lipopolysaccharide
Poly:C: Polycytidylic acid
PolyI:C: Polyinosinic:polycytidylic acid
sAxl: Soluble Axl
sMer: Soluble Mer
TK: Tyrosine kinase
FN III: Fibronectin type III domains
CKD: Chronic kidney disease
CSF: Cerebrospinal fluid
AD: Alzheimer's disease
CIDP: Chronic inflammatory demyelinating polyneuropathy
I/R: Ischemia/reperfusion
TNF-α: Tumor necrosis factor α
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
rGas6: Recombinant Gas6
CLP: Cecal ligation puncture
SOFA: Sequential (sepsis-related) organ failure assessment
ED: Emergency department
qSOFA: Quick SOFA
PCT: Procalcitonin
Gal-3: Galectin-3
sST2: Soluble suppression of tumorigenicity 2
SIRS: Systemic inflammatory response syndrome
ALI: Acute lung injury
TLR4: Toll-like receptor 4
TSLP: Thymic stromal lymphopoietin
TSLPR: TSLP receptor
CCL17: Chemokine (C-C motif) ligand 17
Tregs: Regulatory T cells
Foxp3: Forkhead box P3.

Conflicts of Interest

All the authors state they have no conflicts of interest.

References

[1] M. A. Lemmon and J. Schlessinger, "Cell Signaling by Receptor Tyrosine Kinases," *Cell*, vol. 141, no. 7, pp. 1117–1134, 2010.

[2] C. V. Rothlin, E. A. Carrera-Silva, L. Bosurgi, and S. Ghosh, "TAM receptor signaling in immune homeostasis," *Annual Review of Immunology*, vol. 33, no. 1, pp. 355–391, 2015.

[3] A. Zagórska, P. G. Través, E. D. Lew, I. Dransfield, and G. Lemke, "Diversification of TAM receptor tyrosine kinase function," *Nature Immunology*, vol. 15, no. 10, pp. 920–928, 2014.

[4] R. Ji, L. Meng, X. Jiang et al., "TAM receptors support neural stem cell survival, proliferation and neuronal differentiation," *PLoS One*, vol. 9, no. 12, 2014.

[5] E. Wan, X. Y. Yeap, S. Dehn et al., "Enhanced efferocytosis of apoptotic cardiomyocytes through myeloid-epithelial-reproductive tyrosine kinase links acute inflammation resolution to cardiac repair after infarction," *Circulation Research*, vol. 113, no. 8, pp. 1004–1012, 2013.

[6] M. Sandahl, D. M. Hunter, K. E. Strunk, H. S. Earp, and R. S. Cook, "Epithelial cell-directed efferocytosis in the post-partum mammary gland is necessary for tissue homeostasis and future lactation," *BMC Developmental Biology*, vol. 10, no. 1, p. 122, 2010.

[7] T. Fujimori, A. M. Grabiec, M. Kaur et al., "The Axl receptor tyrosine kinase is a discriminator of macrophage function in the inflamed lung," *Mucosal Immunology*, vol. 8, no. 5, pp. 1021–1030, 2015.

[8] A. Kazeros, B. G. Harvey, B. J. Carolan, H. Vanni, A. Krause, and R. G. Crystal, "Overexpression of apoptotic cell removal receptor MERTK in alveolar macrophages of cigarette smokers," *American Journal of Respiratory Cell and Molecular Biology*, vol. 39, no. 6, pp. 747–757, 2008.

[9] S. K. Mukherjee, A. Wilhelm, and C. G. Antoniades, "TAM receptor tyrosine kinase function and the immunopathology of liver disease," *American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 310, no. 11, pp. G899–G905, 2016.

[10] M. Subramanian, C. D. Hayes, J. J. Thome et al., "An AXL/LRP-1/RANBP9 complex mediates DC efferocytosis and antigen cross-presentation in vivo," *Journal of Clinical Investigation*, vol. 124, no. 3, pp. 1296–1308, 2014.

[11] W.-H. Shao, Y. Zhen, R. A. Eisenberg, and P. L. Cohen, "The Mer receptor tyrosine kinase is expressed on discrete macrophage subpopulations and mainly uses Gas6 as its ligand for uptake of apoptotic cells," *Clinical Immunology*, vol. 133, no. 1, pp. 138–144, 2009.

[12] F. Zhong, Z. Chen, L. Zhang et al., "Tyro3 is a podocyte protective factor in glomerular disease," *JCI Insight*, vol. 3, no. 22, 2018.

[13] H. Wang, Y. Chen, Y. Ge et al., "Immunoexpression of Tyro 3 family receptors—Tyro 3, Axl, and Mer—and their ligand Gas6 in postnatal developing mouse testis," *Journal of Histochemistry & Cytochemistry*, vol. 53, no. 11, pp. 1355–1364, 2005.

[14] T. Deng, Y. Zhang, Q. Chen, K. Yan, and D. Han, "Toll-like receptor-mediated inhibition of Gas6 and ProS expression facilitates inflammatory cytokine production in mouse macrophages," *Immunology*, vol. 135, no. 1, pp. 40–50, 2012.

[15] W. R. Gould, S. M. Baxi, R. Schroeder et al., "Gas6 receptors Axl, Sky and Mer enhance platelet activation and regulate thrombotic responses," *Journal of Thrombosis and Haemostasis*, vol. 3, no. 4, pp. 733–741, 2005.

[16] A. Malawista, X. Wang, M. Trentalange, H. G. Allore, and R. R. Montgomery, "Coordinated expression of tyro3, axl, and mer receptors in macrophage ontogeny," *Macrophage*, vol. 3, 2016.

[17] M. Paolino, A. Choidas, S. Wallner et al., "The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells," *Nature*, vol. 507, no. 7493, pp. 508–512, 2014.

[18] H. M. Seitz, T. D. Camenisch, G. Lemke, H. S. Earp, and G. K. Matsushima, "Macrophages and dendritic cells use different Axl/Mertk/Tyro3 receptors in clearance of apoptotic cells," *The Journal of Immunology*, vol. 178, no. 9, pp. 5635–5642, 2007.

[19] M. A. Wallet, P. Sen, R. R. Flores et al., "MerTK is required for apoptotic cell-induced T cell tolerance," *The Journal of Experimental Medicine*, vol. 205, no. 1, pp. 219–232, 2008.

[20] E. . M. Behrens, P. Gadue, S.-y. Gong, S. Garrett, P. . L. Stein, and P. . L. Cohen, "The mer receptor tyrosine kinase: expression and function suggest a role in innate immunity," *European Journal of Immunology*, vol. 33, no. 8, pp. 2160–2167, 2003.

[21] R. S. Scott, E. J. McMahon, S. M. Pop et al., "Phagocytosis and clearance of apoptotic cells is mediated by MER," *Nature*, vol. 411, no. 6834, pp. 207–211, 2001.

[22] G. Wu, Z. Ma, Y. Cheng et al., "Targeting Gas6/TAM in cancer cells and tumor microenvironment," *Molecular Cancer*, vol. 17, no. 1, p. 20, 2018.

[23] A. M. Pierce and A. K. Keating, "TAM receptor tyrosine kinases: expression, disease and oncogenesis in the central nervous system," *Brain Research*, vol. 1542, pp. 206–220, 2014.

[24] M. Wium, J. Paccez, and L. Zerbini, "The dual role of TAM receptors in autoimmune diseases and cancer: an overview," *Cells*, vol. 7, no. 10, p. 166, 2018.

[25] E. D. Lew, J. Oh, P. G. Burrola et al., "Differential TAM receptor-ligand-phospholipid interactions delimit differential TAM bioactivities," *eLife*, vol. 3, 2014.

[26] F. Alciato, P. P. Sainaghi, D. Sola, L. Castello, and G. C. Avanzi, "TNF-α, IL-6, and IL-1 expression is inhibited by GAS6 in monocytes/macrophages," *Journal of Leukocyte Biology*, vol. 87, no. 5, pp. 869–875, 2010.

[27] V. A. Korshunov, "Axl-dependent signalling: a clinical update," *Clinical Science*, vol. 122, no. 8, pp. 361–368, 2012.

[28] G. Lemke, "Biology of the TAM receptors," *Cold Spring Harbor Perspectives in Biology*, vol. 5, no. 11, 2013.

[29] C. V. Rothlin, S. Ghosh, E. I. Zuniga, M. B. A. Oldstone, and G. Lemke, "TAM receptors are pleiotropic inhibitors of the innate immune response," *Cell*, vol. 131, no. 6, pp. 1124–1136, 2007.

[30] Y. T. Akalu, C. V. Rothlin, and S. Ghosh, "TAM receptor tyrosine kinases as emerging targets of innate immune checkpoint blockade for cancer therapy," *Immunological Reviews*, vol. 276, no. 1, pp. 165–177, 2017.

[31] C. Parinot and E. F. Nandrot, "A comprehensive review of mutations in the MERTK proto-oncogene," *Retinal Degenerative Diseases*, vol. 854, 2016.

[32] G. Lemke and T. Burstyn-Cohen, "TAM receptors and the clearance of apoptotic cells," *Annals of the New York Academy of Sciences*, vol. 1209, no. 1, pp. 23–29, 2010.

[33] T. Kato, "Biological roles of hepatocyte growth factor-Met signaling from genetically modified animals (Review)," *Biomedical Reports*, vol. 7, 2017.

[34] Y. Chen, H. Wang, N. Qi et al., "Functions of TAM RTKs in regulating spermatogenesis and male fertility in mice," *Reproduction*, vol. 138, no. 4, pp. 655–666, 2009.

[35] D. Prasad, C. V. Rothlin, P. Burrola et al., "TAM receptor function in the retinal pigment epithelium," *Molecular and Cellular Neuroscience*, vol. 33, no. 1, pp. 96–108, 2006.

[36] J. L. Duncan, M. M. LaVail, D. Yasumura et al., "An RCS-Like Retinal Dystrophy Phenotype inMerKnockout Mice," *Investigative Opthalmology & Visual Science*, vol. 44, no. 2, p. 826, 2003.

[37] Q. Lu, "Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family," *Science*, vol. 293, no. 5528, pp. 306–311, 2001.

[38] N. Qi, P. Liu, Y. Zhang, H. Wu, Y. Chen, and D. Han, "Development of a spontaneous liver disease resembling autoimmune hepatitis in mice lacking Tyro3, Axl and Mer receptor tyrosine kinases," *PLoS One*, vol. 8, no. 6, p. e66604, 2013.

[39] E. Thorp, T. Vaisar, M. Subramanian, L. Mautner, C. Blobel, and I. Tabas, "Shedding of the Mer tyrosine kinase receptor is mediated by ADAM17 protein through a pathway involving reactive oxygen species, protein kinase Cδ, and p38 mitogen-activated protein kinase (MAPK)," *Journal of Biological Chemistry*, vol. 286, no. 38, pp. 33335–33344, 2011.

[40] M. A. Miller, R. J. Sullivan, and D. A. Lauffenburger, "Molecular pathways: receptor ectodomain shedding in treatment, resistance, and monitoring of cancer," *Clinical Cancer Research*, vol. 23, no. 3, pp. 623–629, 2017.

[41] J. Wu, C. Ekman, A. Jönsen et al., "Increased plasma levels of the soluble Mer tyrosine kinase receptor in systemic lupus erythematosus relate to disease activity and nephritis," *Arthritis Research & Therapy*, vol. 13, no. 2, p. R62, 2011.

[42] L. Xu, F. Hu, H. Zhu et al., "Soluble TAM receptor tyrosine kinases in rheumatoid arthritis: correlation with disease activity and bone destruction," *Clinical & Experimental Immunology*, vol. 192, no. 1, pp. 95–103, 2018.

[43] S. Sather, K. D. Kenyon, J. B. Lefkowitz et al., "A soluble form of the Mer receptor tyrosine kinase inhibits macrophage clearance of apoptotic cells and platelet aggregation," *Blood*, vol. 109, no. 3, pp. 1026–1033, 2006.

[44] J. J. Orme, Y. du, K. Vanarsa et al., "Heightened cleavage of Axl receptor tyrosine kinase by ADAM metalloproteases may contribute to disease pathogenesis in SLE," *Clinical Immunology*, vol. 169, pp. 58–68, 2016.

[45] C. Ekman, J. Stenhoff, and B. Dahlbäck, "Gas6 is complexed to the soluble tyrosine kinase receptor Axl in human blood," *Journal of Thrombosis and Haemostasis*, vol. 8, no. 4, pp. 838–844, 2010.

[46] K. Nagata, K. Ohashi, T. Nakano et al., "Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases," *Journal of Biological Chemistry*, vol. 271, no. 47, pp. 30022–30027, 1996.

[47] N. B. Caberoy, Y. Zhou, and W. Li, "Tubby and tubby-like protein 1 are new MerTK ligands for phagocytosis," *The EMBO Journal*, vol. 29, no. 23, pp. 3898–3910, 2010.

[48] N. B. Caberoy, G. Alvarado, J.-L. Bigcas, and W. Li, "Galectin-3 is a new MerTK-specific eat-me signal," *Journal of Cellular Physiology*, vol. 227, no. 2, pp. 401–407, 2012.

[49] M. E. Baker, F. S. French, and D. R. Joseph, "Vitamin K-dependent protein S is similar to rat androgen-binding protein," *Biochemical Journal*, vol. 243, no. 1, pp. 293–296, 1987.

[50] B. Saposnik, D. Borgel, M. Aiach, and S. Gandrille, "Functional properties of the sex-hormone-binding globulin (SHBG)-like domain of the anticoagulant protein S," *European Journal of Biochemistry*, vol. 270, no. 3, pp. 545–555, 2003.

[51] J. H. M. van der Meer, T. van der Poll, and C. van 't Veer, "TAM receptors, Gas6, and protein S: roles in inflammation and hemostasis," *Blood*, vol. 123, no. 16, pp. 2460–2469, 2014.

[52] C. Maillard, M. Berruyer, C. M. Serre, M. Dechavanne, and P. D. Delmas, "Protein-S, a vitamin K-dependent protein, is a bone matrix component synthesized and secreted by osteoblasts," *Endocrinology*, vol. 130, no. 3, pp. 1599–1604, 1992.

[53] B. Dahlbäck, "The tale of protein S and C4b-binding protein, a story of affection," *Thrombosis and Haemostasis*, vol. 98, no. 07, pp. 90–96, 2007.

[54] F. J. Walker, "Protein S and the regulation of activated protein C," *Seminars in Thrombosis and Hemostasis*, vol. 10, no. 02, pp. 131–138, 1984.

[55] R. S. Robins, C. A. Lemarié, S. Laurance, M. N. Aghourian, J. Wu, and M. D. Blostein, "Vascular Gas6 contributes to thrombogenesis and promotes tissue factor up-regulation after vessel injury in mice," *Blood*, vol. 121, no. 4, pp. 692–699, 2013.

[56] H. Axelrod and K. J. Pienta, "Axl as a mediator of cellular growth and survival," *Oncotarget*, vol. 5, no. 19, pp. 8818–8852, 2014.

[57] K.-S. Wu, Y.-J. Hung, C.-H. Lee, F.-C. Hsiao, and P.-S. Hsieh, "The involvement of GAS6 signaling in the development of obesity and associated inflammation," *International Journal of Endocrinology*, vol. 2015, Article ID 202513, 7 pages, 2015, 2015.

[58] G. Zahuczky, E. Kristóf, G. Majai, and L. Fésüs, "Differentiation and glucocorticoid regulated apopto-phagocytic gene expression patterns in human macrophages. Role of Mertk in enhanced phagocytosis," *PLoS one*, vol. 6, no. 6, p. e21349, 2011.

[59] O. Butovsky, M. P. Jedrychowski, C. S. Moore et al., "Identification of a unique TGF-β-dependent molecular and functional signature in microglia," *Nature Neuroscience*, vol. 17, no. 1, pp. 131–143, 2014.

[60] S. Loges, T. Schmidt, M. Tjwa et al., "Malignant cells fuel tumor growth by educating infiltrating leukocytes to produce the mitogen Gas6," *Blood*, vol. 115, no. 11, pp. 2264–2273, 2010.

[61] Q. Lu, M. Gore, Q. Zhang et al., "Tyro-3 family receptors are essential regulators of mammalian spermatogenesis," *Nature*, vol. 398, no. 6729, pp. 723–728, 1999.

[62] G. Manfioletti, C. Brancolini, G. Avanzi, and C. Schneider, "The protein encoded by a growth arrest-specific gene (gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade," *Molecular and Cellular Biology*, vol. 13, no. 8, pp. 4976–4985, 1993.

[63] G. C. Avanzi, M. Gallicchio, G. Cavalloni et al., "GAS6, the ligand of Axl and Rse receptors, is expressed in hematopoietic tissue but lacks mitogenic activity," *Experimental Hematology*, vol. 25, no. 12, pp. 1219–1226, 1997.

[64] S. Goruppi, E. Ruaro, and C. Schneider, "Gas6, the ligand of Axl tyrosine kinase receptor, has mitogenic and survival activities for serum starved NIH3T3 fibroblasts," *Oncogene*, vol. 12, no. 3, pp. 471–480, 1996.

[65] I. Balogh, S. Hafizi, J. Stenhoff, K. Hansson, and Ë. Dahlbäck, "Analysis of Gas6 in human platelets and plasma," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 6, pp. 1280–1286, 2005.

[66] M. Bellan, G. Pogliani, C. Marconi et al., "Gas6 as a putative noninvasive biomarker of hepatic fibrosis," *Biomarkers in Medicine*, vol. 10, no. 12, pp. 1241–1249, 2016.

[67] M. Bellan, P. P. Sainaghi, M. T. Minh et al., "Gas6 as a predictor of esophageal varices in patients affected by hepatitis C virus related-chronic liver disease," *Biomarkers in Medicine*, vol. 12, no. 1, pp. 27–34, 2018.

[68] K. Staufer, M. Dengler, H. Huber et al., "The non-invasive serum biomarker soluble Axl accurately detects advanced liver fibrosis and cirrhosis," *Cell Death & Disease*, vol. 8, no. 10, p. e3135, 2017.

[69] W. Li, J. Wang, L. Ge, J. Shan, C. Zhang, and J. Liu, "Growth arrest-specific protein 6 (Gas6) as a noninvasive biomarker for early detection of diabetic nephropathy," *Clinical and Experimental Hypertension*, vol. 39, no. 4, pp. 382–387, 2017.

[70] I. J. Lee, B. A. Hilliard, M. Ulas et al., "Monocyte and plasma expression of TAM ligand and receptor in renal failure: links to unregulated immunity and chronic inflammation," *Clinical Immunology*, vol. 158, no. 2, pp. 231–241, 2015.

[71] P. P. Sainaghi, M. Bellan, F. Lombino et al., "Growth arrest specific 6 concentration is increased in the cerebrospinal fluid of patients with Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 55, no. 1, pp. 59–65, 2016.

[72] J. G. Weinger, K. M. Omari, K. Marsden, C. S. Raine, and B. Shafit-Zagardo, "Up-regulation of soluble Axl and Mer receptor tyrosine kinases negatively correlates with Gas6 in established multiple sclerosis lesions," *The American Journal of Pathology*, vol. 175, no. 1, pp. 283–293, 2009.

[73] P. P. Sainaghi, L. Collimedaglia, F. Alciato et al., "Growth arrest specific gene 6 protein concentration in cerebrospinal fluid correlates with relapse severity in multiple sclerosis,"

Mediators of Inflammation, vol. 2013, Article ID 406483, 7 pages, 2013.

[74] M. Bellan, M. Pirisi, and P. P. Sainaghi, "The Gas6/TAM system and multiple sclerosis," *International Journal of Molecular Sciences*, vol. 17, no. 11, p. 1807, 2016.

[75] P. P. Sainaghi, L. Collimedaglia, F. Alciato et al., "Elevation of Gas6 protein concentration in cerebrospinal fluid of patients with chronic inflammatory demyelinating polyneuropathy (CIDP)," *Journal of the Neurological Sciences*, vol. 269, no. 1-2, pp. 138–142, 2008.

[76] C. S. Wu, C. Y. Hu, H. F. Tsai et al., "Elevated serum level of growth arrest-specific protein 6 (Gas6) in systemic lupus erythematosus patients is associated with nephritis and cutaneous vasculitis," *Rheumatology International*, vol. 34, no. 5, pp. 625–629, 2014.

[77] S. Gong, Z. Xu, Y. Liu et al., "Plasma sMer, sAxl and GAS6 levels correlate with disease activity and severity in lupus nephritis," *European Journal of Clinical Investigation*, vol. 49, no. 3, p. e13064, 2019.

[78] C. V. Rothlin and G. Lemke, "TAM receptor signaling and autoimmune disease," *Current Opinion in Immunology*, vol. 22, no. 6, pp. 740–746, 2010.

[79] P. P. Sainaghi, F. Alciato, S. Carnieletto et al., "Gas6 evaluation in patients with acute dyspnea due to suspected pulmonary embolism," *Respiratory Medicine*, vol. 103, no. 4, pp. 589–594, 2009.

[80] L. Llacuna, C. Bárcena, L. Bellido-Martín et al., "Growth arrest-specific protein 6 is hepatoprotective against murine ischemia/reperfusion injury," *Hepatology*, vol. 52, no. 4, pp. 1371–1379, 2010.

[81] L. W. Chen, W. Chen, Z. Q. Hu et al., "Protective effects of growth arrest-specific protein 6 (Gas6) on sepsis-induced acute kidney injury," *Inflammation*, vol. 39, no. 2, pp. 575–582, 2016.

[82] M. D. Giangola, W.-L. Yang, S. R. Rajayer, J. Nicastro, G. F. Coppa, and P. Wang, "Growth arrest-specific protein 6 attenuates neutrophil migration and acute lung injury in sepsis," *Shock*, vol. 40, no. 6, pp. 485–491, 2013.

[83] K. E. Rudd, N. Kissoon, D. Limmathurotsakul et al., "The global burden of sepsis: barriers and potential solutions," *Critical Care*, vol. 22, no. 1, p. 232, 2018.

[84] S. A. Novosad, M. R. P. Sapiano, C. Grigg et al., "Vital signs: epidemiology of sepsis: prevalence of health care factors and opportunities for prevention," *MMWR. Morbidity and Mortality Weekly Report*, vol. 65, no. 33, pp. 864–869, 2016.

[85] J. Rello, F. Valenzuela-Sánchez, M. Ruiz-Rodriguez, and S. Moyano, "Sepsis: a review of advances in management," *Advances in Therapy*, vol. 34, no. 11, pp. 2393–2411, 2017.

[86] M. Singer, C. S. Deutschman, C. W. Seymour et al., "The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)," *JAMA*, vol. 315, no. 8, pp. 801–810, 2016.

[87] A. Rhodes, L. E. Evans, W. Alhazzani et al., "Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016," *Intensive Care Medicine*, vol. 43, no. 3, pp. 304–377, 2017.

[88] M. Jozwiak, O. Hamzaoui, X. Monnet, and J.-L. Teboul, "Fluid resuscitation during early sepsis: a need for individualization," *Minerva Anestesiologica*, vol. 84, no. 8, pp. 987–992, 2018.

[89] A. Beurton, J. L. Teboul, F. Gavelli et al., "The effects of passive leg raising may be detected by the plethysmographic oxygen saturation signal in critically ill patients," *Critical Care*, vol. 23, no. 1, 2019.

[90] M. M. Levy, L. E. Evans, and A. Rhodes, "The surviving sepsis campaign bundle: 2018 update," *Intensive Care Medicine*, vol. 44, no. 6, pp. 925–928, 2018.

[91] J. L. Vincent, R. Moreno, J. Takala et al., "The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure," *Intensive Care Medicine*, vol. 22, no. 7, pp. 707–710, 1996.

[92] C. W. Seymour, V. X. Liu, T. J. Iwashyna et al., "Assessment of clinical criteria for sepsis," *JAMA*, vol. 315, no. 8, pp. 762–774, 2016.

[93] on behalf of GREAT Network, H. Kim, M. Hur, H.-W. Moon, Y.-M. Yun, and S. Di Somma, "Multi-marker approach using procalcitonin, presepsin, galectin-3, and soluble suppression of tumorigenicity 2 for the prediction of mortality in sepsis," *Ann Intensive Care*, vol. 7, no. 1, p. 27, 2017.

[94] AdrenOSS-1 study investigators, A. Mebazaa, C. Geven et al., "Circulating adrenomedullin estimates survival and reversibility of organ failure in sepsis: the prospective observational multinational Adrenomedullin and Outcome in Sepsis and Septic Shock-1 (AdrenOSS-1) study," *Critical Care*, vol. 22, no. 1, 2018.

[95] L. M. Castello, M. Baldrighi, L. Molinari et al., "The role of osteopontin as a diagnostic and prognostic biomarker in sepsis and septic shock," *Cells*, vol. 8, no. 2, p. 174, 2019.

[96] F. Mearelli, N. Fiotti, C. Giansante et al., "Derivation and validation of a biomarker-based clinical algorithm to rule out sepsis from noninfectious systemic inflammatory response syndrome at emergency department admission," *Critical Care Medicine*, vol. 46, no. 9, pp. 1421–1429, 2018.

[97] R. G. Ferreira, L. C. Rodrigues, D. C. Nascimento et al., "Galectin-3 aggravates experimental polymicrobial sepsis by impairing neutrophil recruitment to the infectious focus," *Journal of Infection*, vol. 77, no. 5, pp. 391–397, 2018.

[98] D. Borgel, S. Clauser, C. Bornstain et al., "Elevated growth-arrest-specific protein 6 plasma levels in patients with severe sepsis," *Critical Care Medicine*, vol. 34, no. 1, pp. 219–222, 2006.

[99] S. Gibot, F. Massin, A. Cravoisy et al., "Growth arrest-specific protein 6 plasma concentrations during septic shock," *Critical Care*, vol. 11, no. 1, p. R8, 2007.

[100] C. Ekman, A. Linder, P. Akesson, and B. Dahlbäck, "Plasma concentrations of Gas6 (growth arrest specific protein 6) and its soluble tyrosine kinase receptor sAxl in sepsis and systemic inflammatory response syndromes," *Critical Care*, vol. 14, no. 4, p. R158, 2010.

[101] G. Stalder, Y. A. Que, S. Calzavarini et al., "Study of early elevated Gas6 plasma level as a predictor of mortality in a prospective cohort of patients with sepsis," *PLoS One*, vol. 11, no. 10, p. e0163542, 2016.

[102] L.-C. Yeh, P. W. Huang, K. H. Hsieh et al., "Elevated plasma levels of Gas6 are associated with acute lung injury in patients with severe sepsis," *The Tohoku Journal of Experimental Medicine*, vol. 243, no. 3, pp. 187–193, 2017.

[103] S. Fujishima, et al.S. Gando, S. Daizoh et al., "Infection site is predictive of outcome in acute lung injury associated with severe sepsis and septic shock," *Respirology*, vol. 21, no. 5, pp. 898–904, 2016.

[104] Y.-J. Lee, J. Y. Han, J. Byun et al., "Inhibiting Mer receptor tyrosine kinase suppresses STAT1, SOCS1/3, and NF-κB activation and enhances inflammatory responses in lipopolysaccharide-induced acute lung injury," *Journal of Leukocyte Biology*, vol. 91, no. 6, pp. 921–932, 2012.

[105] J.-Y. Choi, J. Y. Seo, Y.-S. Yoon, Y.-J. Lee, H.-S. Kim, and J. L. Kang, "Mer signaling increases the abundance of the transcription factor LXR to promote the resolution of acute sterile inflammation," *Science Signaling*, vol. 8, no. 365, p. ra21, 2015.

[106] C. Cao, T. Ma, Y.-F. Chai, and S.-T. Shou, "The role of regulatory T cells in immune dysfunction during sepsis," *World Journal of Emergency Medicine*, vol. 6, no. 1, p. 5, 2015.

[107] J. G. Heuer, T. Zhang, J. Zhao et al., "Adoptive transfer of in vitro-stimulated $CD4^+CD25^+$ regulatory T cells increases bacterial clearance and improves survival in polymicrobial sepsis," *The Journal of Immunology*, vol. 174, no. 11, pp. 7141–7146, 2005.

[108] G.-J. Zhao, J. Y. Zheng, J. L. Bian et al., "Growth arrest-specific 6 enhances the suppressive function of $CD4^+CD25^+$ regulatory T cells mainly through Axl receptor," *Mediators of Inflammation*, vol. 2017, Article ID 6848430, 13 pages, 2017.

[109] A. J. Lewis, C. W. Seymour, and M. R. Rosengart, "Current murine models of sepsis," *Surgical Infections*, vol. 17, no. 4, pp. 385–393, 2016.

[110] J. Ni, M. Lin, Y. Jin et al., "Gas6 attenuates sepsis-induced tight junction injury and vascular endothelial hyperpermeability via the Axl/NF-κB signaling pathway," *Frontiers in Pharmacology*, vol. 10, 2019.

Hindawi
Disease Markers
Volume 2019, Article ID 7838195, 9 pages
https://doi.org/10.1155/2019/7838195

Review Article

Gas6/TAM Receptors in Systemic Lupus Erythematosus

Philip L. Cohen[1] **and Wen-Hai Shao**[2]

[1]*Section of Rheumatology, Department of Medicine, Temple University, Philadelphia, PA 19140, USA*
[2]*Division of Immunology, Allergy and Rheumatology, Department of Internal Medicine, College of Medicine, University of Cincinnati, OH 45267, USA*

Correspondence should be addressed to Wen-Hai Shao; shaowi@ucmail.uc.edu

Received 18 March 2019; Revised 14 June 2019; Accepted 25 June 2019; Published 9 July 2019

Guest Editor: Pier P. Sainaghi

Systemic lupus erythematosus (SLE) is a multiorgan autoimmune disease associated with impaired immune system regulation. The exact mechanisms of SLE development remain to be elucidated. TAM receptor tyrosine kinases (RTKs) are important for apoptotic cell clearance, immune homeostasis, and resolution of immune responses. TAM deficiency leads to lupus-like autoimmune diseases. Activation of TAM receptors leads to proteolytic cleavage of the receptors, generating soluble forms of TAM. Circulating TAM receptors have an immunoregulatory function and may also serve as biomarkers for disease prognosis. Here, we review the biological function and signaling of TAM RTKs in the development and pathogenesis of lupus and lupus nephritis. Targeting Gas6/TAM pathways may be of therapeutic benefit. A discussion of potential TAM activation and inhibition in the treatment of lupus and lupus nephritis is included.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by impairment of the regulation of the immune system and the development of immune-mediated inflammation in multiple organs [1]. Lupus nephritis (LN) is a serious complication requiring aggressive immunosuppression. Despite therapy, about 10% of LN patients develop end-stage renal disease [2]. Defective clearance of apoptotic cells is believed to promote the development of SLE by increasing the availability of potential self immunogens in SLE patients [3]. The TAM (Tyro3, Axl, and Mer) receptor tyrosine kinases (RTKs) are membrane proteins that recognize apoptotic cells with the help of the intermediate molecules, Protein S (ProS) and growth arrest-specific 6 (Gas6) [4–7]. The extracellular part of TAM receptors consists of two Ig-like and two fibronectin-type III domains, which can be proteolytically shed from the cells, forming the soluble forms of TAM receptors [8]. Though serving as classic tyrosine kinase membrane receptors activating proliferation and survival, cell adhesion, and migration in malignant cells, TAM receptors have been implicated in innate and adaptive immunities and have been recently shown to play prominent roles in immune regulation [4].

Gas6 and ProS are vitamin K-dependent TAM ligands that have been studied the most, but other TAM ligands have been reported (Tubby, Tulp1, and Galectin-3) [9–11]. Gas6 and ProS have the same domain structure, with the exception of the thrombin cleavage sites presented in ProS. Gas6 can bind to and activate all three TAM receptors, but ProS only activates Tyro3 and Mer [8, 12, 13]. However, it is worthy of note that Gas6 and ProS are also important regulators of thrombosis and many other biological processes [14]. Gas6 is believed to contribute to platelet aggregation [15]. Deficiency of Gas6 prevents venous and arterial thrombosis [14, 16]. Knockout of ProS and Gas6 leads to the loss of Mer-dependent retinal pigment epithelium phagocytosis in mice [17], suggesting a redundant role of TAM ligands and dominant role of Mer in the phagocytosis of photoreceptors.

Here, we review the current literature on immunobiological function of TAM receptors and their ligands in SLE. We discuss the soluble TAM receptors in the context of disease development and prognosis. Finally, we explore

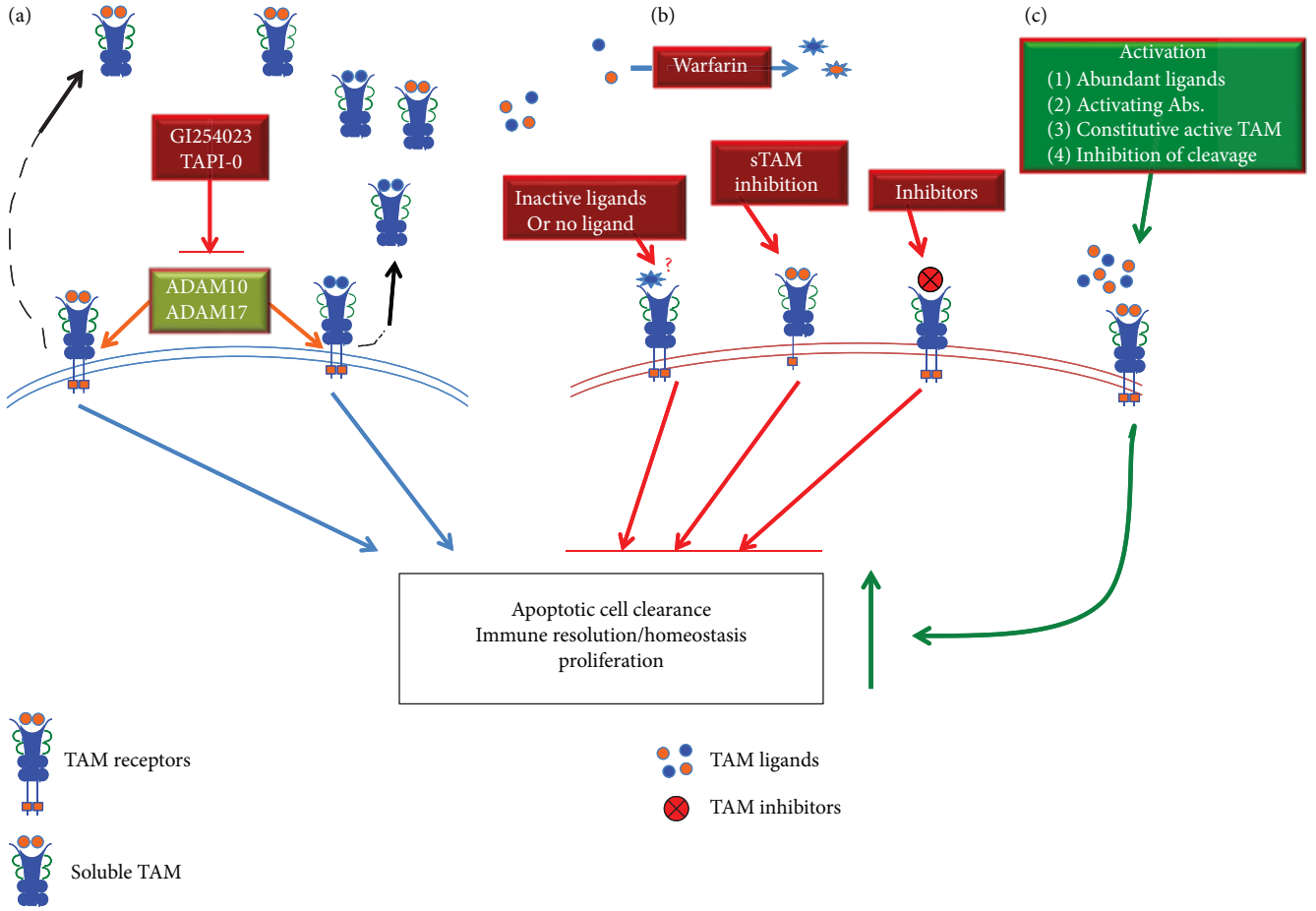


Figure 1: Pathogenic and therapeutic roles of TAM receptors in lupus. (a) Normal TAM functions in lupus are shown in light blue arrows [4–8]. Ligand engagement leads to receptor dimerization and autophosphorylation, which result in the activation of TAM downstream signaling. The effector phase of TAM activation links to apoptotic cell clearance, immune homeostasis, and cell survival/proliferation [8, 21, 35]. TAM activation is reported to be associated with metalloproteinase, ADAM10 and ADAM17, activated cleavage of the receptors [47]. sTAM are released thereby [48–51]. (b) Pathogenic roles of TAM receptors are shown in red arrows. Defects of TAM activation occur in several conditions, including inactivation/exhaustion of the ligands, TAM inhibition, and sTAM-mediated inactivation [48, 54–58]. The consequence of impaired TAM function will be the accumulation of apoptotic debris and breakdown of immune tolerance and autoimmune disease develops over time [36, 39]. (c) Potential TAM-targeted therapeutic roles in lupus are shown in the green box [34, 77–79]. Enhancement of TAM activation can be achieved through exogenous administration with TAM ligands, activating Abs, or inhibition of sTAM generation. Construction of constitutive activated TAM is also on the way.

strategies that target TAM receptors in lupus and lupus nephritis. We will focus mainly on the roles of Axl and Mer in lupus and lupus nephritis. Though Tyro3 expression and function primarily associate with the central nervous system [18–20], we will review the published Tyro3 studies under the scope of immune regulation suggesting a function in the pathogenesis/therapeutics in lupus.

2. TAM Signaling Pathways and Immunobiological Functions: Implication of Function in SLE

Activation of the TAM receptors has been shown to affect a diversity of cellular functions, including survival, proliferation, migration, and phagocytosis (Figure 1). Numerous studies of TAM receptor activation and signaling have been published. However, variable outcomes have resulted in an inconsistent understanding of TAM signaling. A thorough investigation of TAM ligand/receptor specificity and optimal activation was undertaken by the Lemke group [21]. Purified Gas6 and ProS are capable of inducing Tyro3 and Mer phosphorylation, which also allow cross-species ligand-receptor activation. However, Axl could be activated only by Gas6 [21, 22]. Most importantly, when different compounds and combinations of ligands and Phosphatidylserine (PtdSer) were compared, maximal activation of the TAM receptors required the simultaneous presence of ligands, PtdSer, and calcium ions [21]. Interestingly, the widely used goat anti-Mer (AF591) and anti-Axl (AF759) antibodies from R&D Systems induced receptor phosphorylation [23], but blocked receptor-mediated phagocytosis of apoptotic cells [24], simultaneously. Nevertheless, Gas6 and ProS are present in the serum at a concentration of 0.2 nM [25] and

350 nM [26], respectively. Axl can be activated by Gas6 at a concentration as low as 1 nM [21]. The microenvironmental concentration of Gas6 may be higher than 1 nM, especially in inflammatory conditions. It is a mystery why TAM receptors are not constitutively activated *in vivo* by their circulating ligands. One mechanism is probably through complex inhibition. Over 60% of ProS is actually bound to C4b-binding protein [26] and all Gas6 is bound to sAxl [25]. On the other hand, optimal TAM activation engages ligand, PtdSer, and calcium, a condition that can be mostly satisfied with the presence of apoptotic cells but can also occur during platelet and endothelial cell activation. The presence of PtdSer on the surface of apoptotic cells is probably the optimal condition for ligand-induced receptor dimerization, which causes a conformational change in the cytoplasmic domain that activates the tyrosine kinase catalytic activity. It may also be possible that low level phosphorylation of TAM receptors by circulating ligands occurs. Such activation may be important for the maintenance of quiescent stage immune homeostasis. However, the exact mechanism demands in-depth investigation.

Much of the early work on TAM signaling pathways was done with chimeric receptors conjugating a TAM receptor intracellular kinase domain to an extracellular receptor domain not normally expressed in the target cells [8]. However, care must be taken when interpreting the data, as multiple factors may contribute to the final outcome of the signaling cascade, including receptor dimerization, extracellular engagement, and ligand/PtdSer complexes in association with the apoptotic cell presence. Most recent work on TAM signaling focuses on the readout of proliferation, migration, and invasion due to a pivotal role of TAM receptors in cancer metastasis, survival, and therapy resistance [27, 28]. Nevertheless, early work by Rothlin et al. demonstrated that TAM receptor signals control the amplification of TLR signaling. The best-known signaling molecules activated by TAM receptors in this scenario are SOCS1/3 [7], as reviewed elsewhere [4, 6, 29]. TAM receptors are potent suppressors of T-cell dendritic cell (DC) responses [30, 31]. However, the signaling cascade has not been worked out. New discoveries have been pointed to distinct and nonoverlapping roles of Axl and Mer in regulating immune responses [32]. Mer is expressed in many cells and functions in the maintenance of immune homeostasis within tissues. Axl expression is inducible and is responsive to inflammatory conditions [32]. Axl activation leads to marked suppression of *Ifn* mRNA in mice injected with anti-Axl antibodies [23], and similar inhibition was also observed in DCs when Axl is activated by Gas6 [7]. Mer was found to be highly expressed on endothelial cells in mouse kidneys [33]. We found that Mer activation leads to the suppression of LPS signaling in primary glomerular endothelial cells through the upregulation of SOCS3 but not SOCS1 [33]. Axl expression in mesangial cells is promoted largely by transcription factor Sp1, but not Sp3. The activation of Axl in mesangial cells links to Akt activation, leading to mTOR phosphorylation [34]. It seems reasonable to conclude that TAM receptors have distinct patterns of expression and disparate signaling and that their function is thus both tissue- and stress-dependent.

TAM receptors play a critical role in regulating innate immunity and maintaining the efficiency of apoptotic cell clearance. TAM receptor-facilitated recognition of apoptotic cells requires the binding of TAM ligands, as bridging molecules, to PtdSer exposed on the surface of apoptotic cells [8, 35]. TAM receptors are of special significance for macrophage and monocyte recognition of apoptotic cells [35–37], a process thought to be impaired in SLE patients [38]. TAM-facilitated phagocytosis of apoptotic cells releases anti-inflammatory cytokines by the phagocytes and induces immune tolerance by supplying autoantigens in a noninflammatory environment [38]. The importance of the involvement of the TAM receptors in the regulation of immunity has been clearly demonstrated in animal models. Mice lacking Mer only (single knockout) suffer from impaired clearance of infused apoptotic cells and go on to develop moderate lupus-like autoimmunity [36]. Mice lacking both Mer and Axl receptors develop more severe lupus-like pathology. Ablation of all three TAM receptors in mice (TAM triple knockout) results in a broad spectrum of autoimmune disease with high titer of autoantibodies and pathologies affecting multiple organs, including the kidney [39].

TAM receptors actively participate in immune regulation. Early studies by Rothlin et al. revealed that TAM receptors mediate an inhibitory role in TLR signaling through a negative feedback mechanism, which occurs via the induction of SOCS1 and SOCS3 [7]. Further research suggests that activated T cells produce ProS, which signals through TAM receptors on DCs to limit the magnitude of DC activation [31]. Among the three TAM receptors, Mer seems to be the most potent as an immune regulation checkpoint. Mer-Fc protein, used to mimic Mer on DCs, suppresses activation of naïve and antigen-specific memory T cells [30]. When the constitutively activated form of Mer-Fc fusion protein was expressed on 293T cells, PD-L1 transcripts and surface expression were increased. PD-L1 is well known for regulating the balance between T cell activation, tolerance, and immunopathology [40]. Mer also plays a critical role in germinal center (GC) apoptotic cell clearance by tangible body macrophages [41]. Prolonged apoptotic cell accumulation in GCs of Mer-deficient mice results in elevated B cell and $CD4^+$ T_H cell responses, leading to autoantibody production [42]. Tyro3, on the other hand, selectively inhibits type 2 immunity. Accordingly, house dust mite-(HDM-) sensitized Tyro3-KO mice display enhanced type 2 responses, accompanied by increased total and effector memory $CD4^+$ T cells and type 2 cytokines (IL-4 and IL-13) [43]. Axl is the least studied TAM receptor in immune regulation. Most of the studies have focused on its role in the survival and proliferative function of cancer cells resistant to therapy [44, 45]. It seems reasonable to assume that Axl is less important in immune regulation, as Axl-KO mice are viable and healthy and have a normal life span with no gross anatomical defects [46]. However, early studies of TAM immunoregulatory functions were achieved in the TAM triple knockout mice or Axl/Mer-double knockout mice [7, 31]. It is possible that the immune regulatory function of Axl is redundant compared to that of Mer and Tyro3. Axl may be important

in immune regulation only when Mer is deficient or Mer and Tyro3 are both deficient. It is also possible that Axl and Mer heterodimers are important in regulating immune responses, while Axl homodimers lack this function.

3. TAM Ligands and Soluble TAM in SLE Pathogenesis

The heterogeneous features of SLE call for the identification of biomarkers that can quantify disease activity and severity. The extracellular domains (two Ig-like and two fibronectin-III domains) of TAM receptors can be proteolytically cleaved by metalloproteases to yield soluble forms of the receptor (sTAM). A disintegrin and metalloproteinase 10 (ADAM10) and 17 are the two main enzymes responsible for the generation of sTAM [47] (Figure 1). All three TAM receptors are shed from the cells and their soluble forms have been found in plasma, although the exact roles of sTAM remain to be further elucidated. Recent reports have evaluated the plasma concentrations of sTAM and ligands in SLE and SLE nephritis. In general, increased plasma levels of all 3 soluble forms of TAM receptors were reported to correlate with the SLE disease activity index (SLEDAI). However, variable results were reported by different groups.

Among all three TAM receptors, the soluble form of Mer was mostly investigated and constant results were achieved throughout all groups of SLE patients studied. Significantly increased plasma concentration of sMer was reported in SLE patient cohorts from China [48], Sweden [49], UK [50], and Spain [51], compared to age- and sex-matched healthy controls, respectively. These increased plasma sMer levels positively correlated with disease activity and severity measured by the SLEDAI score. Several groups made further association analysis of sMer levels with clinical and serological parameters. A strong association of higher plasma levels of sMer with nephritis was reported by three groups [49, 52, 53]. Zhu et al. studied 108 Chinese SLE patients and found that plasma levels of sMer were significantly elevated in patients with proteinuria compared to those without increased urinary protein [53]. Similarly, Wu et al. found that sMer correlated with the presence of nephritis in a study of 96 Swedish SLE patients [49]. It was subsequently reported that SLE nephritis patients with higher sMer levels tended to suffer from proliferative glomerulonephritis (GN) [52]. Notably, there was a correlation between the concentration of sMer and the presence of autoantibodies [53]. In general, findings pointed to the important function of Mer in macrophage and dendritic cell phagocytosis of apoptotic cells. Increased sMer in the plasma can compete with cell-bound Mer, thus acting as a decoy receptor, resulting in defective phagocytosis, a phenomenon observed in human SLE patients. Excessive apoptotic debris may be a source of self immunogens that together with dangerous stimulating signals released in the process results in autoimmunity.

Significantly elevated concentrations of plasma sAxl in SLE patients were repeatedly reported by different groups to correlate with disease activity and severity in lupus [48, 52, 54, 55] and lupus nephritis [52]. Plasma levels of sAxl followed the same trend as the plasma levels of sMer. Similar functions were also suggested. Soluble forms of Tyro3 have been less studied in SLE patients. Significant positive linear correlations with SLEDAI were reported in two cohorts of SLE patients from Sweden [49] and Spain [51]. However, the increased concentrations of sTyro3 were not related to disease activity parameters (SLEDAI, low C1q, or the presence of nephritis) in Swedish SLE patients [49].

There remain controversies regarding serum levels of Gas6 and ProS in SLE pathogenesis. Recarte-Pelz and colleagues reported a correlation of plasma concentrations of Gas6 and ProS with SLE disease activity, yet Gas6 levels were higher while ProS levels were lower in the SLE patients [51]. Suh et al. found no significant overall differences between the levels of ProS and Gas6 in SLE patients and healthy controls [56]. ProS levels were highly correlated with C3 and C4 levels, and lower ProS levels were found in SLE patients with a history of serositis, neurologic disorder, hematologic disorder, and immunologic disorder [56]. On the other hand, Zhu et al. found that severe SLE patients (SLEDAI $\geq$ 10) showed significantly lower Gas6 levels [48]. Significantly lower Gas6 levels were associated with shrinking lung syndrome in SLE patients in another study [55]. High Gas6 levels were also observed in SLE patients with GN [52]. Altered but not consistent levels of Gas6 and ProS with disease activity in SLE may reflect the important function of the molecules in regulating thrombosis and inflammation. Gas6 is expressed in many tissues, including capillary endothelial cells, vascular smooth muscle cells, and bone marrow cells [14, 16]. Gas6 acts as an acute-phase reactant and is increased during sepsis and pancreatitis [54]. ProS has a critical function in regulating coagulation. Lower free ProS concentrations in plasma are associated with an increased risk of deep venous thromboembolism [57]. Free ProS acts as a cofactor for activated protein C. Nevertheless, plasma concentrations of Gas6 are approximately 1,000-fold lower than those of ProS [58]. In summary, the significance of plasma levels of Gas6 or ProS in SLE patients is complex and may depend on SLE activity and severity and may also be influenced by other clinical parameters, including lupus disease manifestations (lupus nephritis, vasculitis, arthritis, etc.). We observed significantly lower levels of Gas6 in the serum of Axl-KO nephritic mice compared to the WT nephritic mice. Interestingly, the Axl inhibitor-treated nephritic mice also showed significantly lower serum levels of Gas6 in this study (Shao et al. unpublished data). Taken together, Gas6 may serve as a disease diagnostic biomarker for SLE as increased Gas6 levels correlated with SLE severity. Gas6 may also serve as a biomarker for SLE therapeutics, especially in lupus nephritis.

The exact mechanisms regulating sTAM shedding remain unknown. Nevertheless, the upregulation of sTAM in plasma has been suggested by many studies to serve as a biomarker of disease activity and severity in SLE. It may also serve as a marker for disease prognosis. Hilliard et al. [59] found that Mer expression on monocytes of SLE patients receiving prednisone correlated strongly with the dose of corticosteroid. The potential in vivo functions of the soluble TAM receptors can be speculated as follows: (1) interfere with the TAM-mediated clearance of apoptotic cells and

platelet aggregation and (2) form a complex with the ligands to compete with cell-bound receptors, functioning as decoy receptors (Figure 1). These functions have been demonstrated with in vivo experiments. However, it is also possible that sTAM receptors activate cell-bound receptors through the formation of homo- or heterodimers to induce signal transduction pathways. This has not been experimentally approved.

4. Function of TAM RTKs in the Kidney

The critical role of TAM receptors in kidney homeostasis was first implied by Graham et al.'s report of strong Mer expression in renal tissues [60, 61]. Excessive circulating levels of sMer, indicating increased systemic shedding, have been recently related to the severity of nephritis in patients with lupus and the rapidity of renal function decline in patients with chronic kidney disease of variable origin [62]. Interestingly, lupus nephritis patients with higher sMer, sAxl, and Gas6 levels tended to suffer from proliferative GN [52]. We were the first to identify the protective role of Mer in a mouse model of lupus nephritis [63]. Mer-KO mice were much more susceptible to antiglomerular basement membrane-(anti-GBM-) induced nephritis than age- and sex-matched WT mice. The early-onset renal damage in Mer-KO mice was associated with increased inflammatory cytokines, excessive apoptotic cells, and massive infiltration with neutrophils [63]. Observations suggest that the primary function of Mer in glomerular endothelial cells is to mediate phagocytosis of apoptotic cells and to attenuate immune responses through modulation of cytokine production.

The Gas6/Axl axis has been recently extensively studied in the kidney. Although Gas6 and Axl are generally not detected in healthy kidneys, they are strongly upregulated on mouse and human glomerular mesangial cells and tubular cells at sites of inflammation [12, 64–66]. Gas6 activation of the mesangial Axl receptor has been implicated in the development of glomerular damage in several GN, including diabetic nephritis, lupus nephritis, and IgA nephropathy [64, 67, 68]. Gas6 is an autocrine growth factor for mesangial cells [69]. Gas6 and its receptor Axl play a critical role in the development of GN. Dysregulation of circulating Gas6 is associated with renal disease and is inversely proportional to renal function [65]. Significantly increased levels of Gas6 and ProS were found in chronic kidney disease patients compared with normal controls [65]. Warfarin and the extracellular domain of Axl inhibit mesangial cell proliferation [67]. However, Gas6 inhibition with warfarin might affect the coagulation cascades and prevent thrombotic events by diminishing coagulation, because the coagulation cascade is activated in severe human and experimental GN [70]. Furthermore, warfarin also inhibits the function of ProS, which is more critical in regulating coagulation and protein C activation. Previous studies using Gas6-KO mice have shown a pathological role for Gas6 in anti-GBM nephritis and streptozotocin-induced diabetic nephropathy [71, 72]. Loss of Gas6 protected against mesangial cell proliferation and glomerular hypertrophy and improved proteinuria and survival [72, 73]. These studies suggest that inhibitors of the Gas6/Axl pathway may be of therapeutic benefit in these forms of renal injury. Our recent publication reported that Axl contributes to anti-GBM antibody nephritis by promoting glomerular mesangial cell survival and proliferation, which leads to glomerular mesangial hypertrophy [74]. We found that Axl activation led to mTOR phosphorylation, which likely contributes to the proliferation of mesangial and tubular cells [34]. The mTOR pathway is a critical contributor to human lupus and lupus nephritis [75]. Targeting mTOR activation through Axl inhibition may provide a safe therapy, since Axl-deficient mice are viable and appear to be normal compared to the WT mice [76]. In contrast, rapamycin suppresses immune function, which may cause serious side effects. The safety of long-term use of rapamycin remains unclear.

5. Targeting Axl/Mer in Lupus and Lupus Nephritis

Given the body of evidence implicating TAM regulation, activation, and proteolytic cleavage in lupus and lupus nephritis, it is surprising that the therapeutic focus of TAM receptors has yet to be developed. However, approaches have been implicated by work in several directions (Figure 1). Early findings showed reduced LPS-induced sMer in the bronchoalveolar lavage fluid in mice pretreated with an ADAM17 inhibitor [77]. Mohan's group demonstrated that combined inhibition with ADAM10 and ADAM17 rescues the unresponsiveness of lupus-prone splenocytes to Gas6 [78]. A similar rescued phenotype was observed in human PBMC [78]. Thus, restoration of TAM function by targeting sTAM proteases may be a fruitful therapeutic approach in SLE. Studies conducted in the Rothlin lab showed that the addition of recombinant ProS to the ProS-deficient T cell culture rescued the ability of activated $ProS^{-/-}$ T cells to regulate DC function [31]. Though high concentrations of ProS exist in the plasma, the most is in the form of protein complexes [26], limiting its biological function. Administration of free ProS may lead to an *in vivo* approach to enhance T cell-mediated DC activation suppression. However, large amounts of ProS administration may interfere with endogenous ProS homeostasis, indirectly favoring the environment of protein C activation [15]. On the other hand, the amount of free/active ProS is sufficient to control coagulation and remains relatively constant even in situations of inflammation [57, 79]. Further investigation may be needed when pursing this option. Considering the activating potential of certain polyclonal anti-TAM antibodies from R&D Systems, a better approach would be to engineer the antibody to maximize the activating potential yet diminish the blocking activities. TAM receptors would thus be activated to magnify the anti-inflammatory activities, yet preserve phagocytic function. Nevertheless, TAM functions are rather complex and diverse. TAM-mediated immune suppression and efferocytosis have been adopted by cancer cells to their advantage. Promoting TAM function in lupus for therapeutics could possibly result in an undesired favorable environment for tumor development.

We have demonstrated a critical role for the Gas6/Axl pathway in mouse models of lupus nephritis [34, 74]. Targeting the Gas6/Axl pathway is a promising therapeutic strategy for lupus nephritis [12, 69, 74]. Targeting Axl and Mer in the field of cancer research has shown promise, since Axl and Mer overexpression has been linked to cancer cell metastasis, poor survival, and drug resistance [28, 80]. Studies of Axl and Mer in cancer cells not only advance our understanding of TAM receptor signaling and function but also facilitate application of TAM therapeutics in lupus. Over a dozen Axl-targeted therapeutics have been developed in the last decade [81]. Several of them are in active clinical trials now, including Axl small molecular inhibitors (BGB324, TP0903, AVB-S6-500, etc.) and Axl antibody (CAB-AXL-ADC) (for a complete list and status go to https://clinicaltrials.gov). R428 (also called BGB324) is the most selective small molecule inhibitor of Axl and the first kinase inhibitor designed to specifically target Axl [81]. Pharmacologic studies revealed favorable absorption after oral administration of R428 that was accompanied by a dose-dependent reduction in tumor volume [82–84] and extended survival in a mouse model of metastatic breast cancer [85]. We demonstrated significant efficacy of R428-mediated Axl inhibition, with decreased proteinuria and increased survival in mice with anti-GBM-induced nephritis [34], one of the best models for uncovering the molecular and pathological mechanisms that lead to human lupus nephritis [86].

6. Conclusions

TAM receptors are essential for the phagocytosis of apoptotic cells, and TAM activation is associated with immunosuppressive responses. TAM deficiency promotes lupus-like autoimmune diseases in mice. Impaired TAM function is associated with lupus disease activity in humans. Plasma levels of soluble TAM receptors generated by proteolytic cleavage and TAM ligands may serve as potential biomarkers for lupus development and prognosis. Finally, encouraging results have been achieved supporting the therapeutic role of TAM receptors in lupus and lupus nephritis.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

This current work is supported by the Research Innovator Award from the College of Medicine and the Junior Faculty Pilot Award from the Department of Internal Medicine, University of Cincinnati, and NIDDK/NIH grant K01DK095067 and R01DK116789.

References

[1] H. L. Pedersen, K. D. Horvei, D. Thiyagarajan, N. Seredkina, and O. P. Rekvig, "Murine and human lupus nephritis: pathogenic mechanisms and theoretical strategies for therapy," *Seminars in Nephrology*, vol. 35, no. 5, pp. 427–438, 2015.

[2] S. Almaani, A. Meara, and B. H. Rovin, "Update on lupus nephritis," *Clinical Journal of the American Society of Nephrology*, vol. 12, no. 5, pp. 825–835, 2017.

[3] L. E. Munoz, K. Lauber, M. Schiller, A. A. Manfredi, and M. Herrmann, "The role of defective clearance of apoptotic cells in systemic autoimmunity," *Nature Reviews Rheumatology*, vol. 6, no. 5, pp. 280–289, 2010.

[4] C. V. Rothlin and G. Lemke, "TAM receptor signaling and autoimmune disease," *Current Opinion in Immunology*, vol. 22, no. 6, pp. 740–746, 2010.

[5] G. Lemke and C. V. Rothlin, "Immunobiology of the TAM receptors," *Nature Reviews. Immunology*, vol. 8, no. 5, pp. 327–336, 2008.

[6] C. V. Rothlin, E. A. Carrera-Silva, L. Bosurgi, and S. Ghosh, "TAM receptor signaling in immune homeostasis," *Annual Review of Immunology*, vol. 33, no. 1, pp. 355–391, 2015.

[7] C. V. Rothlin, S. Ghosh, E. I. Zuniga, M. B. A. Oldstone, and G. Lemke, "TAM receptors are pleiotropic inhibitors of the innate immune response," *Cell*, vol. 131, no. 6, pp. 1124–1136, 2007.

[8] R. M. A. Linger, A. K. Keating, H. S. Earp, and D. K. Graham, "TAM receptor tyrosine kinases: biologic functions, signaling, and potential therapeutic targeting in human cancer," *Advances in Cancer Research*, vol. 100, pp. 35–83, 2008.

[9] N. B. Caberoy, Y. Zhou, and W. Li, "Tubby and tubby-like protein 1 are new MerTK ligands for phagocytosis," *The EMBO Journal*, vol. 29, no. 23, pp. 3898–3910, 2010.

[10] N. B. Caberoy, G. Alvarado, J. L. Bigcas, and W. Li, "Galectin-3 is a new MerTK-specific eat-me signal," *Journal of Cellular Physiology*, vol. 227, no. 2, pp. 401–407, 2012.

[11] N. B. Caberoy, G. Alvarado, and W. Li, "Tubby regulates microglial phagocytosis through MerTK," *Journal of Neuroimmunology*, vol. 252, no. 1-2, pp. 40–48, 2012.

[12] M. Yanagita, "Gas6, warfarin, and kidney diseases," *Clinical and Experimental Nephrology*, vol. 8, no. 4, pp. 304–309, 2004.

[13] D. K. Graham, D. DeRyckere, K. D. Davies, and H. S. Earp, "The TAM family: phosphatidylserine sensing receptor tyrosine kinases gone awry in cancer," *Nature Reviews. Cancer*, vol. 14, no. 12, pp. 769–785, 2014.

[14] J. H. M. van der Meer, T. van der Poll, and C. van 't Veer, "TAM receptors, Gas6, and protein S: roles in inflammation and hemostasis," *Blood*, vol. 123, no. 16, pp. 2460–2469, 2014.

[15] J. H. Foley and E. M. Conway, "Gas6 gains entry into the coagulation cascade," *Blood*, vol. 121, no. 4, pp. 570-571, 2013.

[16] A. Angelillo-Scherrer, P. G. de Frutos, C. Aparicio et al., "Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis," *Nature Medicine*, vol. 7, no. 2, pp. 215–221, 2001.

[17] T. Burstyn-Cohen, E. D. Lew, P. G. Traves, P. G. Burrola, J. C. Hash, and G. Lemke, "Genetic dissection of TAM receptor-ligand interaction in retinal pigment epithelial cell phagocytosis," *Neuron*, vol. 76, no. 6, pp. 1123–1132, 2012.

[18] R. Akkermann, A. Aprico, A. A. Perera et al., "The TAM receptor Tyro3 regulates myelination in the central nervous system," *Glia*, vol. 65, no. 4, pp. 581–591, 2017.

[19] F. Blades, A. Aprico, R. Akkermann, S. Ellis, M. D. Binder, and T. J. Kilpatrick, "The TAM receptor TYRO3 is a critical regulator of myelin thickness in the central nervous system," *Glia*, vol. 66, no. 10, pp. 2209–2220, 2018.

[20] A. L. Prieto, J. L. Weber, S. Tracy, M. J. Heeb, and C. Lai, "Gas6, a ligand for the receptor protein-tyrosine kinase

Tyro-3, is widely expressed in the central nervous system," *Brain Research*, vol. 816, no. 2, pp. 646–661, 1999.

[21] E. D. Lew, J. Oh, P. G. Burrola et al., "Differential TAM receptor-ligand-phospholipid interactions delimit differential TAM bioactivities," *eLife*, vol. 3, 2014.

[22] W.-I. Tsou, K.-Q. N. Nguyen, D. A. Calarese et al., "Receptor tyrosine kinases, TYRO3, AXL, and MER, demonstrate distinct patterns and complex regulation of ligand-induced activation," *The Journal of Biological Chemistry*, vol. 289, no. 37, pp. 25750–25763, 2014.

[23] A. Zagorska, P. G. Traves, E. D. Lew, I. Dransfield, and G. Lemke, "Diversification of TAM receptor tyrosine kinase function," *Nature Immunology*, vol. 15, no. 10, pp. 920–928, 2014.

[24] W. H. Shao, R. A. Eisenberg, and P. L. Cohen, "The Mer receptor tyrosine kinase is required for the loss of B cell tolerance in the chronic graft-versus-host disease model of systemic lupus erythematosus," *Journal of Immunology*, vol. 180, no. 11, pp. 7728–7735, 2008.

[25] C. Ekman, J. Stenhoff, and B. Dahlback, "Gas6 is complexed to the soluble tyrosine kinase receptor Axl in human blood," *Journal of Thrombosis and Haemostasis*, vol. 8, no. 4, pp. 838–844, 2010.

[26] B. Dahlback, "The tale of protein S and C4b-binding protein, a story of affection," *Thrombosis and Haemostasis*, vol. 98, no. 7, pp. 90–96, 2017.

[27] M. Wium, J. Paccez, and L. Zerbini, "The dual role of TAM receptors in autoimmune diseases and cancer: an overview," *Cell*, vol. 7, no. 10, p. 166, 2018.

[28] A. Verma, S. L. Warner, H. Vankayalapati, D. J. Bearss, and S. Sharma, "Targeting Axl and Mer kinases in cancer," *Molecular Cancer Therapeutics*, vol. 10, no. 10, pp. 1763–1773, 2011.

[29] L. A. J. O'Neill, "TAMpering with toll-like receptor signaling," *Cell*, vol. 131, no. 6, pp. 1039–1041, 2007.

[30] R. Cabezo´n, E. A. Carrera-Silva, G. Flo´rez-Grau et al., "MERTK as negative regulator of human T cell activation," *Journal of Leukocyte Biology*, vol. 97, no. 4, pp. 751–760, 2015.

[31] E. A. Carrera Silva, P. Y. Chan, L. Joannas et al., "T cell-derived protein S engages TAM receptor signaling in dendritic cells to control the magnitude of the immune response," *Immunity*, vol. 39, no. 1, pp. 160–170, 2013.

[32] I. Dransfield and S. Farnworth, "Axl and Mer receptor tyrosine kinases: distinct and nonoverlapping roles in inflammation and cancer?," *Advances in Experimental Medicine and Biology*, vol. 930, pp. 113–132, 2016.

[33] Y. Zhen, F. D. Finkelman, and W. H. Shao, "Mechanism of Mer receptor tyrosine kinase inhibition of glomerular endothelial cell inflammation," *Journal of Leukocyte Biology*, vol. 103, no. 4, pp. 709–717, 2018.

[34] Y. Zhen, I. J. Lee, F. D. Finkelman, and W. H. Shao, "Targeted inhibition of Axl receptor tyrosine kinase ameliorates anti-GBM-induced lupus-like nephritis," *Journal of Autoimmunity*, vol. 93, pp. 37–44, 2018.

[35] G. Lemke and T. Burstyn-Cohen, "TAM receptors and the clearance of apoptotic cells," *Annals of the New York Academy of Sciences*, vol. 1209, no. 1, pp. 23–29, 2010.

[36] P. L. Cohen, R. Caricchio, V. Abraham et al., "Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase," *The Journal of Experimental Medicine*, vol. 196, no. 1, pp. 135–140, 2002.

[37] G. Lemke, "Phosphatidylserine is the signal for TAM receptors and their ligands," *Trends in Biochemical Sciences*, vol. 42, no. 9, pp. 738–748, 2017.

[38] W. H. Shao and P. L. Cohen, "Disturbances of apoptotic cell clearance in systemic lupus erythematosus," *Arthritis Research & Therapy*, vol. 13, no. 1, p. 202, 2010.

[39] Q. Lu, M. Gore, Q. Zhang et al., "Tyro-3 family receptors are essential regulators of mammalian spermatogenesis," *Nature*, vol. 398, no. 6729, pp. 723–728, 1999.

[40] L. M. Francisco, V. H. Salinas, K. E. Brown et al., "PD-L1 regulates the development, maintenance, and function of induced regulatory T cells," *The Journal of Experimental Medicine*, vol. 206, no. 13, pp. 3015–3029, 2009.

[41] Z. S. M. Rahman, W.-H. Shao, T. N. Khan, Y. Zhen, and P. L. Cohen, "Impaired apoptotic cell clearance in the germinal center by Mer-deficient tingible body macrophages leads to enhanced antibody-forming cell and germinal center responses," *Journal of Immunology*, vol. 185, no. 10, pp. 5859–5868, 2010.

[42] T. N. Khan, E. B. Wong, C. Soni, and Z. S. M. Rahman, "Prolonged apoptotic cell accumulation in germinal centers of Mer-deficient mice causes elevated B cell and $CD4^+$ Th cell responses leading to autoantibody production," *Journal of Immunology*, vol. 190, no. 4, pp. 1433–1446, 2013.

[43] P. Y. Chan, E. A. C. Silva, D. de Kouchkovsky et al., "The TAM family receptor tyrosine kinase TYRO3 is a negative regulator of type 2 immunity," *Science*, vol. 352, no. 6281, pp. 99–103, 2016.

[44] E. Rankin and A. Giaccia, "The receptor tyrosine kinase AXL in cancer progression," *Cancers*, vol. 8, no. 11, p. 103, 2016.

[45] Y. Shen, X. Chen, J. He, D. Liao, and X. Zu, "Axl inhibitors as novel cancer therapeutic agents," *Life Sciences*, vol. 198, pp. 99–111, 2018.

[46] H. Axelrod and K. J. Pienta, "Axl as a mediator of cellular growth and survival," *Oncotarget*, vol. 5, no. 19, pp. 8818–8852, 2014.

[47] J. G. Weinger, K. M. Omari, K. Marsden, C. S. Raine, and B. Shafit-Zagardo, "Up-regulation of soluble Axl and Mer receptor tyrosine kinases negatively correlates with Gas6 in established multiple sclerosis lesions," *The American Journal of Pathology*, vol. 175, no. 1, pp. 283–293, 2009.

[48] H. Zhu, X. Sun, L. Zhu et al., "Different expression patterns and clinical significance of mAxl and sAxl in systemic lupus erythematosus," *Lupus*, vol. 23, no. 7, pp. 624–634, 2014.

[49] J. Wu, C. Ekman, A. Jonsen et al., "Increased plasma levels of the soluble Mer tyrosine kinase receptor in systemic lupus erythematosus relate to disease activity and nephritis," *Arthritis Research & Therapy*, vol. 13, no. 2, p. R62, 2011.

[50] L. Ballantine, A. Midgley, D. Harris, E. Richards, S. Burgess, and M. W. Beresford, "Increased soluble phagocytic receptors sMer, sTyro3 and sAxl and reduced phagocytosis in juvenile-onset systemic lupus erythematosus," *Pediatric Rheumatology Online Journal*, vol. 13, no. 1, 2015.

[51] P. Recarte-Pelz, D. Tàssies, G. Espinosa et al., "Vitamin K-dependent proteins GAS6 and protein S and TAM receptors in patients of systemic lupus erythematosus: correlation with common genetic variants and disease activity," *Arthritis Research & Therapy*, vol. 15, no. 2, p. R41, 2013.

[52] S. Gong, Z. Xu, Y. Liu et al., "Plasma sMer, sAxl and GAS6 levels correlate with disease activity and severity in lupus

nephritis," *European Journal of Clinical Investigation*, vol. 49, no. 3, article e13064, 2019.

[53] H. Zhu, X. Sun, L. Zhu et al., "The expression and clinical significance of different forms of Mer receptor tyrosine kinase in systemic lupus erythematosus," *Journal of Immunology Research*, vol. 2014, Article ID 431896, 12 pages, 2014.

[54] C. Ekman, A. Jonsen, G. Sturfelt, A. A. Bengtsson, and B. Dahlback, "Plasma concentrations of Gas6 and sAxl correlate with disease activity in systemic lupus erythematosus," *Rheumatology (Oxford)*, vol. 50, no. 6, pp. 1064–1069, 2011.

[55] T. A. Gheita, I. H. Bassyouni, and R. H. Bassyouni, "Plasma concentrations of growth arrest specific protein 6 and the soluble form of its tyrosine kinase receptor Axl in patients with Systemic lupus erythematosus and Behçets disease," *Journal of Clinical Immunology*, vol. 32, no. 6, pp. 1279–1286, 2012.

[56] C. H. Suh, B. Hilliard, S. Li, J. T. Merrill, and P. L. Cohen, "TAM receptor ligands in lupus: protein S but not Gas6 levels reflect disease activity in systemic lupus erythematosus," *Arthritis Research & Therapy*, vol. 12, no. 4, p. R146, 2010.

[57] P. Garcia de Frutos, P. Fuentes-Prior, B. Hurtado, and N. Sala, "Molecular basis of protein S deficiency," *Thrombosis and Haemostasis*, vol. 98, no. 3, pp. 543–556, 2007.

[58] S. Hafizi and B. Dahlback, "Gas6 and protein S. Vitamin K-dependent ligands for the Axl receptor tyrosine kinase subfamily," *The FEBS Journal*, vol. 273, no. 23, pp. 5231–5244, 2006.

[59] B. A. Hilliard, G. Zizzo, M. Ulas, M. K. Linan, J. Schreiter, and P. L. Cohen, "Increased expression of Mer tyrosine kinase in circulating dendritic cells and monocytes of lupus patients: correlations with plasma interferon activity and steroid therapy," *Arthritis Research & Therapy*, vol. 16, no. 2, p. R76, 2014.

[60] D. K. Graham, T. L. Dawson, D. L. Mullaney, H. R. Snodgrass, and H. S. Earp, "Cloning and mRNA expression analysis of a novel human protooncogene, c-mer," *Cell Growth & Differentiation*, vol. 5, no. 6, pp. 647–657, 1994.

[61] D. K. Graham, G. W. Bowman, T. L. Dawson, W. L. Stanford, H. S. Earp, and H. R. Snodgrass, "Cloning and developmental expression analysis of the murine c-mer tyrosine kinase," *Oncogene*, vol. 10, no. 12, pp. 2349–2359, 1995.

[62] P. Ochodnicky, L. Lattenist, M. Ahdi et al., "Increased circulating and urinary levels of soluble TAM receptors in diabetic nephropathy," *The American Journal of Pathology*, vol. 187, no. 9, pp. 1971–1983, 2017.

[63] W. H. Shao, Y. Zhen, J. Rosenbaum et al., "A protective role of Mer receptor tyrosine kinase in nephrotoxic serum-induced nephritis," *Clinical Immunology*, vol. 136, no. 2, pp. 236–244, 2010.

[64] A. Fiebeler, J. K. Park, D. N. Muller et al., "Growth arrest specific protein 6/Axl signaling in human inflammatory renal diseases," *American Journal of Kidney Diseases*, vol. 43, no. 2, pp. 286–295, 2004.

[65] I. J. Lee, B. Hilliard, A. Swami et al., "Growth arrest-specific gene 6 (Gas6) levels are elevated in patients with chronic renal failure," *Nephrology, Dialysis, Transplantation*, vol. 27, no. 11, pp. 4166–4172, 2012.

[66] M. Yanagita, "The role of the vitamin K-dependent growth factor Gas6 in glomerular pathophysiology," *Current Opinion in Nephrology and Hypertension*, vol. 13, no. 4, pp. 465–470, 2004.

[67] K. Nagai, H. Arai, M. Yanagita et al., "Growth arrest-specific gene 6 is involved in glomerular hypertrophy in the early stage of diabetic nephropathy," *The Journal of Biological Chemistry*, vol. 278, no. 20, pp. 18229–18234, 2003.

[68] K. Nagai, M. Miyoshi, T. Kake et al., "Dual involvement of growth arrest-specific gene 6 in the early phase of human IgA nephropathy," *PLoS One*, vol. 8, no. 6, article e66759, 2013.

[69] M. Yanagita, H. Arai, K. Ishii et al., "Gas6 regulates mesangial cell proliferation through Axl in experimental glomerulonephritis," *The American Journal of Pathology*, vol. 158, no. 4, pp. 1423–1432, 2001.

[70] T. Madhusudhan, B. A. Kerlin, and B. Isermann, "The emerging role of coagulation proteases in kidney disease," *Nature Reviews. Nephrology*, vol. 12, no. 2, pp. 94–109, 2016.

[71] W. Li, J. Wang, L. Ge, J. Shan, C. Zhang, and J. Liu, "Growth arrest-specific protein 6 (Gas6) as a noninvasive biomarker for early detection of diabetic nephropathy," *Clinical and Experimental Hypertension*, vol. 39, no. 4, pp. 382–387, 2017.

[72] M. Yanagita, Y. Ishimoto, H. Arai et al., "Essential role of Gas6 for glomerular injury in nephrotoxic nephritis," *The Journal of Clinical Investigation*, vol. 110, no. 2, pp. 239–246, 2002.

[73] M. Yanagita, K. Ishii, H. Ozaki et al., "Mechanism of inhibitory effect of warfarin on mesangial cell proliferation," *Journal of the American Society of Nephrology*, vol. 10, no. 12, pp. 2503–2509, 1999.

[74] Y. Zhen, S. O. Priest, and W. H. Shao, "Opposing roles of tyrosine kinase receptors Mer and Axl determine clinical outcomes in experimental immune-mediated nephritis," *Journal of Immunology*, vol. 197, no. 6, pp. 2187–2194, 2016.

[75] D. Fernandez and A. Perl, "mTOR signaling: a central pathway to pathogenesis in systemic lupus erythematosus?," *Discovery Medicine*, vol. 9, no. 46, pp. 173–178, 2010.

[76] H. Wang, S. Cnhen, Y. Chen et al., "The role of Tyro 3 subfamily receptors in the regulation of hemostasis and megakaryocytopoiesis," *Haematologica*, vol. 92, no. 5, pp. 643–650, 2007.

[77] J. Y. Choi, H. J. Park, Y. J. Lee et al., "Upregulation of Mer receptor tyrosine kinase signaling attenuated lipopolysaccharide-induced lung inflammation," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 344, no. 2, pp. 447–458, 2013.

[78] J. J. Orme, Y. Du, K. Vanarsa et al., "Heightened cleavage of Axl receptor tyrosine kinase by ADAM metalloproteases may contribute to disease pathogenesis in SLE," *Clinical Immunology*, vol. 169, pp. 58–68, 2016.

[79] O. Criado García, P. Sánchez-Corral, and S. Rodríguez de Córdoba, "Isoforms of human C4b-binding protein. II. Differential modulation of the C4BPA and C4BPB genes by acute phase cytokines," *Journal of Immunology*, vol. 155, pp. 4037–4043, 1995.

[80] G. Wu, Z. Ma, Y. Cheng et al., "Targeting Gas6/TAM in cancer cells and tumor microenvironment," *Molecular Cancer*, vol. 17, no. 1, p. 20, 2018.

[81] S. H. Myers, V. G. Brunton, and A. Unciti-Broceta, "AXL inhibitors in cancer: a medicinal chemistry perspective," *Journal of Medicinal Chemistry*, vol. 59, no. 8, pp. 3593–3608, 2015.

[82] N. Bansal, P. J. Mishra, M. Stein, R. S. DiPaola, and J. R. Bertino, "Axl receptor tyrosine kinase is up-regulated in metformin resistant prostate cancer cells," *Oncotarget*, vol. 6, no. 17, pp. 15321–15331, 2015.

[83] E. D. Fleuren, M. H. Hillebrandt-Roeffen, U. E. Flucke et al., "The role of AXL and the in vitro activity of the receptor

tyrosine kinase inhibitor BGB324 in Ewing sarcoma," *Oncotarget*, vol. 5, no. 24, pp. 12753–12768, 2014.

[84] K. M. Giles, F. C. Kalinowski, P. A. Candy et al., "Axl mediates acquired resistance of head and neck cancer cells to the epidermal growth factor receptor inhibitor erlotinib," *Molecular Cancer Therapeutics*, vol. 12, no. 11, pp. 2541–2558, 2013.

[85] S. J. Holland, A. Pan, C. Franci et al., "R428, a selective small molecule inhibitor of Axl kinase, blocks tumor spread and prolongs survival in models of metastatic breast cancer," *Cancer Research*, vol. 70, no. 4, pp. 1544–1554, 2010.

[86] Y. Du, Y. Fu, and C. Mohan, "Experimental anti-GBM nephritis as an analytical tool for studying spontaneous lupus nephritis," *Archivum Immunologiae et Therapiae Experimentalis*, vol. 56, no. 1, pp. 31–40, 2008.