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INVITED REVIEW

TAM receptors in phagocytosis: Beyond the mere internalization of particles

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Summary

TYRO3, AXL, and MERTK constitute the TAM family of receptor tyrosine kinases, activated by their ligands GAS6 and PROS1. TAMs are necessary for adult homeostasis in the immune, nervous, reproductive, skeletal, and vascular systems. Among additional cellular functions employed by TAMs, phagocytosis is central for tissue health. TAM receptors are dominant in providing phagocytes with the molecular machinery necessary to engulf diverse targets, including apoptotic cells, myelin debris, and portions of live cells in a phosphatidylserine-dependent manner. Simultaneously, TAMs drive the release of anti-inflammatory and tissue repair molecules. Disruption of the TAM-driven phagocytic pathway has detrimental consequences, resulting in autoimmunity, male infertility, blindness, and disrupted vascular integrity, and which is thought to contribute to neurodegenerative diseases. Although structurally and functionally redundant, the TAM receptors and ligands underlie complex signaling cascades, of which several key aspects are yet to be elucidated. We discuss similarities and differences between TAMs and other phagocytic pathways, highlight future directions and how TAMs can be harnessed therapeutically to modulate phagocytosis.

KEYWORDS
homeostasis, inflammation, phagocytosis, TAM receptors

1 | THE MAIN PLAYERS OF TAM SIGNALING: RECEPTORS AND LIGANDS

The TAM pathway is comprised of a unique family of transmembrane receptor tyrosine kinases, and together with their ligands growth-arrest-specific-6 (GAS6) and Protein S (PROS1), they play an essential role in regulating tissue and cellular homeostasis, especially in organs with high cellular turnover. The receptors TYRO3, AXL, and MERTK form the acronym "TAM," and are expressed by almost every cell type across various tissues: These include the immune, reproductive, nervous, skeletal, and vascular systems.[1] The fact that TAM receptors are functionally redundant, and commonly co-expressed in many cells, allows for different ligand–receptor combinations to activate various signaling cascades, tailored to the specific biological context.[2] Both TAM cognate ligands GAS6 and PROS1 are secreted molecules, and may be produced either by TAM-expressing cells or by neighboring cells that do not express the TAM receptors, thus broadening the cell types involved in this pathway. Biologically, TAM signaling superintends various biological functions, among which are proliferation, migration, differentiation, cytoskeletal rearrangements, and anti-inflammatory signaling.[1–4] Some of the functions mentioned above are also linked to the role of TAMs in phagocytosis, the subject of this review.

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2 | COMPONENTS OF THE TAM SIGNALING PATHWAY

Structurally, the TAMs comprise of two amino-terminal extracellular immunoglobulin (Ig)-related domains used for ligand sensing and binding, followed by two fibronectin type III (FNIII) residues, and a single transmembrane domain (Figure 1).[1,5–9] On the intracellular carboxy-terminus, the TAM receptors bear their tyrosine kinase domains which, upon phosphorylation, trigger various intracellular signaling cascades. The TAM receptors were discovered and isolated by independent groups, and subsequently underwent several changes in nomenclature.[1] The identification of their complete cDNA revealed sequence and structural homology,[7,8,10] and allowed their segregation in an independent category of RTKs.[1] They remained orphan receptors for some time, until their ligands were identified in 1995.[11] Although TAM ligands GAS6 and PROS1 share high structural and functional homology, there are also distinctions, including their binding affinities to the TAM receptors. While GAS6 is an agonist for all three receptors, its affinity to AXL is highest, with significantly lower affinity to TYRO3 and MERTK.[11–14] PROS1 activates TYRO3 and MERTK, but not AXL.[15,16] GAS6 and PROS1 are large proteins (~70 kDa), and share similar structure with 42% homology. As shown in Figure 1, their amino-terminus encodes for a Gla domain, followed by four EGF-like repeats. The Gla domain, present in a dozen proteins, is a ~45 amino acids long stretch,[11,17,18] rich in glutamic acid residues (either 11 or 13 glutamic acid residues, depending on the protein and species), which are post-translationally γ-carboxylated in a vitamin K-dependent manner (Figure 1).[19,20] This γ-carboxylation determines TAM ligand bioactivity,[15,16] as explained in detail in the next paragraphs. The carboxy-terminus of PROS1 and GAS6 contains a "sex hormone-binding globulin" (SHBG) domain, itself composed of two laminin G domains. It is this SHBG domain that binds to the Immunoglobulin (Ig) extracellular domain of the TAM receptors (Figure 1).[6,21–23] As receptor tyrosine kinases, the TAMs dimerize upon ligand binding, allowing for either homo- or heterodimerization. Similarly, GAS6 and PROS1 may bind their respective TAMs contributing to active signaling either as homo- or hetero-dimers. To date, the specific ligand–receptor combinations and their respective downstream signaling pathways are mostly undetermined. Being secreted ligands, they can activate TAMs both in an autocrine and paracrine manner. After ligand binding and TAM dimerization, the receptor's tyrosine intracellular domains are phosphorylated, exposing docking sites for several intracellular molecules carrying out signal transduction. The phosphoinositide 3 kinase (PI3K)/AKT pathway, the phospholipase C, ERK1/2, Ras, and MAP kinase activation are commonly activated signaling cascades in most TAM-expressing cells. Moreover, the JAK/STAT signaling pathway may often prevail over the others, determining differential TAM bioactivity and functional diversity.[9,24] TAM activation culminates in the regulation of several physiological processes, including cellular proliferation, survival and growth, immune functions such as cytokines and reactive oxygen species (ROS) release, phagocytosis and efferocytosis.[1,9,25,26] Figure 1 presents a visualization of TAM receptor–ligand interactions, and the intracellular pathways driving phagocytosis, also elaborated below.

Loss of function of any of the TAM receptors in murine models does not lead to any major developmental issue, even in triple Knockout (KO) mice deleted for all three TAMs.[4,27,28] Postnatal triple KO mice, however, develop male sterility, retinal degeneration, autoimmune-like disorders, and neurodegeneration,[24,27–31] indicating that TAM signaling is necessary to preserve homeostasis in adult tissues. GAS6 mutants are generally healthy and do not develop the postnatal phenotypes mentioned for TAM triple KO mice. Reduced GAS6 levels even confer protection against thrombosis,[32] liver disease,[33] and cancer metastasis.[34] The ability of PROS1 to activate the TAM receptors has been under debate for many years.[14,35,36] It was the generation of *Pros1* conditional KO (cKO) mice that identified PROS1 as a TAM agonist in vivo and allowed to reveal its role as a signaling molecule in several different tissues.[31,37–40] PROS1 is mostly known as a potent blood anticoagulant, and, predictably, deleting *Pros1* systemically causes prenatal death due to coagulopathy and impaired vasculogenesis, with severe hemorrhage.[41,42] Despite the functional redundancy of TAM receptors and their ligands, how this pathway regulates extremely diverse cellular functions affecting numerous features of cellular physiology is still incompletely understood. In the following paragraphs, we will focus on the role of TAMs in phagocytosis and inflammation—two important and functionally linked determinants of adult tissue well-being.

3 | TAM RECEPTOR-MEDIATED PHAGOCYTOSIS: THE MECHANISTICS

Phagocytosis has been described and paralleled to a feast, where the phagocyte cell consumes its meal.[43–45] Like in a feast, different diners recognize and prefer different foods. After making a choice and intake of the preferred food, come satiation and digestion. These humanized terms translate molecularly into recognition, contact, uptake and recycling, with specific molecules involved in the recognition (find me signals), contact (eat me signals) and downstream "digestion" activities. Many forms of phagocytosis exist, from uptake of intruders such as bacteria (by neutrophils), through the localized and limited pruning of cell fractions as in photoreceptor outer segments (by retinal pigment epithelium, RPE) and neuronal synapses (by microglia), to ingesting cellular debris (e.g., myelin fragments by microglia) or entirely devouring dead cells (by macrophages). Each form of phagocytosis is not only tailored to the physiological context but also molecularly regulated and executed by specific proteins. In this context, the TAMs were identified for removing apoptotic cells (ACs), a type of phagocytosis termed efferocytosis,[46] but are now also recognized in the localized pruning of portions from viable cells. Efferocytosis is mechanistically very similar to phagocytosis, but functionally very different. Phagocytosis engages molecular players and intracellular signaling cascades aimed at building up the immune response, with antigen presentation as well as release of pro-inflammatory cytokines. On the contrary, efferocytosis is, from the

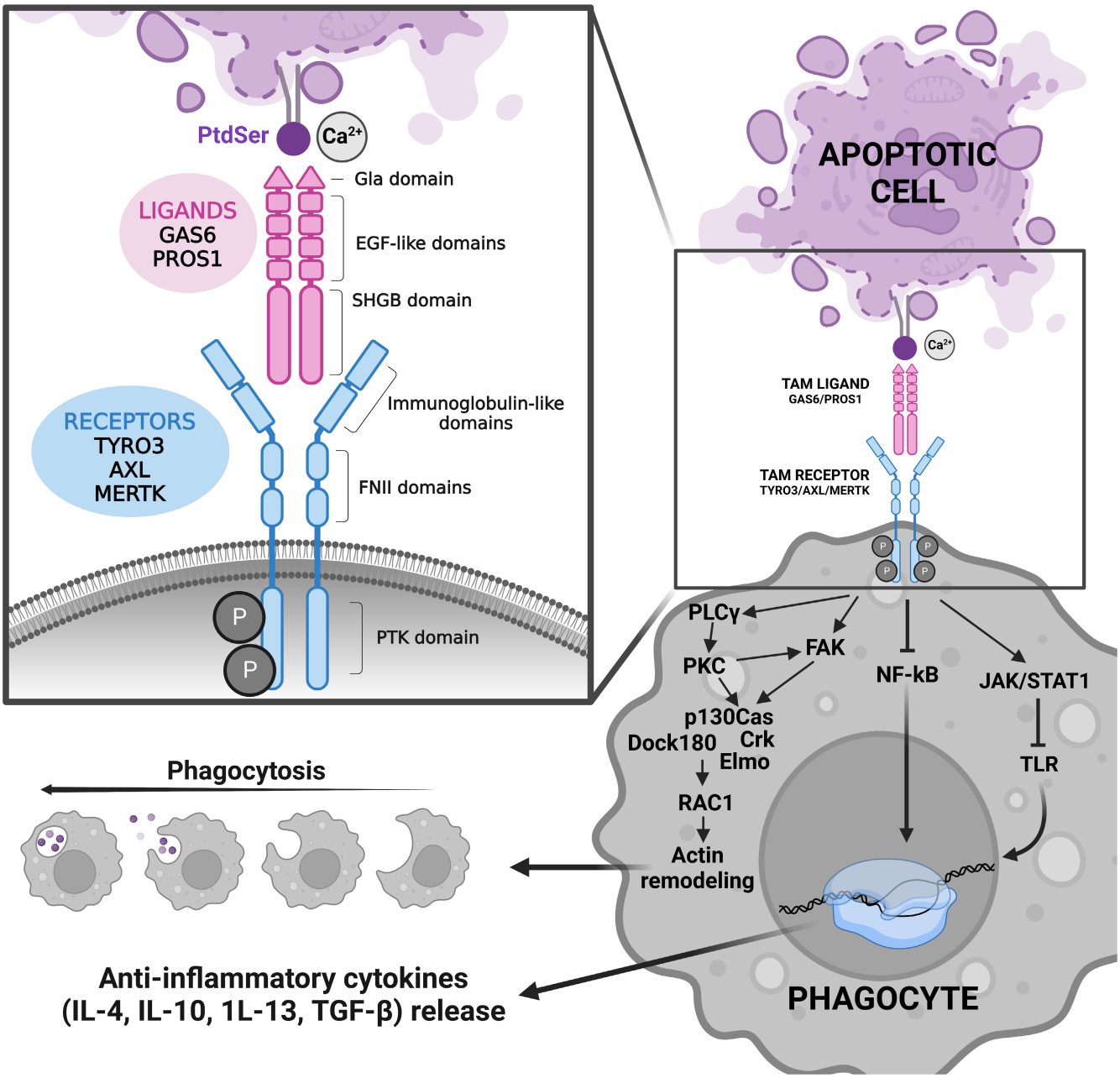

FIGURE 1 TAM receptor–ligand structure, and signaling in phagocytosis. Both ligands and receptors present high structural homology. The TAM ligands GAS6 and PROS1 bind to phosphatidylserine, which has been externalized by apoptotic cells, via the Gla domain at their N-terminus. The Gla domain is followed by four EGF-like repeats, and a SHBG domain on their carboxy-terminus, which constitutes the binding site to the TAM receptors' Ig-domains, on their extracellular N-terminal portion. Subsequently the TAM receptors present two fibronectin type III (FNIII) repeats, the transmembrane spanning portion of the molecule, and the protein-tyrosine kinase (PTK) domains on the intracellular carboxy-terminus (Inset blow-up). The vitamin K-dependent post-translational γ-carboxylation of the Gla domain allows GAS6/PROS1 to dimerize and bind PtdSer on apoptotic cells, in a Ca^{2+}-dependent reaction. GAS6/PROS1 initiate the phagocytic process by acting as bridging ligands between the apoptotic target, and the TAM receptors on the phagocyte. Ligand binding drives receptor dimerization, cross-phosphorylation of the tyrosine domains, and signal transduction via two distinct cascades leading to phagocytosis and anti-inflammatory signaling. Focal adhesion kinase (FAK), or Phospholipase C-γ (PLCγ) through phosphokinase C (PKC), drive CRKII/ELMO/DOCK180-dependent activation of RAC1, in turn mediating actin cytoskeleton rearrangement necessary for phagocytic cup formation and engulfment of the target particle. Simultaneously, through the activation of the JAK/STAT kinases that block TLR, and the inhibition of NF-kB, the TAMs signal for the release of anti-inflammatory and pro-resolving cytokines. TAM-mediated anti-inflammatory signaling diverges from that of phagocytosis and involves nuclear events. Created with BioRender.com.

immune point of view, a "silent" and counter-inflammatory form of clearance, that further signals tissue repair.[47,48] This type of phagocytic engulfment is extremely important for tissues which cannot shed their dead cells to the environment, as done for skin keratinocytes or intestinal epithelium. This would be the case for clearing apoptotic neutrophils within tissues during the resolution phase of

inflammation, and on a daily basis for organs with high cell turnover such as the testes and retina. The testis and retina are tissues with elevated rates of cell turnover at steady state and cannot afford to mount an inflammatory response on a regular basis.

The term "phagocytic synapse" was coined by Goodridge et al. to describe the point of attachment between the phagocyte and an AC to be engulfed, by analogy to the "immunological synapse" describing the specific requirements of contact between an antigen presenting cell (APC) and a T cell.[49] Yet, two key differences exist between the immunological and phagocytic synapses: First, the immunological synapse is necessary to locally concentrate the soluble cytokines needed for effective signaling, whereas the phagocytic synapse is mainly generated by membrane-bound molecules, and it is still unclear whether a physical enclosure is necessary for successful phagocytosis. Second, the immunological synapse is necessary to cage the secreted cytokines and assists in increasing their local concentration to overcome a threshold necessary for a fruitful engagement. By contrast, phagocytosis is mediated by membrane-bound molecules, and therefore, there is no need to concentrate the effector molecules. Finally, the immunological synapse does not usually culminate in the uptake of another cell moiety. Nevertheless, the semblance of the immunological and phagocytic synapses in facilitating both signaling and cell–cell communication is significant. As phagocytic receptors, the TAMs are part of the "phagocytic synapse," which is composed of (1) externalized phosphatidylserine (PtdSer), exposed by the moiety to be engulfed (ACs, myelin, synaptic boutons, photoreceptor outer segments); (2) the professional or non-professional phagocyte, expressing one or more TAM receptors on its outer membrane; (3) the TAM ligands GAS6 and/or PROS1, which act as bridging molecules indirectly linking the phagocyte and target moiety, and promote phagocytosis by stimulating TAM-dependent intracellular downstream signaling (Figure 1).[1,9,26] On the amino-terminal portion of GAS6/PROS1, the vitamin K-dependent gamma-carboxylation of their Gla domain drives ligand dimerization, and binding to PtdSer, the bona fide "eat-me" signal.[11,50–53] This reaction rapidly occurs in a Ca^{2+}-dependent manner and is aborted when calcium ions are removed.[50,54–57] As mentioned above, the gamma-carboxylation of the Gla residues occurs post-translationally, and is necessary for Ca^{2+} docking and the conformational change to catalyze PtdSer binding, as well as for TAM receptor activation. Ligands with non-carboxylated Gla domains, or Gla-less ligands where the Gla residues were altogether truncated, still allow for ligand binding, but not for activation of TAM receptors.[15,16] On the carboxy-terminal, the SHBG domains of the TAM ligands bind to the Ig domains of their receptors, inducing their dimerization, and cross-phosphorylation of the intracellular tyrosine residues (Figure 1). TAM receptor activation triggers intracellular signaling cascades leading to actin cytoskeletal rearrangements necessary for formation of the phagocytic cup, as well as for the final internalization of the target particle.[1,26] Phagocytosis is, like many other biological systems, a multi-step process that constitutes an important regulatory mechanism to avoid off-target phagocytosis, ensure that only particles exposing "eat-me" signals take part in the phagocytic synapse, and ultimately that the TAMs are fully activated to efficiently clear and degrade whatever must be engulfed. Interestingly, a rigorous density of PtdSer must be externalized on the particle membrane to fully activate the TAM system.[58] Once TAMs initiate phagocytosis, a simultaneous series of anti-inflammatory signaling cascades are initiated in the phagocytes. Such TAM-dependent anti-inflammatory pathways were shown in dendritic cells (DCs) and macrophages, shifting their pro-inflammatory profile to an overall anti-inflammatory one. This included release of tissue repair-inducing cytokines, inhibition of Toll-like receptors, and the arrest of the innate immune response.[1,16,24,28,37,59–61]

It is noteworthy to mention that while PtdSer is required for recognition of the apoptotic particles, its exposure alone is not sufficient to promote engulfment by macrophages. Segawa et al. generated viable cells which overexpress and present PtdSer at comparable levels to those of ACs. They showed that these viable cells—exposing PtdSer to the same level of ACs—are not recognized by macrophages and not internalized until apoptosis was induced in these cells (Figure 2).[62] These results point to additional factors other than PtdSer, which allow macrophages to identify and recognize ACs, avoiding massive off-target phagocytosis of viable cells. On the contrary, even exposure of PtdSer in the presence of TAMs and their ligands would not guarantee engulfment. For example, bacteria and fungi present PtdSer, which was shown to promote their virulence,[63] but TAM-expressing immune cells encountering such pathogens do not exploit TAMs for bacterial or fungal uptake and elimination (Figure 2).[64,65] Ligand availability is not assumed to be a limiting factor, as TAM ligands effectively function to activate TAM receptors in tissues and in vitro in the presence of other PtdSer-presenting particles. Moreover, TAM receptors expressed on the surfaces of macrophages, lymphocytes, endothelial cells, microglia, and many other cell types which are continuously exposed to TAM agonists are not constitutively activated, indicating this pathway is tightly regulated. Part of this regulation occurs at the ligand level by means of biochemical modifications, such as the as the vitamin K dependent γ-carboxylation of Gla domains. Another biochemical modification that is thought to lend specificity in the context of ACs is ligand oxidation and oligomerization. Uehara and Shacter showed that upon binding to PtdSer, PROS1 undergoes oxidation of cysteine residues, and this reaction promotes formation of intermolecular disulphide bonds and oligomerization of PROS1. This sequence of events leads to more effective MERTK activation on macrophages.[66] Together, the requirement for a series of complex and localized post-translational modifications contributes to the regulation and fine tuning of TAM signaling. It may be reasonable to speculate that additional unknown factors guide the decision of whether or not to activate TAM signaling (Figure 2).

4 | TAMS COUPLE PHAGOCYTOSIS WITH ANTI-INFLAMMATORY SIGNALING

Among the multitude of cellular functions they supervise, the TAMs are also infallible participants of immune homeostasis maintenance

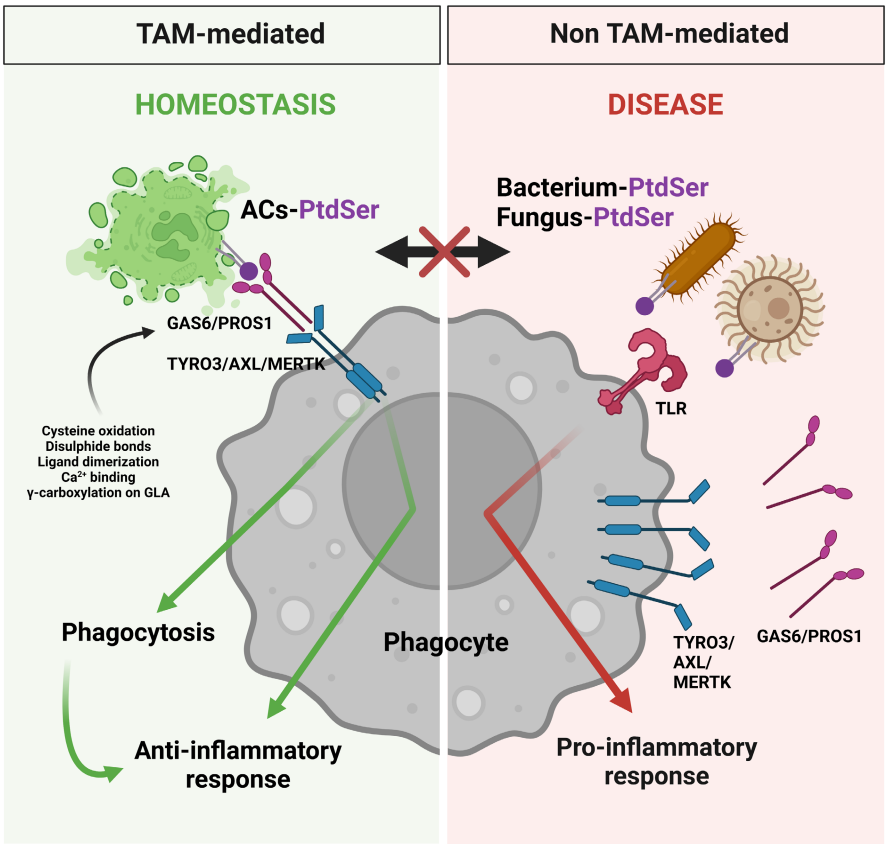

FIGURE 2 Biochemical regulation and specificity of TAM-mediated phagocytosis. Engulfment of different PtdSer-expressing particles employs distinct signaling molecules which have opposite effects, and are tailored to their specific biological contexts. The "eat-me" signal PtdSer functions in a versatile manner and is able to trigger different biological responses in phagocytes, depending on the type of target that exposes PtdSer, and the desired physiological outcome. Under healthy conditions (left), TAMs mediate engulfment of apoptotic cells (ACs) by phagocytes in a mechanism that is biochemically tightly regulated. The post-translational γ-carboxylation on the Gla domain of GAS6 and PROS1, and the presence of calcium ions lead to PtdSer binding and ligand dimerization, and is also necessary for TAM receptor activation. Further, it was shown that PtdSer induces oxidation of PROS1 cysteine residues, leading to oligomerization through disulphide bond formation. These biochemical (and possibly additional unidentified) modifications provide local regulation and allow for TAM ligands to bridge and bind the apoptotic moiety and the phagocyte, while activating TAM signaling at the same time. TAM activation in phagocytes not only leads to clearing ACs, but also activates anti-inflammatory signals, which help to maintain homeostasis, suppressing elevated immune responses. However, when the PtdSer-expressing molecules are expressed by infectious agents (right) such as bacteria or fungi, TAMs are not engaged. Instead, the toll-like receptors (TLR) and other pro-inflammatory responses are recruited, activating the immune system, even in the presence of TAM receptors and ligands. Created with BioRender.com.

and restoration. The TAM system lies in between the innate and adaptive immunity, suppressing the build-up of immune responses by releasing tissue repair mediators.[60] One of the anti-inflammatory activities of the TAMs is clearance of ACs,[64,67–69] which would induce inflammation if the cell corpses remain in the tissue. Evolution has provided TAM signaling with the ability to simultaneously promote efferocytosis by signaling cytoskeletal rearrangements to form the phagocytic cup as well as to curb the inflammatory response through the release of the anti-inflammatory and resolving cytokines IL-4, IL-10, IL-13, and TGF-β.[3,59,70] This way, the same receptor mediates two seemingly independent cellular functions, which are in fact coordinated to maintain immune homeostasis and tissue health. At the molecular level, Tibrewal et al. found that for MERTK, the phosphorylation of tyrosine residue 867 ($MERTK^{Tyr867}$) controls cytoskeletal rearrangements that are required for phagocytosis. Mutating tyrosine 867 abrogated engulfment, but did not affect MERTK-dependent anti-inflammatory function following LPS stimulation, thereby providing a molecular basis for dissociating engulfment from anti-inflammatory functions. This study further demonstrated that the MERTK-dependent anti-inflammatory pathway is a post-nuclear event, involving transcriptional suppression of NFκB-dependent responses.[71] Thus, the active inhibition of inflammatory signaling, as well as efferocytic signaling by TAMs are distinct both functionally and at the molecular level. Engulfment-associated cytoskeletal rearrangement also depends on MERTK phosphorylating and activating focal adhesion kinase at Tyr861 (FAK^{Tyr861}), driving its binding to the intracellular domain of the β5 subunit of integrin αvβ5. Like MERTK, integrin αvβ5 functions as a transmembrane engulfment receptor, activated by MFGE8 (Milk Fat Globule and EGF Factor 8) which, like GAS6 and PROS1 binds PtdSer on ACs, essentially functioning as a bridging ligand.[72] MERTK also induces $p130^{CAS}$ phosphorylation in a αvβ5-dependent manner, to further activate the downstream

effectors CrKII/Dock180/ELMO/Rac1 leading to cytoskeletal rearrangements and phagocytosis[73] (Figure 1). Thus, PtdSer-induced MERTK and PtdSer-induced αvβ5 downstream pathways converge intracellularly and synergize to activate, perhaps amplify, the cytoskeletal rearrangements necessary for engulfment.

While the anti-inflammatory function can be triggered by TAMs without prior particle internalization,[24,74] it is not clear whether AC uptake in vivo would *necessarily* be coupled to and stimulate anti-inflammatory signaling. Furthermore, whether phagocytosis of different moieties such as ACs, myelin, the outer segments of photoreceptors, or apoptotic bodies would result in similar anti-inflammatory engagement remains unknown. Rothlin et al. showed that in DCs, the anti-inflammatory function of AXL is dependent on the type I interferon receptor and STAT1 (IFNAR/STAT1) signaling, highlighting that TAM heterodimerization with non-TAM receptors can influence TAM function.[24] Future research may reveal that heterodimerization with other receptors, or interactions with other modifying proteins present in their vicinity in lipid rafts may guide in steering downstream TAM pathways. In this respect, AXL also signals as a heterodimer with epidermal growth factor receptor (EGFR), promoting drug resistance and survival in head and neck cancer.[75]

When triple KO mice lacking all TAM receptors were first reported in 2001, these mice exhibited severe autoimmunity and hyper-inflammation, even in the absence of experimental inflammatory stimulus.[28] However, more recent studies have found that some of these spontaneous inflammatory phenotypes, such as arthritis, are not as prominent,[76] possibly due to improved cleanliness of modern institutional vivariums. This suggests that TAM receptors act in response to specific inflammatory stimuli rather than constantly regulating inflammation. In agreement, most in vitro studies have investigated the TAM anti-inflammatory function using pre-stimulated cells. For instance, CpG, poly(I:C), or LPS are commonly used in vitro to prime DCs and macrophages, to demonstrate TAM activation dampens inflammation. This enforced the concept that TAMs come into action mediating their anti-inflammatory function following a stimulus that has breached homeostasis. However, the notion that TAMs may continuously function "backstage" to regulate inflammation even at steady state and without any prior stimulation is supported by two recent independent studies. Maimon et al. showed that ablating PROS1 from macrophages was enough to abstain MERTK activation in naïve macrophages in vitro, rendering BMDMs hyper-inflamed at the cell level. This seems to be the case also in vivo, where ablation of PROS1 from myeloid cells led to inflamed lungs in otherwise healthy, non-challenged mice.[74] A more recent report by Mercau et al. (further discussed below) revealed that MERTK inactivation leads to retinal inflammation even before the onset of high retinal turnover, indicating that in the absence of prior challenge, continuous action of TAMs is needed in order to maintain homeostasis. Moreover, because the high retinal turnover commences only later in life, this early time window allowed to differentiate between the anti-inflammatory and phagocytic functions of TAMs in vivo.[77]

5 | TAM SIGNALING IN HOMEOSTATIC PHAGOCYTOSIS

In a human body, several hundred billion cells undergo programmed cell death, or apoptosis, on a daily basis, in the effort of getting rid of old, poorly functional, or simply superfluous cells.[72] This type of cellular death is preponderant in the homeostatic turnover of tissues, and beneficial for making room for younger and healthier cells.[52,72] Life-long, high cell turnover routine processes include spermatogenesis, adult neurogenesis, and hematopoiesis, when millions of cells become apoptotic before completing their differentiation into mature sperm cells, neurons, and immune cells, respectively. In order to prevent disease, ACs must be approached and engulfed by tissue-resident phagocytes, which are also in charge of suppressing the inflammatory response at the same time.[78] The importance of TAM-mediated phagocytosis to the healthy maintenance in these tissues is discussed in the next sections and summarized in Figure 3.

6 | TAM-MEDIATED PHAGOCYTOSIS BY SERTOLI CELLS

An impressive apoptotic rate is observed in the epithelium of the seminiferous tubules of the testes, where spermatogenesis yields hundreds of millions of immature germ cells, of which less than one-third complete maturation and become functional spermatozoa.[79] The entire spermatogenesis process is sustained by the multifunctional Sertoli cells, which "nurse" the spermatogenic cells throughout their development by maintaining hormonal and ion balance, providing nutrition and metabolism regulation.[80] Importantly, the Sertoli cells are also the phagocytes specific to the testes. They engulf those germ cells that present abnormalities and failed to differentiate, as well as foreign antigens.[81,82] One Sertoli cell is often in contact with 30–40 germ cells at different developmental stages. Therefore, the massive apoptotic wave observed during spermatogenesis is essential not only to eliminate excess germ cells, but also to maintain a healthy germ-Sertoli cells ratio.[79,82–84] Unsurprisingly, efficient phagocytosis by Sertoli cells is fundamental for healthy spermatogenesis and male fertility,[79,81,82] and this mechanism is driven by the TAM receptors in a PtdSer-dependent way.[27,81,85] In the testis, Sertoli cells express all three TAM receptors while another cell type in the testes, the Leydig cells, are the source of the ligands GAS6 and PROS1.[27,86] This creates a network of cell interactions that includes Sertoli, Leydig, and germ cells, and maintains the correct functionality of the male reproductive system (Figure 3).[87]

Among the first observed phenotypes of TAM-dependent faulty phagocytosis was male infertility.[1,27] Approximately 10^7 sperm cells per gram of testicular tissue are produced daily, of which around 75% die before reaching maturity. While necessary to maintain a balanced sperm Sertoli cell ratio,[79,83] the products of such massive apoptosis must be rapidly cleared to avoid release

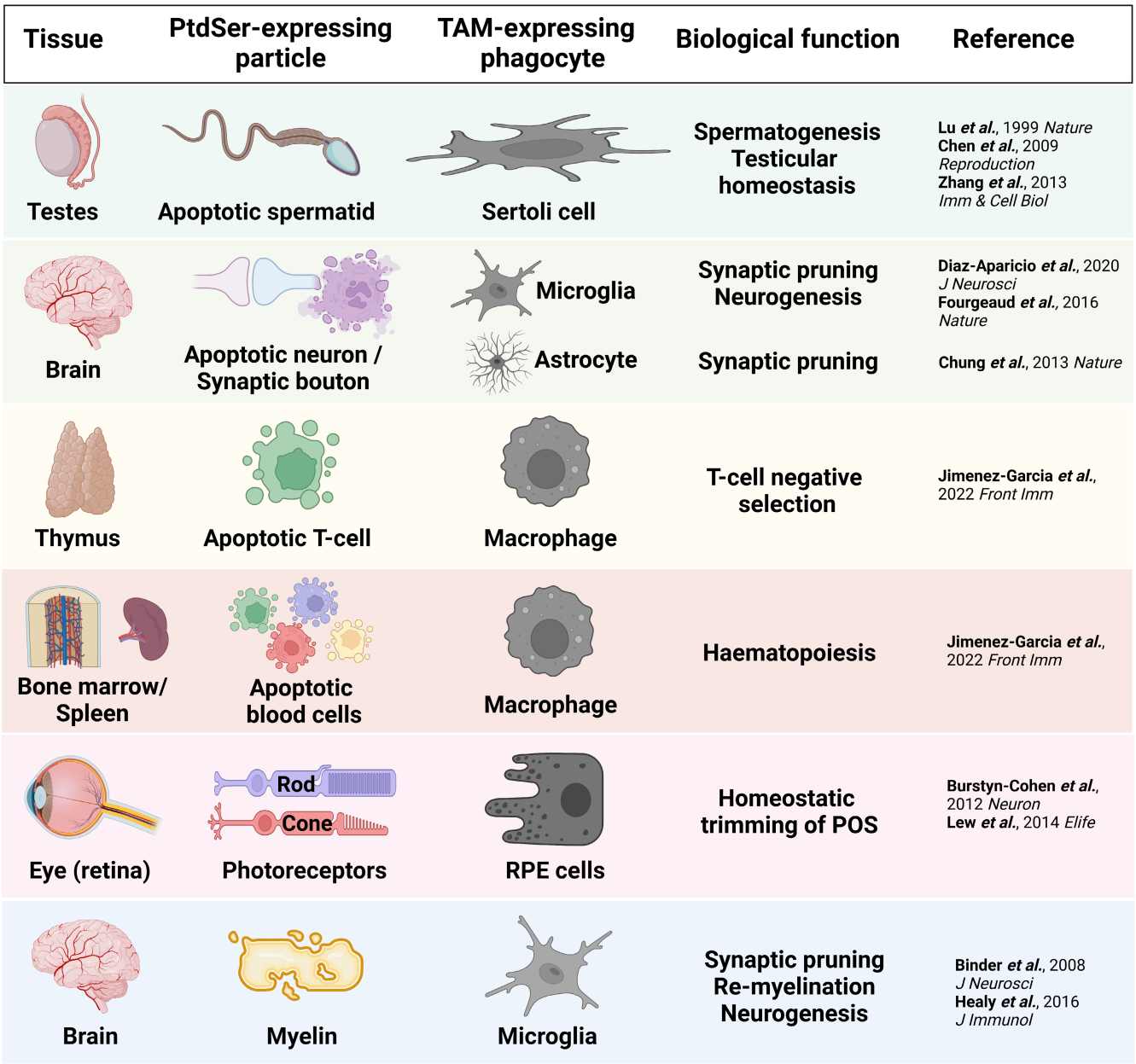

FIGURE 3 TAM-mediated phagocytosis in homeostasis. A schematic summary of unceasing phagocytic events undertaken by TAM signaling to maintain steady state. Depicted are the variety of phosphatidylserine (PtdSer)-presenting phagocytic targets in different tissues and their corresponding TAM-expressing phagocytes in the context of their ultimate biological function, along with the reporting studies. Synaptic pruning by microglia and astrocytes, as well as POS trimming differ, as they present engulfment of portions of live cells, whereas the other events involve the uptake of bona fide apoptotic elements. RPE, retinal pigment epithelium, POS, photoreceptor outer segments. Created with BioRender.com.

of toxins and reactive oxygen species which would affect healthy spermatogenesis.[81,88] The simultaneous deletion of all three TAM receptors causes infertility due to their inactivation in Sertoli cells. In mice lacking functional TAM signaling, seminiferous tubule cell architecture is damaged, sperm and spermatogenesis are absent, testes are shrunk and present heightened inflammation characterized by increased infiltration of T-cell lymphocytes and inflammatory macrophages (Figure 4).[88,89] As uncleared apoptotic cells accumulate in the testes, certain phenotypes progressively worsen over time. These include inflammation, the presence of autoantibodies against germ cell antigens, and the breach of the blood-testicular barrier. Zhang et al. characterized the time course of the inflammatory phenotype within the testis of TAM-deficient mice.[88] Notably, these inflammatory and autoimmune features were absent prior to apoptotic spermatid accumulation, suggesting that inflammation is a secondary reaction to the failed clearance of apoptotic germ cells.[88] Interestingly, single or double ablation of any combination of TAMs is not enough to cause sterility, indicating that the three receptors are either functionally redundant, or must work together to maintain spermatogenesis.[27,89] However,

ablating *Mertk* together with either *Axl* or *Tyro3* (*Tyro3*$^{-/-}$*Mertk*$^{-/-}$ or *Axl*$^{-/-}$*Mertk*$^{-/-}$) is enough to cause a major reduction in testis weight and sperm counts. This suggests that MERTK may be the dominant TAM member mediating Sertoli cell phagocytosis, though its ablation alone is not sufficient by itself to cause infertility.[27,89] A gene expression array performed in murine Sertoli cells deficient for all TAMs revealed that compared to TAM-expressing controls, *Cd36* was among the significantly downregulated genes, and found to be implicated in phagocytosis by Sertoli,[90] as well as other cell types.[91,92] Together with *N-cadherin*, an important mediator of Sertoli cell-spermatid adhesion,[93] these genes were identified as the main candidates to contribute to infertility.[89] The

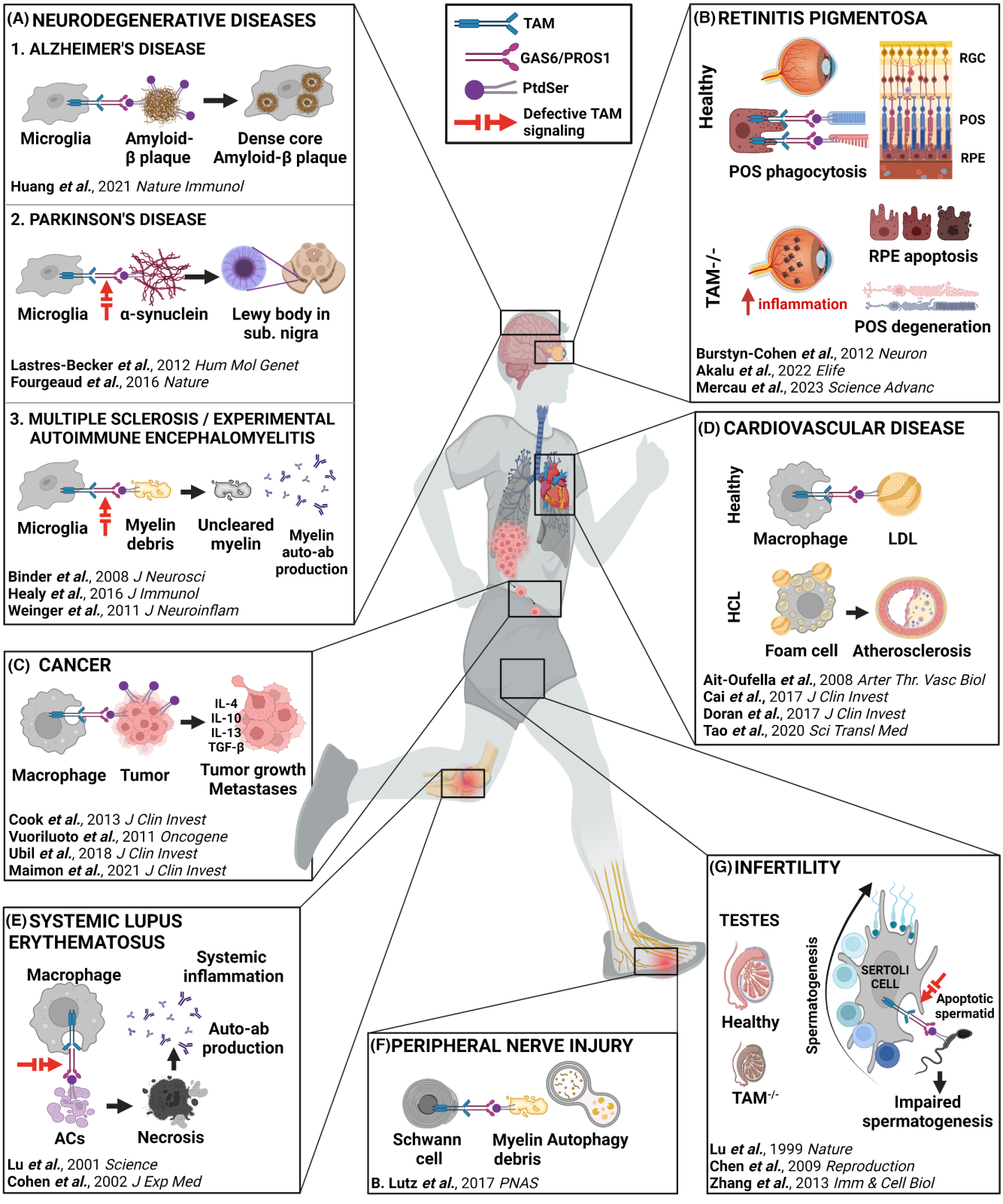

FIGURE 4 Ramifications of TAM-mediated phagocytosis in disease. (A) In neurodegenerative disorders, phagocytosis through the TAMs has different implications. In Alzheimer's disease (AD), microglial engulfment of Aβ plaques results in the formation of dense-core plaques in the phagocytes, which are either unable to process them, or die and release the load. On the contrary, in Parkinson's disease (PD), phagocytosis promotes the clearance of α-synuclein protein aggregates, and absence of TAM signaling speeds up Lewy bodies formation in the substantia nigra. In multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), defective myelin uptake exacerbates autoimmunity and inflammation, which inhibits remyelination and aggravates disease progression. (B) In the retina, retinal pigment epithelium (RPE) cells trim the outer segments of rods and cones through TAMs. Ablating TAM receptors results in early elevated RPE inflammation and degeneration of photoreceptors, leading to vision loss in mice and retinitis pigmentosa in humans. (C) In the tumor microenvironment, elevated cell turnover attracts TAM-mediated phagocytosis by macrophages, but also activates anti-inflammatory agents, thereby creating conditions favorable for tumor growth and progression. (D) In hypercholesterolemic (HCL) conditions, TAM-mediated LDL uptake leads to lipid accumulation in macrophages that turn into foam cells and drive the formation of atherosclerotic plaques in blood vessels. This mechanism is further aggravated by immune cell activation, infiltration, and accumulation, leading to atherosclerosis, and in severe cases to myocardial infarction. (E) Systemically, altered phagocytosis due to TAM loss of function leads to accumulation of cellular debris, persistent inflammation, and autoimmune disorders, as in systemic lupus erythematosus (SLE). Absence of TAM signaling was found to develop into Lupus-like clinical manifestation in mice. (F) In a mouse model of peripheral nerve injury, Schwann cells perform autophagy by using the TAMs to uptake degraded myelin. (G) Sertoli cells of the testes utilize the TAMs to phagocytose unviable sperm cells during spermatogenesis. Disruption of such signaling leads to apoptotic sperm cells accumulation, reduced testes size, and infertility. Created with BioRender.com.

discovery of the critical role fulfilled by TAMs and their ligands in the testes paved the road for further studies on their mediation of phagocytosis in other organs.

7 | TAM-MEDIATED PHAGOCYTOSIS IN HEMATOPOIETIC ORGANS

The thymus and bone marrow are another relevant example of organs with elevated cell turnover. Massive apoptotic cell death occurs in the developing thymus, in a process known as negative selection of the T cells.[94,95] During the clonal expansion of thymocytes, autoreactive T cells must be eliminated to prevent autoimmune disorders, as well as hypo-reactive and inert T cells, as these are incompetent to carry out an immune response.[61,96–98] Though to a lesser extent, the negative selection in the thymus continues for a small window of time postnatally, as the organ reduces its size and function. With puberty, both in humans and in mice the thymus undergoes involution, loses its cellular architecture, and produces less T cells as the organism ages.[99–101] The TAM system, although prevalently regulating adult homeostatic mechanisms,[1] was recently found to be critical for the clearance of the negatively selected thymocytes during murine postnatal development (Figure 3). Jimenez-García and colleagues demonstrated that loss of MERTK and AXL results in decreased phagocytic activity of macrophages, resulting in dramatic accumulation of dead T cells. Furthermore, *Axl/Mertk* double mutant mice display a 50% reduction in F4/80^{+}CD11b^{lo} macrophages, which normally express MERTK and are highly efferocytic in the bone marrow and thymus, impairing apoptotic thymocyte clearance.[61] These macrophages are also responsible for continuously phagocytosing erythrocytes and their heme-iron components. In the absence of AXL and MERTK, the remaining macrophage subpopulations downregulate engulfment molecules such as CD163, further aggravating their phagocytic inefficiency.[61] These data highlight the previously unrecognized role of TAM signaling in maintaining the health and function of hematopoietic organs.

8 | TAM-MEDIATED PHAGOCYTOSIS BY RETINAL PIGMENT EPITHELIAL CELLS IN THE EYE

In the retina, phagocytosis is performed by the retinal pigment epithelial (RPE) cells. In addition to their phagocytic properties, for which RPE are considered professional epithelial phagocytes, they undertake numerous functions necessary for retinal viability.[102] However, instead of engulfing ACs, RPE cells ingest and metabolize the outer segments of viable photoreceptors (Figure 3). The photoreceptor outer segments (POS) constitute the outermost layer of the retina, responsible for light absorption.[103] This phagocytosis is programmed to occur daily with light onset on a circadian basis, throughout life. POS are subjected to high intensity light during the day, inducing photo-deterioration of proteins and lipids and generating toxic oxidative products of phototransduction. Like for clearing apoptotic spermatids, the uptake of POS by RPE cells is thought to be extremely important for removal of these toxic waste products, and to maintain healthy conditions for photoreceptor viability allowing vision.[102–104] Lu et al. were the first to report that TAM triple mutant mice were blind due to massive photoreceptor degeneration (Figure 4).[27] A couple of years later, *Mertk* was appointed as the sole accountable gene causing visual loss in the Royal College of Surgeons (RCS) rat model of retinitis pigmentosa,[105] as well as in mice,[30,105] and humans.[106,107] MERTK was found to be expressed on the apical microvilli of RPE cells, where it functions as an essential regulator of POS phagocytosis. TYRO3 acts similarly; however, its deletion alone is not enough to cause blindness, and its complete expression depends on MERTK levels.[108] With respect to the ligands, the observation that only the removal of *both* GAS6 and PROS1 recapitulates *Mertk*$^{-/-}$ phenotypes was a major breakthrough in the TAM field for two main reasons: First, it provided the very first in vivo evidence of PROS1 being a functional ligand of TAMs, ending a hot debate as to the relevance of PROS1 as a TAM agonist.[14,35,36] Second, GAS6 and PROS1 seem to act interchangeably, but are absolutely required for POS engulfment by RPE cells, and for maintaining retinal

homeostasis.[31] The involvement of TAMs in RPE-mediated uptake of ROS diverts from the mainstream theme where TAMs uptake ACs which expose PtdSer. However, the molecular basis remains, with the outer segments of photoreceptors locally externalizing PtdSer in a circadian rhythm, thereby providing PtdSer availability for PROS1 and GAS6 to bridge between the POS presenting PtdSer and the TAM-expressing RPE in a viable cell.[109] Hence, TAM function in the retina diverges from their traditional role in efferocytosis (Figure 3).

Retinitis pigmentosa is a group of genetic disorders leading to the degeneration of rod and cone photoreceptors. As a result, light cannot be detected and transformed into visual information.[110] This leads to peripheral blindness, which progressively advances to tunnel vision and central vision loss.[111,112] *Mertk* is highly expressed by RPE cells,[108] and is a major regulator of POS phagocytosis.[105,107,108,113] The retinal degeneration associated with MERTK mutants observed in rat, mice, and humans shaped the concept that failed phagocytosis of POS leads to retinal degeneration. For this reason, retinitis pigmentosa in MERTK-deficient patients has been largely attributed to the impaired phagocytosis by RPE. However, not all rodents and humans carrying mutations in the *Mertk* gene showed similar blindness severity, and in some cases showed no phenotype at all, hinting at the possibility that genetic variants of *Mertk*, or potentially of *Tyro3* which is also expressed by RPE may modify the disease course.[114,115] In support of this hypothesis, a careful analysis of the popularly studied $Mertk^{-/-}$ mice[27] (henceforth referred to as $Mertk^{-/-V1}$, according to Akalu et al.)[115] revealed hypomorphic expression of *Tyro3* in the RPE, and identified variability among different genetic backgrounds.[114–116] Two new *Mertk* mutants that were generated ($Mertk^{-/-V2}$ and $Mertk^{-/-V3}$) did not exhibit retinitis pigmentosa, unless crossed to *Tyro3* mutant mice ($Mertk^{-/-V2}$ $Tyro3^{-/-V2}$ mice), identifying TYRO3 as a disease modifier affecting early-onset photoreceptor degeneration.[114,115]

A major breakthrough in understanding the role of *Mertk* in the pathophysiology of retinal degeneration was recently made by Mercau et al., after observing elevated inflammation in the RPE of $Mertk^{-/-V2}$ mice as early as postnatal Day 10 (P10), a time point at which there are no signs of retinal degeneration, and, most importantly, phagocytosis of POS is minimal. In their work analyzing P10 mice, Mercau et al. show that MERTK deficiency causes early and unresolved inflammation manifested by monocytes infiltration and pronounced microglial activation.[116] Moreover, when RPE phagocytosis is severely inhibited in integrin β5 null mice, no gross retinal degeneration was observed,[117] suggesting that lack of phagocytosis alone is not sufficient to drive retinal degeneration, and pointing to the observed RPE inflammation as a potential cause for retinal degeneration (Figure 4). The authors hypothesized that if inflammation would underlie retinal degeneration in $Mertk^{-/-V1}$ mice, then pharmacological treatment curbing this inflammation is expected to ameliorate retinal degeneration. To test their speculation, Mercau et al. characterized inflammation in the RPE of $Mertk^{-/-}Tyro3^{-/-}$ deficient mice, and found elevated pro-inflammatory intracellular pathways culminating with the release of interleukin-6 (IL-6), type I and II interferons (IFNs) and tumor necrosis factor–α (TNF-α), which are mediated by the Janus kinase 1/2 (JAK1/2). Indeed, treating these mice with the anti-inflammatory drug Ruxolitinib, a JAK1/2 inhibitor, partially protected the retina from degeneration, further validating inflammation as the driving cause of the retinitis pigmentosa-like retinal degeneration observed in $Mertk^{-/-}$ mice.[116] Thus, rather than improving phagocytosis, targeting the mediators of inflammation which are activated in the absence of TAM signaling should be investigated as a better approach and important therapeutic line to help retinitis pigmentosa patients. Hence, by characterizing the different time points at which inflammation and phagocytosis occur, Mercau et al. show that the retina provides an in vivo model to segregate the anti-inflammatory function of MERTK from its phagocytic role, and provides new evidence for a net anti-inflammatory role for MERTK until POS engulfment becomes evident, at P10.

9 | TAM-MEDIATED PHAGOCYTOSIS IN THE BRAIN

Although accounting for only 10%–15% of total brain cells, microglia are the first-line guardians of the central nervous system (CNS).[118] They perform innate immune functions including phagocytosis and host defense from pathogens,[118,119] as well as strictly glial-related activities, such as support for neurons and their circuits.[120–122] Microglia function as sentinel cells and continuously scan the brain parenchyma both in homeostasis and disease.[118,123] In homeostatic conditions, microglia are busy phagocytosing newborn neurons that do not complete their maturation, and are therefore excluded from developing functional synapses. Indeed, among all the neurons that are generated during neurodevelopment and adult neurogenesis, only a fraction is incorporated within the neural networks.[124] The remaining undergo apoptosis and are rapidly cleared by microglia.[119,124,125] A similar mechanism is observed in the adult brain, when neurogenesis decreases significantly, but is retained and confined in two neurogenic niches, the subgranular zone of the hippocampus, and the subventricular zone, lateral to the ventricles.[126,127] This clearance of apoptotic newborn neurons by microglia is mediated by AXL and MERTK in both adult neurogenic niches (Figure 3).[128] Moreover, mature synapses are continuously remodeled by experience and learning, sometimes generating connections that are no longer used.[129,130] Supernumerary synapses are therefore eliminated as synaptic bodies that are engulfed by microglia in order to maintain proper signal transduction, and strengthen new or existing neural circuits.[131,132] Microglia are commonly defined as the macrophages specific to the CNS, and although during acute inflammation peripheral monocytes infiltrate the brain and become morphologically indistinguishable from the local resident microglia, these two populations still retain genetic and molecular differences.[133] In the attempt to identify such differences, Butovsky et al. performed gene expression analysis and reported that the TAM signaling genes *Gas6*, *Pros1*, and *Mertk* were significantly enriched in murine microglia compared to peripheral monocytes. Similarly, these three genes were exclusively expressed by human adult, and, though to a lower

extent, fetal microglia, and absent in other immune cells,[134] suggesting the dominance of TAM signaling in regulating different aspects of microglial function. Microglia use MERTK to ingest synapses, in a mechanism that is not only confined to the mere digestion of the synapse, but in turn negatively regulates neurogenesis through their phagocytic secretome, creating a feedback loop that ultimately regulates adult hippocampal plasticity and maintains brain homeostasis (Figure 3).[135]

Microglia are the professional phagocytes for the CNS,[118] but are not the only cell type performing phagocytosis in the brain. Interestingly, astrocytes have been reported to engulf synapses too.[136] Most abundant among glial cells,[137] astrocytes are key to brain homeostasis by maintaining blood–brain barrier integrity, ion balance, synaptic transmission, neurotransmitters uptake, and synaptogenesis.[138–140] Synapse elimination by astrocytes occurs during development and in adulthood, and is driven by the PROS1/ MERTK pathway, in parallel to the MEGF10 pathway, also initiated by PtdSer. Inhibition of both pathways had a stronger effect on astrocyte engulfment, indicating these two PtdSer pathways are distinct and function in parallel, allowing for developmental and adult synaptic pruning and neural network refinements[136] (Figure 3).

Another important source of PtdSer in the brain is myelin, a lipid-rich membrane ensheathing neuronal axons, both protecting axonal tracts and allowing for rapid movement (saltatory conduction) of the neural impulse.[141] Free myelin results from disrupted synapses, dead neurons, and the degeneration of the myelin-producing oligodendrocytes. The clearance of such debris is crucial to promote homeostatic synapse refinement and synaptogenesis. The presence of PtdSer in myelin licenses myelin as membrane targets for TAM-mediated phagocytosis in the CNS.[142,143] Similarly, the Schwann cells and oligodendrocytes of the peripheral nervous system (PNS) use the TAM pathways to phagocytose myelin, though more in an injury context rather than during homeostasis (Figure 4).[144]

Diseases in the nervous system generate degraded myelin, as in the case of the autoimmune disease multiple sclerosis (MS) (Figure 4). MS patients produce autoantibodies against their own myelin sheaths,[145] progressing with infiltration of T cells, activation of microglia and astrocytes, and further entry of monocytes.[146] Immune responses lead to inflammation and demyelination, loss of oligodendrocytes, and degeneration of axonal and motor neurons,[146–148] also culminating in accumulation of myelin debris. These must be rapidly cleared to prevent further inflammation, allow for tissue repair, and promote remyelination.[149–151] The professional phagocytes in the inflamed brain comprise of the local proliferating and activated microglia, as well as monocyte-derived macrophages (MDMs) that infiltrate the CNS from the vasculature.[118,123,152,153] *Mertk* expression was altered in MS patients' myeloid cells, resulting in defective myelin phagocytosis by MDMs and a pro-inflammatory environment in human brains.[143,154] Consistent with decreased MERTK activation, lower levels of PROS1 were found in the plasma of MS patients.[155] MERTK-mediated engulfment of myelin by human microglia was maximal following their exposure to TGF-β, also accompanied by a significant upregulation of MERTK, PROS1, and GAS6. Myelin uptake also led to reduced inflammation through the release of IL-10,[143] pointing to mechanistic similarities to the uptake of ACs.[156] Moreover, studies in mice treated with the demyelinating agent Cuprizone show that *Gas6 KO* mice develop more severe demyelination lesions, and fail to efficiently remyelinate upon Cuprizone withdrawal, indicating that GAS6 is protective against demyelination, most likely through MERTK and AXL.[157–159] In line with these findings, administration of GAS6 enhanced clearance of myelin debris and improved remyelination.[158] These findings are of high relevance for the amelioration of MS symptoms: The pharmacological manipulation of MERTK to exploit its anti-inflammatory activity through phagocytosis may be employed to boost remyelination in MS patients. The challenge will be to find an effective treatment to promote debris clearance while maintaining the right balance between immune stimulation and suppression.

A common pathological hallmark of neurodegenerative disorders is the aggregation of insoluble and indigestible oligomers and larger protein aggregates, which, if left uncleared, contribute to the development of chronic inflammation and oxidative stress, driving mechanisms of neurodegeneration.[160,161] Alzheimer's disease (AD) (Figure 4) is a neurodegenerative disorder characterized by the progressive accumulation of such insoluble protein oligomers and aggregates known as Amyloid beta (Aβ) plaques. Patients show dementia, memory loss, and inability to communicate and perform daily tasks.[161] Although the contribution of Aβ plaques to AD is still under debate,[162] their association with disease focuses effort on understanding their role in AD pathogenesis. Aβ plaques are enriched in exposed PtdSer,[163] anticipating a role for the TAM ligands and receptors. AXL has been associated with AD pathogenesis: RNA-sequencing (RNA-seq), and single-cell RNA-seq (scRNA-seq) revealed upregulated *Axl* expression in microglia associated with human Aβ plaques and in the microglia of the 5xFAD murine AD model, respectively.[164,165] AXL's soluble ectodomain (sAXL) bound to GAS6 in the cerebrospinal fluid (CSF) is a biomarker and prognostic factor in AD patients.[166] Moreover, using the *APP/PS1* mouse model of AD, Huang and colleagues demonstrated that microglia employ the TAM system to detect and phagocytose Aβ plaques, mainly through MERTK and AXL, with GAS6 as the putative bridging ligand. $Axl^{-/-}Mertk^{-/-}$ microglia are unresponsive to Aβ fibrils, lacking recognition, proliferation and motility orientation, and ultimately also lack phagocytic clearance.[163] Contrary to expectations, the authors observed that faulty TAM-mediated microglial phagocytosis of Aβ correlated with the deposition of fewer dense-core plaques.[163] This unexpected observation may be explained by three independent studies which show that functional microglia contribute to Aβ plaque compaction: The first reports that Aβ engulfed by microglia are released as packed clusters to the extracellular space due to toxicity and death of the microglia, and this deposition contributes to Aβ plaque growth.[167] Two additional studies find that microglia depletion reduces accumulation of Aβ plaques,[168,169] thus supporting an active role for TAM-mediated microglial phagocytosis of Aβ in AD pathology. PROS1 was recently identified as a novel microglia-derived biomarker in the hippocampi and serum of AD model mice,

also found at elevated levels in the sera of AD patients.[170] It is not clear whether PROS1 is involved in AD pathology or is upregulated as part of an intrinsic anti-inflammatory and repair mechanism to restore homeostasis. More research is needed to clarify how the involvement of TAMs and their ligands in amyloid plaque engulfment and in regulating inflammation is linked to AD.

Parkinson's disease (PD) (Figure 4) is another neurodegenerative disease where alpha-synuclein (α-SYN) forms small soluble oligomers and larger aggregates within subcellular inclusions called Lewy bodies.[171] Inflammation and gliosis also come into play in PD, often with deleterious consequences,[172] raising the question of impaired TAM function. In a mouse model of PD, induced by the introduction of human α-SYN in *Nrf2*$^{-/-}$ mouse brains, disease progressed as α-SYN aggregates accumulated. This was accompanied by microglial inflammation and attenuated expression of *Mertk* and *Axl*.[172] Another study in mice which utilized a late-onset hereditary form of PD, driven by a point mutation of the human α-SYN gene (*SNCA*^{A53T}) reports the upregulation of both AXL and its soluble form (sAXL), documented to be markers of inflammation.[128] Interestingly, such upregulation was exclusively coupled with an increase in expression of the microglial activation marker IBA1, especially in the spinal cord, but also in the brain. Survival of PD mice carrying the *SNCA*^{A53T} mutation was slightly extended following A*xl* and *Mertk* ablation. This was explained by the possible TAM-dependent phagoptotic engulfment, worsening the disease.[128]

10 | THE TAM RECEPTORS PERFORM PHAGOPTOSIS

In the previous paragraphs, the importance of TAM-dependent phagocytosis in preventing secondary necrosis, elevated inflammation, and the development of serious disorders was discussed. Nonetheless, like many biological mechanisms, phagocytosis must be regulated to avoid uncontrolled cell clearance, including that of viable cells. As a general rule, phagocytosis is secondary to initiation of cell death, as externalization of the classic eat-me signal PtdSer is induced by apoptotic pathways.[51] However, possibly due to a subtoxic insult, live cells may express "eat me" signals, or lose the expression of "don't eat me" signals, promoting their engulfment. This type of primary phagocytosis was characterized for the first time by Brown et al., and named *phagoptosis*. Phagoptosis may lead to the clearance of stressed, but otherwise viable cells, and further exacerbate disease development.[173] For example, after brain ischemia, the affected regions suffer from low-oxygen levels and excessive glutamate release, which cause extensive neuronal death.[174] In such a scenario, uptake of those dead neurons within the area of ischemia is crucial, and beneficial for tissue repair. In the neighboring areas, however, neurons may be stressed, yet functional. In such subtoxic conditions, cells can transiently expose PtdSer,[175] and be engulfed alive, causing severe neuronal loss.[174] Surprisingly, sparing these stressed, but viable neurons from clearance has proved to be beneficial, with rats showing reduced motor deficits and overall improved outcome in a brain ischemia/stroke model.[176] The transient upregulation of *Mertk* in microglia and macrophages at the same time phagoptosis occurred, suggested MERTK may be involved in this process. Consistently, the uptake of stressed neurons was reduced by 60% in *Mertk* mutants, which showed reduced brain atrophy and improved motor functions. In parallel, in vitro studies demonstrated that *Mertk* mutant microglia engulfed a smaller number of glutamate-stressed neurons. Taken together, these data suggest that MERTK-mediated phagoptotic cell clearance may constitute a new driver of brain pathology.[176] Therefore, inhibiting MERTK to prevent the engulfment of stressed, yet viable neurons may help the recovery after mild ischemia. Similarly, Fourgeaud et al., showed that ACs clearance through the TAMs not always prevents disease development and point to phagoptotic engulfment of stressed, but live motor neurons by AXL and MERTK as a possible mechanism worsening PD symptoms, and speeding animal death.[128] In conclusion, the TAM receptors are components of the phagocytic mis-targeting that characterizes phagoptosis. Therefore, exploiting the TAM system at specific physiological contexts is an interesting therapeutic approach to improve symptoms and prevent neurodegeneration.

11 | CARDIOVASCULAR DISEASES

Atherosclerosis is the most frequent type of cardiovascular diseases (CVD) and leading cause of death worldwide.[177] It is caused by the accumulation of cholesterol in the arteries, creating masses of inflamed lipid material that builds up atherosclerotic lesions. With time, these become big enough to obstruct the blood flow leading to myocardial infarction (MI) and stroke.[177,178] Both tissue-resident and monocyte-derived macrophages play an important role in the development, or recession, of atherosclerotic plaques.[177] When low-density lipoprotein (LDL) levels are high (hypercholesterolemia, HCL), tissue-resident macrophages are unable to handle the heavy load of lipids through canonical pathways (autophagy or storage), leading to lipid aggregation and atherosclerosis initiation. The overloaded tissue-resident macrophages try unsuccessfully to discharge the lipid overload, and become so called "foam cells",[179] which subsequently undergo apoptosis and necroptosis.[177,180] In the early stages of this process, efferocytosis by other macrophages prevents plaque growth. However, in later stages, foam cells downregulate *Mertk*, rendering efferocytosis inefficient (Figure 4).[181] As a consequence, MDMs are recruited by pro-inflammatory stimuli, and atherosclerotic plaques grow.[177,181] Grafting *Mertk*$^{+/+}$ *or Mertk*$^{-/-}$ BM into atherosclerotic mice showed that macrophages derived from *Mertk*$^{-/-}$ BM were unable to efficiently clean up ACs, increasing necrosis and speeding up lesion development.[182,183] Similarly, Knock-down (KD) of *Mertk* in a model of advanced atherosclerosis showed increased formation of necrotic plaques due to defective MERTK-dependent efferocytosis of ACs.[184] Mechanistic studies suggested that tissue-resident macrophages may be unable to perform efferocytosis in advanced stages of lesion formation due to their increased expression of Ca^{2+}/calmodulin-dependent protein kinase γ (CaMKIIγ), which suppresses ATF6, a transcription

factor regulating liver X receptor-α (LXRα), which is a *Mertk*-inducing transcription factor. This study reveals a macrophage CaMKIIγ/ATF6/LXRα/MERTK pathway as key in development of cardiovascular disease, and highlights that MERTK inactivity not only leads to ACs accumulation, but also prevents the phagocytosis-dependent release of resolving cytokines.[185] This pathway has been recently targeted in a pre-clinical model study by Tao et al.: Introducing small interfering RNA (siRNA) nanoparticles targeting murine *CaMKIIγ*, *Mertk* expression in macrophages increased. This resulted in efficient ACs phagocytosis and reduced necrotic plaques, constituting a promising targetable pathway.[186] In human patients, the titer of the soluble form of MERTK (sMERTK) may serve as a marker for cardiovascular disease, as it was found to positively correlate with necrosis of plaques and symptoms typical of ischemia and stroke.[183] Taken together, these studies highlight the importance of MERTK-mediated efferocytosis within atherosclerotic plaques, and identify novel therapeutic targets to prevent atherosclerosis progression.

12 | SYSTEM-WIDE IMPLICATIONS OF DEFECTIVE TAM RECEPTOR-MEDIATED PHAGOCYTOSIS

Given that the TAM receptors are implicated both in clearing phagocytic cells in numerous organs, and in engulfing different moieties, some implications of TAM-driven phagocytosis are system-wide. This is the case for autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and Sjogren syndrome (SS). SLE is characterized by chronic inflammation triggered by defective clearance of ACs in the lymph nodes germinal center, and the production of autoantibodies against nuclear antigens and double-stranded DNA. It affects several different organs including skin, joints, brain, blood vessels, lungs, and kidneys[187] (Figure 4). Predictably, TAM receptor KO mice develop lupus-like clinical manifestations, including systemic autoimmunity and enlarged lymph nodes.[28,67] Plasma levels of secreted forms of AXL and MERTK (sAXL and sMERTK, respectively), as well as GAS6, are reported to be increased in adult onset SLE patients, and to correlate with renal symptoms severity, specifically glomerulonephritis.[188,189] Similarly, sMERTK levels, although not sAXL, sTYRO3 and GAS6, were found to correspond to active disease in Juvenile SLE (JSLE) patients.[190] Elevated levels of the cleaved forms of TAM receptors imply they may bind their ligands and function as competitors with cell-bound TAMs, inhibiting phagocytosis.[191] Moreover, high titers of autoantibodies against PROS1 were found in SLE patients, which may neutralize PROS1 from activating MERTK-mediated efferocytosis.[192–194] Taken together, these data on human patients show that sTAM levels in serum may serve not only as new biomarkers for SLE activity and prognosis, but also represent great therapeutic targets.[191] RA is the most common autoimmune disorder, manifesting itself as chronic synovial fluid inflammation, and resulting in cartilage and bone damage.[195] TAM receptors are expressed in several locations of the synovial tissue. Akin to SLE pathogenesis, recent findings similarly revealed that sTYRO3, sMERTK and sAXL levels are elevated in the synovial fluids of RA patients. However, no differences were found in GAS6 expression.[196,197] Interestingly sTYRO3 levels positively correlated with increased secretion of pro-inflammatory cytokines in the joints, identifying TYRO3, among the other TAM components, as an important potential candidate promoting RA pathogenesis and severity.[196] In SS, autoimmunity affects salivary and lacrimal glands, where elevated apoptosis and defective efferocytosis are observed. Monocytes isolated from SS model mice exhibit decreased MERTK signaling and defective efferocytosis, with elevated levels of sMERTK in plasma, and similar inflammatory profiles to *Mertk*$^{-/-}$ mice.[198] Despite the lack of evidence of TAMs involvement in SS, several studies reported low levels of TAM receptors mRNAs in mononuclear cells of SS patients' blood, together with high sMERTK and autoantibodies titter.[199] Interestingly, monocytes isolated from SS patients were reported to perform inefficient efferocytosis compared to those from healthy controls, hinting at their involvement in SS through defective cell clearance and reduced inhibition of IFN signaling.[200] Such conditions contribute to defective efferocytosis and a pro-inflammatory environment that ultimately exacerbates autoimmunity.

If the TAM anti-inflammatory ability is favorable in the context of most pathologies discussed so far, it is not always beneficial in cancer. The tumor microenvironment (TME) is characterized by high cell turnover and is therefore abundant with PtdSer-expressing cells: intra-tumor ACs, but also viable PtdSer-expressing tumor cells and tumor-associated endothelial cells.[57,201] PROS1 and GAS6 are attracted to the high presence of PtdSer, bridging between PtdSer-expressing cells and TAM-expressing phagocytes, promoting phagocytosis and anti-inflammatory signaling, altogether favoring tumor growth (Figure 4). Phagocytosis through the TAMs shifts the pro-inflammatory status of macrophages secreting TNF-α, IL-6, IL-1β, and nitric oxide (NO), which are strong activators of the immune response, to anti-inflammatory, known to secrete resolving cytokines (IL-4, IL-10, IL-13, TGF-β). These events provide for a tumor-supportive environment and feed its growth.[57,74,202,203] A large body of literature indicate that TAM signaling (receptors and ligands) are overexpressed in numerous cancers where they contribute to tumor proliferation, metastatic ability, and immune evasion-all driving poor prognosis.[57,204–208] It is likely that there is a positive selection for PtdSer-expressing cells in the TME, allowing these cells to survive and contribute to tumor survival and promote epithelial to mesenchymal transition (EMT), as demonstrated in mouse models for breast,[209] brain,[210] and lung cancers.[74] Given that efferocytosis is anti-inflammatory,[48,211] TAM-driven clearance of ACs within tumors may contribute to an immune-suppressed and cancer-promoting environment. Thus, pharmacological treatments tackling cancer should therefore aim at inhibiting TAM-mediated phagocytosis in the TME.[57]

13 | CONCLUDING REMARKS

TAM receptors and their ligands are often functionally redundant, as mentioned above. Yet, this pathway accurately initiates a multitude

of intracellular signaling cascades, each within its specific physiological context. As briefly mentioned, TAMs also initiate additional non-phagocytic cellular functions such as proliferation, migration, and cell fate decisions with implications ranging from stem cell biology to cancer signaling.[2,57,206] In this respect, how TAMs acquire their functional specificity in a variety of biological contexts is still not fully understood.

Now, we know that ACs are not the only targets recognized and engulfed by TAMs, as PtdSer-exposing live cells, or portions thereof (synapses, POS), as well as myelin, are also engulfed by phagocytes through TAMs. This way, the same system is employed to clear up different particles that may affect tissue health, and simultaneously dampen the inflammatory response. Disruption of TAM signaling causes particle accumulation and heightened inflammation, resulting in tissue degeneration, as shown in many organs including the brain, retina, testes, joints, and blood vessels. On the contrary, TAM-phagocytosis is not necessarily beneficial in the tumor and stroke micro-environments.

However, TAMs are not universal executers of phagocytosis, as they are not activated by bacteria or fungi, even if all components seem to be in place (Figure 2). How then is this specificity regulated? Additional open questions are whether TAMs similarly recognize the different PtdSer-exposing moieties, regardless of their size and content? Engulfment alone introduces quite a few challenges to the phagocyte. Following recognition of the target, uptake requires cytoskeletal changes to generate the phagocytic cup leading to internalization. Once internalized, digesting a bulk of biomass is probably challenging, especially since ACs carry toxic waste products. In this respect, Anwar et al. showed that MERTK also promotes survival of macrophages under oxidative stress conditions, mimicking AC uptake.[212] Despite many similarities to phagocytosis of pathogen-infected ACs, efferocytosis is anti-inflammatory. Remarkably, TAMs converge cytoskeletal rearrangement, anti-inflammatory signaling, and survival pathways. Linking survival and anti-inflammatory signaling to phagocytosis in general, and to efferocytosis in particular, is evolutionarily beneficial, as it allows optimization of cell resources and shortens the response time. Moreover, activation of protective pathways safeguards from the potentially detrimental consequences of engulfing the toxic cargo, enabling successful accomplishment of efferocytosis. This is particularly important for postmitotic phagocytes such as RPE and Sertoli cells.

Understanding the specificity of TAM function is only beginning to be revealed. While MERTK mainly functions at steady state, AXL is recruited following stress induction,[213] but how this is regulated is still unknown. Initial reports point to functional variability among the ligands in regulation of adult neural stem cell proliferation,[2] and in POS phagocytosis in the retina.[214]

Moreover, in order to cope efficiently with the toxic products of cell death, evolution generated different families of receptors, other than the TAMs, to perform phagocytosis in a PtdSer-dependent way.[215] These include integrins, the brain-specific angiogenesis inhibitors (BAI) subfamily of GPCRs, and the T cell/transmembrane, immunoglobulin, and the mucin (TIM) family.[26,30,216–218] Whether the many PtdSer receptors that have evolved are unique or functionally interchangeable was addressed by Penberthy et al., who asked whether BAI1 overexpression would compensate for MERTK loss of function in the testis and retina. Interestingly, although BAI1 overexpression was able to rescue ACs build-up in the testes, it was not sufficient to rescue photoreceptor degeneration in the retina.[215] The authors hypothesized that this difference is due to the fact that the uptake of POS by RPE is not a traditional corpse clearing event, as is apoptotic spermatid clearance. However, a more recent research now revealed that retinal degeneration in *Mertk*$^{-/-}$ mice is due to early inflammation rather than lack of POS uptake.[116] Nevertheless, given that both BAI1 and MERTK have anti-inflammatory functions when dealing with homeostatic clearance of ACs,[78,219] these pathways are clearly distinct and not completely redundant. Similarly, clearing degraded myelin by the two PtdSer engulfment receptors MERTK and MEGF10 was not redundant.[136] Such experiments reveal that despite significant molecular and mechanistic similarity, TAMs and other phagocytosis receptors are not simply redundant, or interchangeable, illuminating functional difference and hence the need for numerous PtdSer-recognizing phagocytosis receptors.

By virtue of the expression on virtually every cell and tissue, TAM receptors and their ligands are major regulators of adult homeostasis, though they also play important roles during development. Their quintessential homeostasis-related functions are efferocytosis, discovered in the nineties, and anti-inflammatory signaling. Clearly, the development of therapeutics targeting the TAMs is of high interest and has great potential to treat diseases involving system-wide, or organ-specific, implications. However, to best harness this system, different aspects of TAM-mediated phagocytosis still remain to be elucidated.

AUTHOR CONTRIBUTIONS

T. B-C and R. F. conceived and wrote the manuscript. R.F prepared the figures. T. B-C edited the manuscript and figures.

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REFERENCES

1. Lemke G. Biology of the TAM receptors. *Cold Spring Harb Perspect Biol*. 2013;5(11):1-17. doi:10.1101/cshperspect.a009076
2. Burstyn-Cohen T, Hochberg A. TAM signaling in the nervous system. *Brain Plast*. 2021;7(1):33-46. doi:10.3233/bpl-210125
3. Rothlin CV, Carrera-Silva EA, Bosurgi L, Ghosh S. TAM receptor signaling in immune homeostasis. *Annu Rev Immunol*. 2015;33:355-391.
4. Burstyn-Cohen T. TAM receptor signaling in development. *Int J Dev Biol*. 2017;61(3–5):215-224. doi:10.1387/ijdb.160285tb
5. Heiring C, Dahlbäck B, Muller YA. Ligand recognition and homophilic interactions in Tyro3: structural insights into the Axl/Tyro3 receptor tyrosine kinase family. *J Biol Chem*. 2004;279(8):6952-6958. doi:10.1074/jbc.M311750200
6. Sasaki T, Knyazev PG, Clout NJ, et al. Structural basis for Gas6-Axl signalling. *EMBO J*. 2006;25(1):80-87. doi:10.1038/sj.emboj.7600912
7. O'Bryan JP, Frye RA, Cogswell PC, et al. Axl, a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. *Mol Cell Biol*. 1991;11(10):5016-5031. doi:10.1128/mcb.11.10.5016-5031.1991
8. Lai C, Lemke G. An extended family of protein-tyrosine kinase genes differentially expressed in the vertebrate nervous system. *Neuron*. 1991;6(5):691-704. doi:10.1016/0896-6273(91)90167-X
9. Lemke G, Rothlin CV. Immunobiology of the TAM receptors. *Nat Rev Immunol*. 2008;8(5):327-336.
10. Graham DK, Salzberg DB, Kurtzberg J, et al. Ectopic expression of the proto-oncogene Mer in pediatric T-cell acute lymphoblastic leukemia. *Clin Cancer Res*. 2006;12(9):2662-2669. doi:10.1158/1078-0432.CCR-05-2208
11. Stitt TN, Conn G, Goret M, et al. The anticoagulation factor protein S and its relative, Gas6, are ligands for the tyro 3/Axl family of receptor tyrosine kinases. *Cell*. 1995;80(4):661-670. doi:10.1016/0092-8674(95)90520-0
12. Ohashi K, Nagata K, Toshima J, et al. Stimulation of sky receptor tyrosine kinase by the product of growth arrest-specific gene 6. *J Biol Chem*. 1995;270(39):22681-22684. doi:10.1074/jbc.270.39.22681
13. Mark MR, Chen J, Glenn Hammonds R, Sadick M, Godowsk PJ. Characterization of Gas6, a member of the superfamily of G domain-containing proteins, as a ligand for Rse and Axl. *J Biol Chem*. 1996;271(16):9785-9789. doi:10.1074/jbc.271.16.9785
14. Nagata K, Ohashi K, Nakano T, et al. Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, sky, and Mer receptor tyrosine kinases. *J Biol Chem*. 1996;271(47):30022-30027. doi:10.1074/jbc.271.47.30022
15. Tsou WI, Nguyen KQN, Calarese DA, et al. Receptor tyrosine kinases, TYRO3, AXL, and MER, demonstrate distinct patterns and complex regulation of ligand-induced activation. *J Biol Chem*. 2014;289(37):25750-25763. doi:10.1074/jbc.M114.569020
16. Lew ED, Oh J, Burrola PG, et al. Differential TAM receptor-ligand-phospholipid interactions delimit differential TAM bioactivities. *Elife*. 2014;3:e03385. doi:10.7554/eLife.03385
17. Manfioletti G, Brancolini C, Avanzi G, Schneider C. 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. *Mol Cell Biol*. 1993;13(8):4976-4985. doi:10.1128/mcb.13.8.4976-4985.1993
18. Letunic I, Khedkar S, Bork P. SMART: recent updates, new developments and status in 2020. *Nucleic Acids Res*. 2021;49:D458-D460. doi:10.1093/nar/gkaa937
19. Li T, Chang CY, Jin DY, Lin PJ, Khvorova A, Stafford DW. Identification of the gene for vitamin K epoxide reductase. *Nature*. 2004;427(6974):541-544. doi:10.1038/nature02254
20. Rost S, Fregin A, Ivaskevicius V, et al. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. *Nature*. 2004;427(6974):537-541. doi:10.1038/nature02214
21. Evenäs P, Dahlbäck B, García de Frutos P. The first laminin G-type domain in the SHBG-like region of protein S contains residues essential for activation of the receptor tyrosine kinase sky. *Biol Chem*. 2000;381:199-209.
22. Tanabe K, Nagata K, Ohashi K, Nakano T, Arita H, Mizuno K. Roles of γ-carboxylation and a sex hormone-binding globulin-like domain in receptor-binding and in biological activities of Gas6. *FEBS Lett*. 1997;408(3):306-310. doi:10.1016/S0014-5793(97)00448-1
23. Sasaki T, Knyazev PG, Cheburkin Y, et al. Crystal structure of a C-terminal fragment of growth arrest-specific protein Gas6: receptor tyrosine kinase activation by laminin G-like domains. *J Biol Chem*. 2002;277(46):44164-44170. doi:10.1074/jbc.M207340200
24. Rothlin CV, Ghosh S, Zuniga EI, Oldstone MBA, Lemke G. TAM receptors are pleiotropic inhibitors of the innate immune response. *Cell*. 2007;131(6):1124-1136. doi:10.1016/j.cell.2007.10.034
25. Rothlin CV, Lemke G. TAM receptor signaling and autoimmune disease. *Curr Opin Immunol*. 2010;22(6):740-746. doi:10.1016/j.coi.2010.10.001
26. Lemke G, Burstyn-Cohen T. TAM receptors and the clearance of apoptotic cells. *Ann N Y Acad Sci*. 2010;1209(1):23-29. doi:10.1111/j.1749-6632.2010.05744.x
27. Lu Q, Gore M, Zhang Q, et al. Tyro-3 family receptors are essential regulators of mammalian spermatogenesis. *Nature*. 1999;398(6729):723-728.
28. Lu Q, Lemke G. Homeostatic regulation of the immune system by receptor tyrosine kinases of the tyro 3 family. *Science*. 2001;293(5528):306-311. doi:10.1126/science.1061663
29. Lemke G, Lu Q. Macrophage regulation by tyro 3 family receptors. *Curr Opin Immunol*. 2003;15(1):31-36. doi:10.1016/S0952-7915(02)00016-X
30. Duncan JL, LaVail MM, Yasumura D, et al. An RCS-like retinal dystrophy phenotype in Mer knockout mice. *Invest Ophthalmol Vis Sci*. 2003;44(2):826-838. doi:10.1167/iovs.02-0438
31. Burstyn-Cohen T, Lew ED, Través PG, Burrola PG, Hash GC, Lemke G. Genetic dissection of TAM receptor-ligand interaction in retinal pigment epithelial cell phagocytosis. *Neuron*. 2012;76(6):1123-1132.
32. Angelillo-Scherrer A, de Frutos PG, Aparicio C, et al. Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis. *Nat Med*. 2001;7:215-221.
33. Fourcot A, Couchie D, Chobert MN, et al. Gas6 deficiency prevents liver inflammation, steatohepatitis, and fibrosis in mice. *Am J Physiol Gastrointest Liver Physiol*. 2011;300(6):1043-1053. doi:10.1152/ajpgi.00311.2010
34. Loges S, Schmidt T, Tjwa M, et al. Malignant cells fuel tumor growth by educating infiltrating leukocytes to produce the mitogen Gas6. *Blood*. 2010;115(11):2264-2273.
35. Godowski PJ, Mark MR, Chen J, Sadick MD, Raab H, Hammonds RG. Reevaluation of the roles of protein S and gas6 as ligands for the receptor tyrosine kinase Rse/tyro 3. *Cell*. 1995;82(3):355-358.
36. Hall MO, Prieto AL, Obin MS, et al. Outer segment phagocytosis by cultured retinal pigment epithelial cells requires Gas6. *Exp Eye Res*. 2001;73(4):509-520. doi:10.1006/exer.2001.1062
37. Lumbroso D, Soboh S, Maimon A, Schif-Zuck S, Ariel A, Burstyn-Cohen T. Macrophage-derived protein S facilitates apoptotic polymorphonuclear cell clearance by resolution phase macrophages and supports their reprogramming. *Front Immunol*. 2018;9:10-13. doi:10.3389/fimmu.2018.00358
38. Zelentsova K, Talmi Z, Abboud-Jarrous G, et al. Protein S regulates neural stem cell quiescence and neurogenesis. *Stem Cells*. 2017;35(3):679-693. doi:10.1002/stem.2522

39. Zelentsova-Levytskyi K, Talmi Z, Abboud-Jarrous G, Capucha T, Sapir T, Burstyn-Cohen T. Protein S negatively regulates neural stem cell self-renewal through Bmi-1 signaling. *Front Mol Neurosci*. 2017;10:1-14. doi:10.3389/fnmol.2017.00124
40. Tabib Y, Jaber NS, Nassar M, et al. Cell-intrinsic regulation of murine epidermal Langerhans cells by protein S. *Proc Natl Acad Sci USA*. 2018;115(25):E5736-E5745. doi:10.1073/pnas.1800303115
41. Burstyn-Cohen T, Heeb MJ, Lemke G. Lack of protein S in mice causes embryonic lethal coagulopathy and vascular dysgenesis. *J Clin Investig*. 2009;119(10):2942-2953. doi:10.1172/JCI39325
42. Saller F, Brisset AC, Tchaikovski SN, et al. Generation and phenotypic analysis of protein S–deficient mice. *Blood*. 2009;114(11):2307-2314.
43. Ravichandran KS. "Recruitment signals" from apoptotic cells: invitation to a quiet meal. *Cell*. 2003;113(7):817-820. doi:10.1016/S0092-8674(03)00471-9
44. Ravichandran KS, Lorenz U. Engulfment of apoptotic cells: signals for a good meal. *Nat Rev Immunol*. 2007;7(12):964-974. doi:10.1038/nri2214
45. Ravichandran KS. Beginnings of a good apoptotic meal: the find-me and eat-me signaling pathways. *Immunity*. 2011;35(4):445-455. doi:10.1016/j.immuni.2011.09.004
46. DeCathelineau AM, Henson PM. The final step in programmed cell death: phagocytes carry apoptotic cells to the grave. *Essays Biochem*. 2003;39:105-117. doi:10.1042/bse0390105
47. Henson PM. Cell removal: efferocytosis. *Annu Rev Cell Dev Biol*. 2017;33:127-144. doi:10.1146/annurev-cellbio-111315-125315
48. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-β, PGE2, and PAF. *J Clin Investig*. 1998;101(4):890-898. doi:10.1172/JCI1112
49. Goodridge HS, Reyes CN, Becker CA, et al. Activation of the innate immune receptor Dectin-1 upon formation of a 'phagocytic synapse'™. *Nature*. 2011;472(7344):471-475. doi:10.1038/nature10071
50. Anderson HA, Maylock CA, Williams JA, Paweletz CP, Shu H, Shacter E. Serum-derived protein S binds to phosphatidylserine and stimulates the phagocytosis of apoptotic cells. *Nat Immunol*. 2003;4(1):87-91. doi:10.1038/ni871
51. Ravichandran KS. Find-me and eat-me signals in apoptotic cell clearance: progress and conundrums. *J Exp Med*. 2010;207(9):1807-1817. doi:10.1084/jem.20101157
52. Nagata S, Hanayama R, Kawane K. Autoimmunity and the clearance of dead cells. *Cell*. 2010;140(5):619-630. doi:10.1016/j.cell.2010.02.014
53. Lemke G. Phosphatidylserine is the signal for TAM receptors and their ligands. *Trends Biochem Sci*. 2017;42(9):738-748. doi:10.1016/j.tibs.2017.06.004
54. Stenflo J. Contributions of Gla and EGF-like domains to the function of vitamin K-dependent coagulation factors. *Crit Rev Eukaryot Gene Expr*. 1999;9(1):59-88.
55. Huang M, Furie BC, Furie B. Crystal structure of the calcium-stabilized human factor IX Gla domain bound to a conformation-specific anti-factor IX antibody. *J Biol Chem*. 2004;279(14):14338-14346. doi:10.1074/jbc.M314011200
56. Dransfield I, Zagórska A, Lew ED, Michail K, Lemke G. Mer receptor tyrosine kinase mediates both tethering and phagocytosis of apoptotic cells. *Cell Death Dis*. 2015;6(2):1-10. doi:10.1038/cddis.2015.18
57. Burstyn-Cohen T, Maimon A. TAM receptors, phosphatidylserine, inflammation, and cancer. *Cell Commun Signaling*. 2019;17(1):156. doi:10.1186/s12964-019-0461-0
58. Tietjen GT, Gong Z, Chen CH, et al. Molecular mechanism for differential recognition of membrane phosphatidylserine by the immune regulatory receptor Tim4. *Proc Natl Acad Sci USA*. 2014;111(15):E1463-E1472. doi:10.1073/pnas.1320174111
59. Bosurgi L, Cao YG, Cabeza-Cabrerizo M, et al. Macrophage function in tissue repair and remodeling requires IL-4 or IL-13 with apoptotic cells. *Science*. 2017;356(6342):1072-1076. doi:10.1126/science.aai8132
60. Carrera Silva EA, Chan PY, Joannas L, et al. T cell-derived protein S engages TAM receptor signaling in dendritic cells to control the magnitude of the immune response. *Immunity*. 2013;39(1):160-170. doi:10.1016/j.immuni.2013.06.010
61. Jiménez-García L, Mayer C, Burrola PG, Huang Y, Shokhirev MN, Lemke G. The TAM receptor tyrosine kinases Axl and Mer drive the maintenance of highly phagocytic macrophages. *Front Immunol*. 2022;13:1-20. doi:10.3389/fimmu.2022.960401
62. Segawa K, Suzuki J, Nagata S. Constitutive exposure of phosphatidylserine on viable cells. *Proc Natl Acad Sci USA*. 2011;108(48):19246-19251. doi:10.1073/pnas.1114799108
63. Cassilly CD, Reynolds TB. PS, it's complicated: the roles of phosphatidylserine and phosphatidylethanolamine in the pathogenesis of Candida albicans and other microbial pathogens. *J Fungi*. 2018;4(1):28. doi:10.3390/jof4010028
64. Scott RS, McMahon EJ, Pop SM, et al. Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature*. 2001;411(6834):207-211. doi:10.1038/35075603
65. Williams BJC, Craven RR, Earp HS, Kawula TH, Matsushima GK. TAM receptors are dispensable in the phagocytosis and killing of bacteria. *Cell Immunol*. 2009;259(2):128-134. doi:10.1016/j.cellimm.2009.06.006
66. Uehara H, Shacter E. Auto-oxidation and oligomerization of protein S on the apoptotic cell surface is required for Mer tyrosine kinase-mediated phagocytosis of apoptotic cells. *J Immunol*. 2008;180(4):2522-2530.
67. Cohen PL, Caricchio R, Abraham V, et al. Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase. *J Exp Med*. 2002;196(1):135-140. doi:10.1084/jem.20012094
68. Seitz HM, Camenisch TD, Lemke G, Earp HS, Matsushima GK. Macrophages and dendritic cells use different Axl/Mertk/Tyro3 receptors in clearance of apoptotic cells. *J Immunol*. 2007;178(9):5635-5642.
69. Bosurgi L, Bernink JH, Cuevas VD, et al. Paradoxical role of the proto-oncogene Axl and Mer receptor tyrosine kinases in colon cancer. *Proc Natl Acad Sci USA*. 2013;110(32):13091-13096. doi:10.1073/pnas.1302507110
70. Grommes C, Lee CYD, Wilkinson BL, et al. Regulation of microglial phagocytosis and inflammatory gene expression by Gas6 acting on the Axl/Mer family of tyrosine kinases. *J Neuroimmune Pharmacol*. 2008;3(2):130-140. doi:10.1007/s11481-007-9090-2
71. Tibrewal N, Wu Y, D'Mello V, et al. Autophosphorylation docking site Tyr-867 in Mer receptor tyrosine kinase allows for dissociation of multiple signaling pathways for phagocytosis of apoptotic cells and down-modulation of lipopolysaccharide-inducible NF-κB transcriptional activation. *J Biol Chem*. 2008;283(6):3618-3627. doi:10.1074/jbc.M706906200
72. Arandjelovic S, Ravichandran KS. Phagocytosis of apoptotic cells in homeostasis. *Nat Immunol*. 2015;16(9):907-917. doi:10.1038/ni.3253
73. Wu Y, Singh S, Georgescu MM, Birge RB. A role for Mer tyrosine kinase in αvβ5 integrin-mediated phagocytosis of apoptotic cells. *J Cell Sci*. 2005;118(3):539-553.
74. Maimon A, Levi-Yahid V, Ben-Meir K, et al. Myeloid cell–derived PROS1 inhibits tumor metastasis by regulating inflammatory and immune responses via IL-10. *J Clin Investig*. 2021;131(10):1-20. doi:10.1172/JCI126089
75. Elkabets M, Pazarentzos E, Juric D, et al. AXL mediates resistance to PI3Kα inhibition by activating the EGFR/PKC/mTOR Axis in head and neck and esophageal squamous cell carcinomas. *Cancer Cell*. 2015;27(4):533-546. doi:10.1016/j.ccell.2015.03.010

76. Waterborg CEJ, Koenders MI, van Lent PLEM, van der Kraan PM, van de Loo FAJ. Tyro3/Axl/Mertk-deficient mice develop bone marrow edema which is an early pathological marker in rheumatoid arthritis. *PLoS One*. 2019;13(10):1-13. doi:10.1371/journal.pone.0205902
77. Mercau ME, Akalu YT, Mazzoni F, et al. Inflammation of the retinal pigment epithelium drives early-onset photoreceptor degeneration in Mertk-associated retinitis pigmentosa. *Sci Adv*. 2023;9(3):1-19. doi:10.1126/sciadv.ade9459
78. Nagata S. Autoimmune diseases caused by defects in clearing dead cells and nuclei expelled from erythroid precursors. *Immunol Rev*. 2007;220(1):237-250. doi:10.1111/j.1600-065X.2007.00571.x
79. Asadi A, Ghahremani R, Abdolmaleki A, Rajaei F. Role of sperm apoptosis and oxidative stress in male infertility: a narrative review. *Int J Reprod Biomed*. 2021;19(6):493-504. doi:10.18502/ijrm.v19i6.9371
80. Da NF, Hao SL, Yang WX. Multiple signaling pathways in Sertoli cells: recent findings in spermatogenesis. *Cell Death Dis*. 2019;10(8):541. doi:10.1038/s41419-019-1782-z
81. Kawasaki Y, Nakagawa A, Nagaosa K, Shiratsuchi A, Nakanishi Y. Phosphatidylserine binding of class B scavenger receptor type I, a phagocytosis receptor of testicular sertoli cells. *J Biol Chem*. 2002;277(30):27559-27566. doi:10.1074/jbc.M202879200
82. Elliott MR, Zheng S, Park D, et al. Unexpected requirement for ELMO1 in clearance of apoptotic germ cells in vivo. *Nature*. 2010;467(7313):333-337. doi:10.1038/nature09356
83. Print CG, Loveland KL. Germ cell suicide: new insights into apoptosis during spermatogenesis. *Bioessays*. 2000;22(5):423-430. doi:10.1002/(SICI)1521-1878(200005)22:5<423::AID-BIES4>3.0.CO;2-0
84. Lysiak JJ, Turner SD, Turner TT. Molecular pathway of germ cell apoptosis following ischemia/reperfusion of the rat testis. *Biol Reprod*. 2000;63(5):1465-1472. doi:10.1095/biolreprod63.5.1465
85. Xiong W, Chen Y, Wang H, et al. Gas6 and the tyro 3 receptor tyrosine kinase subfamily regulate the phagocytic function of Sertoli cells. *Reproduction*. 2008;135(1):77-87. doi:10.1530/REP-07-0287
86. Wang H, Chen Y, Ge Y, et al. Immunoexpression of tyro 3 family receptors – tyro 3, Axl, and Mer – and their ligand Gas6 in postnatal developing mouse testis. *J Histochem Cytochem*. 2005;53(11):1355-1364. doi:10.1369/jhc.5A6637.2005
87. Skinner MK. Cell-cell interactions in the testis. *Endocr Rev*. 1991;12(1):45-77. doi:10.1210/edrv-12-1-45
88. Zhang Y, Li N, Chen Q, et al. Breakdown of immune homeostasis in the testis of mice lacking Tyro3, Axl and Mer receptor tyrosine kinases. *Immunol Cell Biol*. 2013;91(6):416-426. doi:10.1038/icb.2013.22
89. Chen Y, Wang H, Qi N, et al. Functions of TAM RTKs in regulating spermatogenesis and male fertility in mice. *Reproduction*. 2009;138(4):655-666. doi:10.1530/REP-09-0101
90. Gillot I, Jehl-Pietr C, Gounon P, et al. Germ cells and fatty acids induce translocation of CD36 scavenger receptor to the plasma membrane of Sertoli cells. *J Cell Sci*. 2005;118(14):3027-3035. doi:10.1242/jcs.02430
91. Philips JA, Rubin EJ, Perrimon N. Drosophila RNAi screen reveals CD36 family member required for mycobacterial infection. *Science*. 2005;309(5738):1251-1253. doi:10.1126/science.1116006
92. Erdman LK, Cosio G, Helmers AJ, Gowda DC, Grinstein S, Kain KC. CD36 and TLR interactions in inflammation and phagocytosis: implications for malaria. *J Immunol*. 2009;183(10):6452-6459.
93. Newton SC, Blaschuk OW, Millette CF. N-cadherin mediates sertoli cell-spermatogenic cell adhesion. *Dev Dyn*. 1993;197(1):1-13. doi:10.1002/aja.1001970102
94. Shortman K, Egerton M, Spangrude GJ, Scollay R. The generation and fate of thymocytes. *Semin Immunol*. 1990;2(1):3-12.
95. Surh CD, Sprent J. T-cell apoptosis detected in situ selection in the thymus. *Nature*. 1994;372:100-103.
96. Palmer E. Negative selection – clearing out the bad apples from the T-cell repertoire. *Nat Rev Immunol*. 2003;3(5):383-391. doi:10.1038/nri1085
97. Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. *Annu Rev Immunol*. 2003;21:139-176. doi:10.1146/annurev.immunol.21.120601.141107
98. Klein L, Kyewski B, Allen PM, Hogquist KA. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat Rev Immunol*. 2014;14(6):377-391. doi:10.1038/nri3667
99. Torroba M, Zapata AG. Aging of the vertebrate immune system. *Microsc Res Tech*. 2003;62(6):477-481. doi:10.1002/jemt.10409
100. Shanley DP, Aw D, Manley NR, Palmer DB. An evolutionary perspective on the mechanisms of immunosenescence. *Trends Immunol*. 2009;30(7):374-381. doi:10.1016/j.it.2009.05.001
101. Cowan JE, Takahama Y, Bhandoola A, Ohigashi I. Postnatal involution and counter-involution of the thymus. *Front Immunol*. 2020;11:1-11. doi:10.3389/fimmu.2020.00897
102. Strauss O. The retinal pigment epithelium in visual function. *Physiol Rev*. 2005;85(3):845-881. doi:10.1152/physrev.00021.2004
103. Bok D. The retinal pigment epithelium: a versatile partner in vision. *J Cell Sci*. 1993;106(SUPPL. 17):189-195. doi:10.1242/jcs.1993.supplement_17.27
104. Nguyen-legros J, National I, Sante D, Medicale R. Renewal of photoreceptor outer segments and their phagocytosis the retinal pigment epithelium. Published Online 2000.
105. D'Cruz PM, Yasumura D, Weir J, et al. Mutation of the receptor tyrosine kinase gene Mertk in the retinal dystrophic RCS rat. *Hum Mol Genet*. 2000;9(4):645-651. doi:10.1093/hmg/9.4.645
106. Tschernutter M, Jenkins SA, Waseem NH, et al. Clinical characterisation of a family with retinal dystrophy caused by mutation in the Mertk gene. *Br J Ophthalmol*. 2006;90(6):718-723. doi:10.1136/bjo.2005.084897
107. Gal A, Li Y, Thompson DA, et al. Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. *Nat Genet*. 2000;26(3):270-271. doi:10.1038/81555
108. Prasad D, Rothlin CV, Burrola P, et al. TAM receptor function in the retinal pigment epithelium. *Mol Cell Neurosci*. 2006;33(1):96-108. doi:10.1016/j.mcn.2006.06.011
109. Ruggiero L, Connor MP, Chen J, Langen R, Finnemann SC. Diurnal, localized exposure of phosphatidylserine by rod outer segment tips in wild-type but not Itgb5−/− or Mfge8−/− mouse retina. *Proc Natl Acad Sci USA*. 2012;109(21):8145-8148. doi:10.1073/pnas.1121101109
110. Marigo V. Programmed cell death in retinal degeneration: targeting apoptosis in photoreceptors as potential therapy for retinal degeneration. *Cell Cycle*. 2007;6(6):652-655. doi:10.4161/cc.6.6.4029
111. Hartong D, Berson E, Dryja T. Retinitis pigmentosa prevalence and inheritance patterns. *Lancet*. 2006;368:1795-1809.
112. Ferrari S, Di Iorio E, Barbaro V, Ponzin D, Sorrentino F, Parmeggiani F. Retinitis pigmentosa: genes and disease mechanisms. *Curr Genomics*. 2011;12(4):238-249. doi:10.2174/138920211795860107
113. Nandrot E, Dufour E. Mertk in daily retinal phagocytosis: a history in the making. *Adv Exp Med Biol*. 2010;664:133-140.
114. Vollrath D, Yasumura D, Benchorin G, et al. Tyro3 modulates Mertk-associated retinal degeneration. *PLoS Genet*. 2015;11(12):1-20. doi:10.1371/journal.pgen.1005723
115. Akalu YT, Mercau ME, Ansems M, et al. Tissue-specific modifier alleles determine Mertk loss-of-function traits. *Elife*. 2022;11:1-27. doi:10.7554/eLife.80530
116. Mercau ME, Akalu YT, Mazzoni F, et al. Inflammation of the retinal pigment epithelium drives early-onset photoreceptor degeneration in Mertk-associated retinitis pigmentosa. *Sci Adv*. 2023;9(3):1-19. doi:10.1126/sciadv.ade9459

117. Nandrot EF, Kim Y, Brodie SE, Huang X, Sheppard D, Finnemann SC. Loss of synchronized retinal phagocytosis and age-related blindness in mice lacking αvβ5 integrin. *J Exp Med*. 2004;200(12):1539-1545. doi:10.1084/jem.20041447
118. Prinz M, Priller J. Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. *Nat Rev Neurosci*. 2014;15(5):300-312. doi:10.1038/nrn3722
119. Tremblay MÈ, Stevens B, Sierra A, Wake H, Bessis A, Nimmerjahn A. The role of microglia in the healthy brain. *J Neurosci*. 2011;31(45):16064-16069. doi:10.1523/JNEUROSCI.4158-11.2011
120. Streit WJ, Conde JR, Fendrick SE, Flanary BE, Mariani CL. Role of microglia in the central nervous system's immune response. *Neurol Res*. 2005;27(7):685-691. doi:10.1179/016164105x49463a
121. Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci*. 2007;10(11):1387-1394. doi:10.1038/nn1997
122. Neumann H, Kotter MR, Franklin RJM. Debris clearance by microglia: an essential link between degeneration and regeneration. *Brain*. 2009;132(2):288-295. doi:10.1093/brain/awn109
123. Prinz M, Jung S, Priller J. Microglia biology: one century of evolving concepts. *Cell*. 2019;179(2):292-311. doi:10.1016/j.cell.2019.08.053
124. Sierra A, Encinas JM, Deudero JJP, et al. Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell*. 2010;7(4):483-495. doi:10.1016/j.stem.2010.08.014
125. Paolicelli RC, Bolasco G, Pagani F, et al. Synaptic pruning by microglia is necessary for normal brain development. *Science*. 2011;333(6048):1456-1458. doi:10.1126/science.1202529
126. Ehninger D, Kempermann G. Neurogenesis in the adult hippocampus. *Cell Tissue Res*. 2008;331(1):243-250. doi:10.1007/s00441-007-0478-3
127. Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, et al. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nat Med*. 2019;25(4):554-560. doi:10.1038/s41591-019-0375-9
128. Fourgeaud L, Través PG, Tufail Y, et al. TAM receptors regulate multiple features of microglial physiology. *Nature*. 2016;532(7598):240-244. doi:10.1038/nature17630.TAM
129. Yu X, Wang G, Gilmore A, et al. Accelerated experience-dependent pruning of cortical synapses in ephrin-A2 knockout mice. *Neuron*. 2014;80(1):64-71. doi:10.1016/j.neuron.2013.07.014.Accelerated
130. Lichtman JW, Colman H. Synapse elimination and indelible memory. *Neuron*. 2000;25(2):269-278. doi:10.1016/S0896-6273(00)80893-4
131. Schafer DP, Lehrman EK, Kautzman AG, et al. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron*. 2012;74(4):691-705.
132. Schafer DP, Lehrman EK, Stevens B. The "quad-partite" synapse: microglia-synapse interactions in the developing and mature CNS. *Glia*. 2013;61(1):24-36. doi:10.1002/glia.22389
133. Márquez-Ropero M, Benito E, Plaza-Zabala A, Sierra A. Microglial corpse clearance: lessons from macrophages. *Front Immunol*. 2020;11:1-17. doi:10.3389/fimmu.2020.00506
134. Butovsky O, Jedrychowski MP, Moore CS, et al. Identification of a unique TGF-β-dependent molecular and functional signature in microglia. *Nat Neurosci*. 2014;17(1):131-143. doi:10.1038/nn.3599
135. Diaz-Aparicio I, Paris I, Sierra-Torre V, et al. Microglia actively remodel adult hippocampal neurogenesis through the phagocytosis secretome. *J Neurosci*. 2020;40(7):1453-1482. doi:10.1523/JNEUROSCI.0993-19.2019
136. Chung WS, Clarke LE, Wang GX, et al. Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. *Nature*. 2013;504(7480):394-400. doi:10.1038/nature12776.Astrocytes
137. Freeman MR. Specification and morphogenesis of astrocytes. *Science*. 2010;330(6005):774-778. doi:10.1126/science.1190928
138. Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci*. 2005;6(8):626-640. doi:10.1038/nrn1722
139. Abbott NJ, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci*. 2006;7(1):41-53. doi:10.1038/nrn1824
140. Lee J, Kim SW, Kim KT. Region-specific characteristics of astrocytes and microglia: a possible involvement in aging and diseases. *Cell*. 2022;11(12):1902. doi:10.3390/cells11121902
141. Bean BP. The action potential in mammalian central neurons. *Nat Rev Neurosci*. 2007;8(6):451-465. doi:10.1038/nrn2148
142. Binder MD, Kilpatrick TJ. TAM receptor signalling and demyelination. *Neurosignals*. 2009;17(4):277-287. doi:10.1159/000231894
143. Healy LM, Perron G, Won SY, et al. MerTK is a functional regulator of myelin phagocytosis by human myeloid cells. *J Immunol*. 2016;196(8):3375-3384. doi:10.4049/jimmunol.1502562
144. Lutz AB, Chung WS, Sloan SA, et al. Schwann cells use TAM receptor-mediated phagocytosis in addition to autophagy to clear myelin in a mouse model of nerve injury. *Proc Natl Acad Sci USA*. 2017;114(38):E8072-E8080. doi:10.1073/pnas.1710566114
145. Genain CP, Cannella B, Hauser SL, Raine CS. Identification of autoantibodies associated with myelin damage in multiple sclerosis. *Nat Med*. 1999;5(2):170-175. doi:10.1038/5532
146. Shafit-Zagardo B, Gruber RC, DuBois JC. The role of TAM family receptors and ligands in the nervous system: from development to pathobiology. *Pharmacol Ther*. 2018;188:97-117. doi:10.1016/j.pharmthera.2018.03.002
147. Lassmann H, Brück W, Lucchinetti CF. The immunopathology of multiple sclerosis: an overview. *Brain Pathol*. 2007;17(2):210-218. doi:10.1111/j.1750-3639.2007.00064.x
148. Kister I, Bacon TE, Chamot E, et al. Natural history of multiple sclerosis symptoms. *Int J MS Care*. 2013;15(3):146-158. doi:10.7224/1537-2073.2012-053
149. Wang KC, Koprivica V, Kim JA, et al. Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. *Nature*. 2002;417(6892):941-944. doi:10.1038/nature00867
150. Kotter MR, Li WW, Zhao C, Franklin RJM. Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. *J Neurosci*. 2006;26(1):328-332. doi:10.1523/JNEUROSCI.2615-05.2006
151. Olah M, Amor S, Brouwer N, et al. Identification of a microglia phenotype supportive of remyelination. *Glia*. 2012;60(2):306-321. doi:10.1002/glia.21266
152. Ransohoff RM, Cardona AE. The myeloid cells of the central nervous system parenchyma. *Nature*. 2010;468(7321):253-262. doi:10.1038/nature09615
153. Yamasaki RR, Haiyan L, Butovsky O, Nobuhiko O, et al. Differential roles of microglia and monocytes in the inflamed central nervous system. *J Exp Med*. 2014;211(8):1533-1549. doi:10.1084/jem.20132477
154. Healy LM, Jang JH, Won SY, et al. MerTK-mediated regulation of myelin phagocytosis by macrophages generated from patients with MS. *Neurol Neuroimmunol Neuroinflamm*. 2017;4(6):1-7. doi:10.1212/NXI.0000000000000402
155. Ma GZM, Giuffrida LL, Gresle MM, et al. Association of plasma levels of protein S with disease severity in multiple sclerosis. *Mult Scler J Exp Transl Clin*. 2015;1:2055217315596532. doi:10.1177/2055217315596532
156. Healy LM, Perron G, Won SY, et al. MerTK is a functional regulator of myelin phagocytosis by human myeloid cells. *J Immunol*. 2016;196(8):3375-3384.
157. Binder MD, Cate HS, Prieto AL, et al. Gas6 deficiency increases oligodendrocyte loss and microglial activation in response to cuprizone-induced demyelination. *J Neurosci*. 2008;28(20):5195-5206. doi:10.1523/JNEUROSCI.1180-08.2008

158. Tsiperson V, Li X, Schwartz GJ, Raine CS, Shafit-Zagardo B. GAS6 enhances repair following cuprizone-induced demyelination. *PLoS One*. 2010;5(12):1-9. doi:10.1371/journal.pone.0015748
159. Weinger JG, Brosnan CF, Loudig O, 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. *J Neuroinflammation*. 2011;8:1-18. doi:10.1186/1742-2094-8-49
160. Braak H, de Vos RAI, Jansen ENH, et al. Neuropathological Hallmarks of Alzheimer's and Parkinson's Diseases. *Progress in Brain Research*. Vol 117. Elsevier Science BV; 1998.
161. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimer's and Dementia: Translational Research and Clinical Interventions*. 2018;4:575-590. doi:10.1016/j.trci.2018.06.014
162. Makin S. The amyloid hypothesis on trial. *Nature*. 2018;559:4-7.
163. Huang Y, Happonen KE, Burrola PG, et al. Microglia use TAM receptors to detect and engulf amyloid β plaques. *Nat Immunol*. 2021;22(5):586-594. doi:10.1038/s41590-021-00913-5
164. Keren-Shaul H, Spinrad A, Weiner A, et al. A unique microglia type associated with restricting development of Alzheimer's disease. *Cell*. 2017;169(7):1276-1290.e17. doi:10.1016/j.cell.2017.05.018
165. Yin Z, Raj D, Saiepour N, et al. Immune hyperreactivity of Aβ plaque-associated microglia in Alzheimer's disease. *Neurobiol Aging*. 2017;55:115-122. doi:10.1016/j.neurobiolaging.2017.03.021
166. Sainaghi PP, Bellan M, Lombino F, et al. Growth arrest specific 6 concentration is increased in the cerebrospinal fluid of patients with Alzheimer's disease. *J Alzheimers Dis*. 2017;55(1):59-65.
167. Baik H, Kang S, Son SM, Jung IM. Microglia contributes to plaque growth by cell death due to uptake of amyloid β in the brain of Alzheimer's disease mouse model. *Glia*. 2016;64(12):2274-2290.
168. Sosna J, Philipp S, Albay RI, et al. Early long-term administration of the CSF1R inhibitor PLX3397 ablates microglia and reduces accumulation of intraneuronal amyloid, neuritic plaque deposition and pre-fibrillar oligomers in 5XFAD mouse model of Alzheimer's disease. *Mol Neurodegener*. 2018;13(1):11. doi:10.1186/s13024-018-0244-x
169. Spangenberg E, Severson PL, Hohsfield LA, et al. Sustained microglial depletion with CSF1R inhibitor impairs parenchymal plaque development in an Alzheimer's disease model. *Nat Commun*. 2019;10(1):3758. doi:10.1038/s41467-019-11674-z
170. Kim DK, Han D, Park J, et al. Deep proteome profiling of the hippocampus in the 5XFAD mouse model reveals biological process alterations and a novel biomarker of Alzheimer's disease. *Exp Mol Med*. 2019;51(11):1-17. doi:10.1038/s12276-019-0326-z
171. Kalia LV, Kalia SK, McLean PJ, Lozano AM, Lang AE. α-Synuclein oligomers and clinical implications for Parkinson disease. *Ann Neurol*. 2013;73(2):155-169.
172. Lastres-Becker I, Ulusoy A, Innamorato NG, et al. α-Synuclein expression and Nrf2 deficiency cooperate to aggravate protein aggregation, neuronal death and inflammation in early-stage Parkinson's disease. *Hum Mol Genet*. 2012;21(14):3173-3192. doi:10.1093/hmg/dds143
173. Brown GC, Neher JJ. Eaten alive! Cell death by primary phagocytosis: "phagoptosis". *Trends Biochem Sci*. 2012;37(8):325-332. doi:10.1016/j.tibs.2012.05.002
174. Brown GC, Neher JJ. Microglial phagocytosis of live neurons. *Nat Rev Neurosci*. 2014;15(4):209-216. doi:10.1038/nrn3710
175. Mari C, Karabiyikoglu M, Goris ML, Tait JF, Yenari MA, Blankenberg FG. Detection of focal hypoxic-ischemic injury and neuronal stress in a rodent model of unilateral MCA occlusion/reperfusion using radiolabeled annexin V. *Eur J Nucl Med Mol Imaging*. 2004;31(5):733-739. doi:10.1007/s00259-004-1473-5
176. Neher JJ, Emmrich JV, Fricker M, Mander PK, Théry C, Brown GC. Phagocytosis executes delayed neuronal death after focal brain ischemia. *Proc Natl Acad Sci USA*. 2013;110(43):E4098-E4107. doi:10.1073/pnas.1308679110
177. Susser LI, Rayner KJ. Through the layers: how macrophages drive atherosclerosis across the vessel wall. *J Clin Investig*. 2022;132(9):1-9. doi:10.1172/JCI157011
178. Björkegren JLM, Lusis AJ. Atherosclerosis: recent developments. *Cell*. 2022;185(10):1630-1645. doi:10.1016/j.cell.2022.04.004
179. Yu XH, Fu YC, Zhang DW, Yin K, Tang CK. Foam cells in atherosclerosis. *Clin Chim Acta*. 2013;424:245-252. doi:10.1016/j.cca.2013.06.006
180. Gofman JW, Lindgren F, Elliott H, et al. The role of lipids and lipoproteins in atherosclerosis. *Science*. 1950;111(2877):166-186. doi:10.1126/science.111.2877.166
181. Decker C, Sadhu S, Fredman G. Pro-resolving ligands orchestrate phagocytosis. *Front Immunol*. 2021;12:1-11. doi:10.3389/fimmu.2021.660865
182. Ait-Oufella H, Pouresmail V, Simon T, et al. Defective mer receptor tyrosine kinase signaling in bone marrow cells promotes apoptotic cell accumulation and accelerates atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2008;28(8):1429-1431. doi:10.1161/ATVBAHA.108.169078
183. Cai B, Thorp EB, Doran AC, et al. MerTK receptor cleavage promotes plaque necrosis and defective resolution in atherosclerosis. *J Clin Investig*. 2017;127(2):564-568. doi:10.1172/JCI90520
184. Thorp E, Cui D, Schrijvers DM, Kuriakose G, Tabas I. Mertk receptor mutation reduces efferocytosis efficiency and promotes apoptotic cell accumulation and plaque necrosis in atherosclerotic lesions of Apoe−/− mice. *Arterioscler Thromb Vasc Biol*. 2008;28(8):1421-1428. doi:10.1161/ATVBAHA.108.167197
185. Doran AC, Ozcan L, Cai B, et al. CAMKIIγ suppresses an efferocytosis pathway in macrophages and promotes atherosclerotic plaque necrosis. *J Clin Investig*. 2017;127(11):4075-4089. doi:10.1172/JCI94735
186. Tao W, Yurdagul A, Kong N, et al. SiRNA nanoparticles targeting CaMKIIγ in lesional macrophages improve atherosclerotic plaque stability in mice. *Sci Transl Med*. 2020;12(553):eaay1063. doi:10.1126/SCITRANSLMED.AAY1063
187. Muñoz LE, Lauber K, Schiller M, Manfredi AA, Herrmann M. The role of defective clearance of apoptotic cells in systemic autoimmunity. *Nat Rev Rheumatol*. 2010;6(5):280-289. doi:10.1038/nrrheum.2010.46
188. Bellan M, Quaglia M, Nerviani A, et al. Increased plasma levels of Gas6 and its soluble tyrosine kinase receptors Mer and Axl are associated with immunological activity and severity of lupus nephritis. *Clin Exp Rheumatol*. 2021;39(1):132-138. doi:10.55563/clinexprheumatol/xyylza
189. Gong S, Xu Z, Liu Y, et al. Plasma sMer, sAxl and GAS6 levels correlate with disease activity and severity in lupus nephritis. *Eur J Clin Invest*. 2019;49(3):1-11. doi:10.1111/eci.13064
190. Liphaus BL, Lima L, Palmeira P, Silva CA, Goldenstein-Schainberg C, Carneiro-Sampaio M. Increased sMer, but not sAxl, sTyro3, and Gas6 relate with active disease in juvenile systemic lupus erythematosus. *Clin Rheumatol*. 2020;39(2):509-514. doi:10.1007/s10067-019-04799-5
191. Cohen PL, Shao WH. Gas6/TAM receptors in systemic lupus erythematosus. *Dis Markers*. 2019;2019:1-9. doi:10.1155/2019/7838195
192. Nojima J, Kuratsune H, Suehisa E, et al. Association between the prevalence of antibodies to beta(2)-glycoprotein I, prothrombin, protein C, protein S, and annexin V in patients with systemic lupus erythematosus and thrombotic and thrombocytopenic complications. *Clin Chem*. 2001;47(6):1008-1015.
193. Song KS, Park YS, Kim HK. Prevalence of anti-protein S antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum*. 2000;43(3):557-560. doi:10.1002/1529-0131(200003)43:3<557::AID-ANR11>3.0.CO;2-O

194. Guermazi S, Regnault V, Gorgi Y, Ayed K, Lecompte KD. Further evidence for the presence of anti-protein S autoantibodies in patients with systemic lupus erythematosus. *Blood Coagul Fibrinolysis*. 2000;11(5):491-498.
195. Scott DL, Wolfe F, Huizinga TWJ. Rheumatoid arthritis. *Lancet*. 2010;376(9746):1094-1108. doi:10.1016/S0140-6736(10)60826-4
196. Vullings J, Vago JP, Waterborg CEJ, et al. Selective increment of synovial soluble TYRO3 correlates with disease severity and joint inflammation in patients with rheumatoid arthritis. *J Immunol Res*. 2020;2020:1-10. doi:10.1155/2020/9690832
197. Zheng L, Xu L, Hu F, et al. Elevated expression of TAM receptor tyrosine kinase in synovial fluid and synovial tissue of rheumatoid arthritis. *Clin Exp Immunol*. 2022;209(3):270-279. doi:10.1093/cei/uxac071
198. Witas R, Peck AB, Ambrus JL, Nguyen CQ. Sjogren's syndrome and tam receptors: a possible contribution to disease onset. *J Immunol Res*. 2019;2019:1-12. doi:10.1155/2019/4813795
199. Qin B, Wang J, Ma N, et al. The association of Tyro3/Axl/Mer signaling with inflammatory response, disease activity in patients with primary Sjögren's syndrome. *Joint Bone Spine*. 2015;82(4):258-263. doi:10.1016/j.jbspin.2015.01.008
200. Hauk V, Fraccaroli L, Grasso E, et al. Monocytes from Sjögren's syndrome patients display increased vasoactive intestinal peptide receptor 2 expression and impaired apoptotic cell phagocytosis. *Clin Exp Immunol*. 2014;177(3):662-670. doi:10.1111/cei.12378
201. Utsugi T, Schroit AJ, Connor J, Bucana CD, Fidler IJ. Elevated expression of phosphatidylserine in the outer membrane leaflet of human tumor cells and recognition by activated human blood monocytes. *Cancer Res*. 1991;51(11):3062-3066.
202. Cook RS, Jacobsen KM, Wofford AM, et al. MerTK inhibition in tumor leukocytes decreases tumor growth and metastasis. *J Clin Investig*. 2013;123(8):3231-3242. doi:10.1172/JCI67655
203. Ubil E, Caskey L, Holtzhausen A, Hunter D, Story C, Earp HS. Tumor-secreted Pros1 inhibits macrophage M1 polarization to reduce antitumor immune response. *J Clin Investig*. 2018;128(6):2356-2369. doi:10.1172/JCI97354
204. Hutterer M, Knyazev P, Abate A, et al. Axl and growth arrest-specific gene 6 are frequently overexpressed in human gliomas and predict poor prognosis in patients with glioblastoma multiforme. *Clin Cancer Res*. 2008;14(1):130-138. doi:10.1158/1078-0432.CCR-07-0862
205. Nguyen MK, Jeon O, Krebs MD, Schapira D, Alsberg E. Sustained localized presentation of RNA interfering molecules from in situ forming hydrogels to guide stem cell osteogenic differentiation. *Biomaterials*. 2014;35(24):6278-6286. doi:10.1016/j.biomaterials.2014.04.048
206. Graham DK, Deryckere D, Davies KD, Earp HS. The TAM family: phosphatidylserine-sensing receptor tyrosine kinases gone awry in cancer. *Nat Rev Cancer*. 2014;14(12):769-785. doi:10.1038/nrc3847
207. Pierce AM, Keating AK. TAM receptor tyrosine kinases: expression, disease and oncogenesis in the central nervous system. *Brain Res*. 2014;1542:206-220. doi:10.1016/j.brainres.2013.10.049
208. Rea K, Pinciroli P, Sensi M, et al. Novel Axl-driven signaling pathway and molecular signature characterize high-grade ovarian cancer patients with poor clinical outcome. *Oncotrget*. 2015;6(31):30859-30875. doi:10.18632/oncotarget.5087
209. Vuoriluoto K, Haugen H, Kiviluoto S, et al. Vimentin regulates EMT induction by slug and oncogenic H-Ras and migration by governing Axl expression in breast cancer. *Oncogene*. 2011;30(12):1436-1448. doi:10.1038/onc.2010.509
210. Debruyne DN, Bhatnagar N, Sharma B, et al. ALK inhibitor resistance in ALKF1174L-driven neuroblastoma is associated with AXL activation and induction of EMT. *Oncogene*. 2016;35(28):3681-3691. doi:10.1038/onc.2015.434
211. Fadok VA, Bratton DL, Henson PM. Phagocyte receptors for apoptotic cells: recognition, uptake, and consequences. *J Clin Investig*. 2001;108(7):957-962. doi:10.1172/jci14122
212. Al A, Keating AK, Joung D, et al. Mer tyrosine kinase (MerTK) promotes macrophage survival following exposure to oxidative stress. *J Leucoc Biol*. 2009;86(1):73-79.
213. Zagórska A, Través PG, Lew ED, Dransfield I, Lemke G. Diversification of TAM receptor tyrosine kinase function. *Nat Immunol*. 2014;15(10):920-928. doi:10.1038/ni.2986
214. Nandrot E. Opposite roles of MerTK ligands Gas6 and protein S during retinal phagocytosis. *Adv Exp Med Biol*. 2018;1074:577-583.
215. Penberthy KK, Rival C, Shankman LS, et al. Context-dependent compensation among phosphatidylserine-recognition receptors. *Sci rep*. 2017;7(1):1-14. doi:10.1038/s41598-017-15191-1
216. Miyanishi M, Tada K, Koike M, Uchiyama Y, Kitamura T, Nagata S. Identification of Tim4 as a phosphatidylserine receptor. *Nature*. 2007;450(7168):435-439. doi:10.1038/nature06307
217. Park D, Tosello-Trampont AC, Elliott MR, et al. BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature*. 2007;450(7168):430-434. doi:10.1038/nature06329
218. Albert ML, Kim JI, Birge RB. CrkII-Dock180-Rac1 complex for phagocytosis of apoptotic cells. *Nat Cell Biol*. 2000;2:899-905.
219. Mathema VB, Na-Bangchang K. Regulatory roles of brain-specific angiogenesis inhibitor 1(BAI1) protein in inflammation, tumorigenesis and phagocytosis: a brief review. *Crit Rev Oncol Hematol*. 2017;111:81-86. doi:10.1016/j.critrevonc.2017.01.006

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