Immunobiology of the TAM receptors

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Abstract | Recent studies have revealed that the TAM receptor protein tyrosine kinases — TYRO3, AXL and MER — have pivotal roles in innate immunity. They inhibit inflammation in dendritic cells and macrophages, promote the phagocytosis of apoptotic cells and membranous organelles, and stimulate the maturation of natural killer cells. Each of these phenomena may depend on a cooperative interaction between TAM receptor and cytokine receptor signalling systems. Although its importance was previously unrecognized, TAM signalling promises to have an increasingly prominent role in studies of innate immune regulation.

Natural killer (NK) cells
NK cells are lymphoid cells capable of lysing bacteria- and virus-infected cells, as well as many tumour cells, without prior sensitization. They have important roles in combating infections, in the immune surveillance of cancer and in host-versus-graft rejection.

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Receptor protein tyrosine kinases (PTKs) are cell-surface transmembrane receptors that contain a regulated PTK activity within their cytoplasmic domains. They function as sensors for extracellular ligands, the binding of which triggers receptor dimerization and activation of the receptor's kinase. This leads to the recruitment, phosphorylation and activation of multiple downstream signalling proteins, which ultimately change the physiology of cells. Although there are only 58 receptor PTK genes in the human genome[1] (see the human kinome website), the signal transduction cascades initiated by receptor PTK activation control diverse cellular processes — from cell differentiation to cell death. Well-known receptor PTK subfamilies include the ERBB receptors, which have essential roles in cardiac and neural development and the progression of some forms of breast cancer[2]; and the ephrin receptors, which are required for tissue morphogenesis and the patterning of neuronal connections in the developing brain[3].

The focus of this Review, the TAM group, was among the last receptor PTK subfamilies to be identified, and the biological roles of its three members — TYRO3, AXL and MER — remained uncharacterized for several years. Largely through the analysis of engineered loss-of-function mutants in mice, these roles have become increasingly apparent. They reflect a specific requirement for TAM signalling in settings in which fully differentiated cells, tissues and organs must be maintained in the face of continuous challenge, turnover and renewal. In humans, ongoing homeostatic regulation of this sort must be carried out, frequently on a daily basis, for decades. Although an essential role for TAM regulation of tissue homeostasis is evident in the adult nervous, reproductive and vascular systems, it is in the regulation of the innate immune response where the TAM receptors have especially profound effects.

In this Review, we highlight the central roles that TAM signalling has in the intrinsic inhibition of the inflammatory response to pathogens by dendritic cells (DCs) and macrophages; during phagocytosis of apoptotic cells by these same cells; and in the maturation and killing activity of natural killer (NK) cells. We also discuss how, in many or all of these settings, TAM receptors depend on and interact with cytokine receptors.

TAM receptors and ligands

The three TAM receptors, TYRO3, AXL and MER, were identified as a distinct receptor PTK subfamily in 1991 (REFS 4,5). Subsequent cloning of full-length cDNAs by multiple laboratories resulted in a profusion of different names for the receptors, but TYRO3, AXL and MER are now the official NCBI designations. Similar to class 1 and class 2 cytokine receptors (such as the interleukin-2 and type I interferon receptors), the TAM receptors are specific to chordates, and this receptor PTK subfamily was among the last to appear in evolution[6]. The extracellular, ligand-binding regions of the TAMs have a defining arrangement of two immunoglobulin-related domains and two fibronectin type III repeats, each in tandem. These are followed by a single-pass transmembrane domain, and a catalytically competent, cytoplasmic PTK (FIG. 1). The TAM receptors are most closely related to RON (also known as CD136, MST1R), the PTK receptor for macrophage-stimulating protein, and to MET, the hepatocyte growth-factor receptor[1]. Similar to all other receptor PTKs, the TAMs seem to signal as dimers[7] (FIG. 1).

The two ligands that bind to and activate the TAM receptors remain the subject of investigation. When originally cloned, TYRO3, AXL and MER were orphans, in that their ligands were unknown and could not be predicted. A series of biochemical and cell-culture experiments identified two closely related proteins

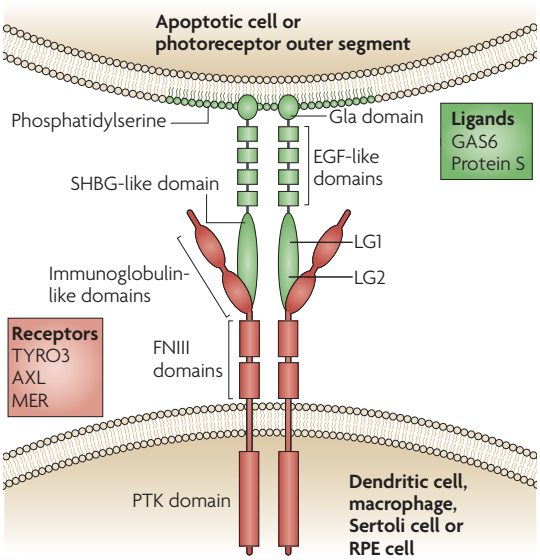


Figure 1 | **TAM receptors and their ligands.** TYRO3 (also known as BRT, DTK, RSE, SKY and TIF), AXL (also known as ARK, TYRO7 and UFO) and MER (also known as EYK, NYM and TYRO12) are receptor protein tyrosine kinases (PTKs) that are expressed by dendritic cells, macrophages and immature natural killer (NK) cells of the immune system, Sertoli cells of the testis, retinal pigment epithelial (RPE) cells of the eye, and several other cell types. TAM receptor dimers bind to their two ligands, growth-arrest-specific 6 (GAS6) and protein S, through interaction between the two N-terminal immunoglobulin-like domains of the receptors and the two C-terminal laminin G (LG) regions, which together make up the SHBG (sex hormone binding globulin) domain, of the ligands. (The solved X-ray crystal structure of the GAS6 SHBG domain bound to the immunoglobulin domains of AXL[7] reveals that both ligand and receptor crystallize as dimers.) Via their N-terminal Gla domains, GAS6 and protein S then bind to phosphatidylserine that is displayed on the extracellular surface of the plasma membranes of apoptotic cells or on the outer segments of photoreceptors. EGF, epidermal growth factor; FNIII, fibronectin type III.

— growth-arrest-specific 6 (GAS6) and protein S — as TAM agonists[8] (FIG. 1). GAS6 was found to bind and activate — that is, stimulate the tyrosine autophosphorylation of — all three receptors, albeit with markedly different affinities (AXL≥TYRO3>>MER)[9]. Although protein S was initially reported to be a specific agonist for TYRO3 (REF. 8), more recent studies have demonstrated that in cells in which TYRO3 is co-expressed with MER, protein S is also a potent MER agonist[10]. An important issue with regard to published ligand–receptor interaction studies is that most have involved the assay of single agonists against single receptors. However, both TAM ligands and TAM receptors heterodimerize (C.V.R. and G.L., unpublished observations), and heterodimeric ligand–receptor binding and activation profiles are, based on what is known for other receptor PTKs, almost certain to differ from those of homodimers. A recent analysis has demonstrated that the ability of protein S to stimulate both the phagocytosis of apoptotic cells by macrophages (see later) and the autophosphorylation of MER in macrophages, requires protein S dimerization[11].

In this Review, we generally discuss GAS6 and protein S interchangeably. It is important to emphasize, however, that these proteins have different patterns of expression in mammalian tissues (Supplementary information S1 (table)) and different bioactivities. In addition to its role as a TAM ligand, for example, protein S has an important, TAM-independent activity as a blood anticoagulant — an activity that is not exhibited by GAS6. Protein S, which is present at relatively high levels (~300 nM) in the serum, serves as an essential cofactor for activated protein C, a protease that degrades factor Va and factor VIIIa, and thereby inhibits blood coagulation[12].

GAS6 and protein S share the same distinctive arrangement of structural motifs. Each protein has a ~60 amino-acid Gla domain at its amino terminus, a region rich in glutamic acid residues that are γ-carboxylated in a vitamin-K-dependent reaction. (Gla-domain-containing proteins are prominent components of the blood coagulation cascade.) These Gla domains bind the phospholipid phosphatidylserine[13], and this is an important feature of the *in vivo* function of GAS6 and protein S[14–17]. The Gla domain is followed by four epidermal growth factor (EGF)-like modules, and then by two tandem laminin G domains that are related to those of the sex hormone binding globulin (SHBG) (FIG. 1). This SHBG-like module is both necessary and sufficient for TAM receptor binding and activation, whereas the Gla domain is dispensable for these activities[7,18]. Overall, GAS6 and protein S share ~42% amino-acid identity.

Biological roles of TAM signalling

TAM receptors are broadly expressed in cells of the mature immune, nervous, reproductive and vascular systems (BOX 1; Supplementary information S1 (table)). Usually, there is expression of more than one TAM receptor in a given cell type, such as in DCs; and co-expression of all three receptors, as occurs in Sertoli cells of the testis, is not uncommon. Many TAM-positive cells also express one or both TAM ligands. In contrast to most other receptor PTKs, expression of the TAMs is substantially upregulated postnatally, and is maintained at relatively high levels in adult tissues. Although cDNAs encoding the TAM receptors have been cloned repeatedly from tumour cells, and TAM receptors, particularly AXL, have been implicated in cancer progression[19], genuine insights into the biological roles of these receptors were only obtained when mice lacking TYRO3, AXL, MER or GAS6 were generated and analysed[20–25].

Because knockout of each of the TAM genes still produced viable and fertile mice, and because the *Tyro3* and *Mer* genes are linked in the mouse genome, it was possible to generate knockout mice that were deficient for all three TAM receptors[20]. Remarkably, even these triple knockout mice were found to be viable at birth, and to be superficially normal for the first several weeks thereafter. This notwithstanding, mice lacking all three TAM receptors were found to develop a plethora of debilitating phenotypes, all of which appeared to be degenerative in nature[20,21].

Toll-like receptors (TLRs). Pattern-recognition receptors that recognize molecules — such as the lipopolysaccharide of bacterial cell walls, the unmethylated CpG-containing deoxynucleotides of bacterial DNA and the double-stranded RNAs of viruses — that are broadly shared by pathogens but not by host cells. Pathogen activation of TLRs initiates the innate immune response in dendritic cells and macrophages.

SOCS proteins (Suppressor of cytokine signalling proteins). SOCS proteins inhibit STAT (signal-transducer and activator of transcription) phosphorylation by binding and inhibiting JAKs (Janus-family kinases) and/or competing with STATs for phosphotyrosine binding sites on cytokine receptors. They also inhibit signal transducers downstream of Toll-like receptor activation.

Beginning at about 3 weeks after birth, for example, germ cells in the testis were observed to die in increasing numbers, such that by three months after birth, the seminiferous tubules in the TAM-deficient mutants were depleted of spermatogonia, spermatocytes, spermatids and mature sperm[20]. Another dramatic phenotype was observed in the retinae of TAM-deficient mice, where, beginning at about the same time, photoreceptors were seen to die by apoptosis. By 2–3 months of age, essentially all photoreceptors were lost from these mice[20]. As discussed below, these two apparently unrelated degenerative phenotypes are in fact directly linked, in that they both result from the inability of TAM-deficient cells to properly phagocytose apoptotic cells and membranes.

Autoimmunity in TAM mutant mice

Although the TAMs have important roles in the nervous, reproductive and vascular systems (see BOX 1), perhaps the most serious consequences of mutations of the TAM genes are seen in the immune system. Also at around 3 weeks after birth, the peripheral lymphoid organs of the TAM triple mutant mice begin to grow at elevated rates, such that by 6 months of age, the spleens and lymph nodes of these mice are often 10 times the weight of those from wild-type mice. This is due to the expansion of both myeloid- and lymphoid-cell populations, and colonies of lymphocytes are eventually observed in essentially all tissues of the triple mutant animals[21]. T and B cells, as well as macrophages and DCs, are constitutively activated in these mice: they express elevated levels of both acute and chronic activation markers, such as CD95 and CD44 (in B cells), CD25 (in T cells), and MHC class I and II, CD86 and IL-12 (in peritoneal macrophages and $CD11c^+$ splenic DCs)[21,25]. The TAM receptors are not expressed by most lymphocytes, and multiple lines of evidence demonstrate that the lymphocyte activation seen in the TAM-deficient mice is due primarily to the loss of TAM receptor function in antigen-presenting cells (APCs).

Not surprisingly, the TAM-deficient mice eventually develop broad-spectrum autoimmune disease[21]. In addition to the enlargement of peripheral lymphoid organs, clinical manifestations of autoimmunity include swollen joints and footpads, skin lesions, blood vessel haemorrhages and IgG deposits in kidney glomeruli. Humoral manifestations include high titres of circulating antibody to multiple autoantigens, including double-stranded DNA (dsDNA), a variety of plasma membrane phospholipids and collagen[21]. As for most other features of TAM-deficient mice, a clear gene dosage effect is seen, with mice deficient in a single TAM receptor exhibiting milder phenotypes, such as lower dsDNA-specific autoantibody titres, than mice deficient in two TAM receptors, and with mice deficient in all three receptors exhibiting the most severe phenotypes[21]. These gene dosage effects are in keeping with the co-expression of more than one TAM receptor in most, if not all, TAM-positive cells. In addition to developing autoimmunity, TAM-deficient mice and APCs are hyper-responsive to endotoxins, as monitored by the production of pro-inflammatory cytokines[25,26]. Most $Mer^{-/-}$ mice, for example, die from a dose of lipopolysaccharide (LPS) that is otherwise non-lethal in wild-type mice[26].

TAM inhibition of inflammation

The autoimmune disease that develops in the TAM mutant mice is likely to result from the loss of TAM regulation of two related phenomena: the innate inflammatory response to pathogens by DCs and macrophages, and the phagocytosis of apoptotic cells by these APCs. Recent analyses of signal transduction events have provided important insights into these two phenomena.

As for any dynamic system, the innate immune response must be carefully regulated — a mechanism that turns it off must be tied to a mechanism that turns it on. A rapid inflammatory response to bacteria, viruses and other disease-causing pathogens, for example, is crucial to their defeat. Yet unrestrained signalling by Toll-like receptors (TLRs) and cytokine receptors in DCs and macrophages generates a chronic inflammatory milieu that can lead to disease and even death. A recent study has shown that the TAM receptors function as pivotal inhibitors that prevent this dysregulation from occurring[25].

Consistent with the observations in TAM-deficient mice that are outlined earlier, TLR activation of wild-type DCs has been found to be potently inhibited by prior incubation with either GAS6 or protein S[25,27]. TAM-mediated inhibition is seen irrespective of whether the TLR activated is TLR3, TLR4 or TLR9; and multiple points in TLR signal transduction cascades — including the activation of the p38 mitogen-activated protein kinase (MAPK), extracellular-signal-regulated kinase 1 (ERK1)/ERK2, nuclear factor-κB (NF-κB), tumour-necrosis factor (TNF)-receptor-associated factor 3 (TRAF3) and TRAF6 — are inhibited. TAM receptor signalling has also been found to inhibit TLR-induced production of pro-inflammatory cytokines — including TNF, interleukin-6 (IL-6), IL-12 and type I interferons (IFNs)[25].

This inhibition requires new gene expression, and among the most important of the inhibitory genes that are induced by the TAMs are the suppressor of cytokine signalling (SOCS) proteins SOCS1 and SOCS3 (REF. 25). SOCS proteins have been studied extensively, and have for many years been known to be induced through the activation of cytokine receptors, most notably by type I IFN receptor (IFNAR) activation[28,29]. This induction was thought to represent a classic negative-feedback loop, as

Box 1 | TAM receptor signalling in other tissues and organs

In addition to the roles discussed in this Review, TAM receptor signalling has been shown have important regulatory roles in vascular smooth-muscle homeostasis[92–94], in platelet function and thrombus stabilization[23,95,96], in erythropoiesis[97] and in cancer development and progression[19,98,99]. TAM receptors are also implicated in the regulation of osteoclast function[100,101], in the control of oligodendrocyte cell survival[102,103], and in the infection of dendritic cells and other cells by Ebola and Marburg viruses[104,105]. In many of these instances, the primary downstream TAM signalling pathway appears to be the phosphoinositide 3-kinase–AKT pathway[95,97,99,102,103], rather than the Janus kinase–STAT (signal transducer and activator of transcription) pathway that is highlighted in this Review.

SOCS1 and SOCS3 inhibit JAK (Janus kinase)–STAT (signal transducer and activator of transcription) signalling downstream of cytokine receptors. However, the induction of SOCS proteins by IFNAR activation has recently been shown to proceed through and be dependent on TAM receptors[25].

As the transcription of SOCS genes is known to be driven by STAT transcription factors, the activation (that is, the tyrosine phosphorylation) of these transcription factors in response to TAM signalling was also examined[25]. It was found that STAT1, but not STAT2 or STAT3, is activated directly by TAM activation in DCs. Moreover, studies in *Stat1*$^{-/-}$ DCs demonstrated that TAM-mediated inhibition of TLR-activated inflammatory responses requires STAT1. That STAT1 is normally activated downstream of cytokine receptors[30], notably IFNAR, prompted an examination of the role that this receptor might have in TAM signalling. Using *Ifnar1*$^{-/-}$ DCs, it was found that all aspects of TAM-mediated inhibition of inflammation also require IFNAR[25]. Consistent with this, the R1 subunit of IFNAR and AXL can be reciprocally co-immunoprecipitated from wild-type DCs, demonstrating a physical association between cytokine and TAM receptors that parallels their physiological association. Together, these results indicate that TAM receptors bind to and usurp the IFNAR–STAT1 complex, and thereby switch it from a signalling complex that initiates and amplifies inflammation to one that inhibits inflammation[25].

That the TAM system inhibits inflammation in DCs suggests that, in principle, this system should not be fully engaged at the onset of a TLR-initiated immune response, and further, that some feature of the system — for example, TAM receptor or ligand expression — should be upregulated subsequent to TLR and cytokine receptor engagement. This is indeed the case — *AXL* mRNA and protein levels are markedly elevated upon treatment of DCs with either TLR agonists (LPS, CpG-containing oligonucleotides or polyI:C (polyinosinic–polycytidylic acid)) or with IFNα (REFS 25,31). Both of these forms of AXL upregulation depend on the presence of both STAT1 and IFNAR[25].

Taken together, these results illuminate a tri-partite cycle of inflammation, for which the ultimate, previously unrecognized regulatory arm consists of TAM-mediated inhibition (FIG. 2). The integration of TAM signalling into such a regulatory cycle has significant implications for both the understanding and treatment of human immune system disorders (see later).

STATs
(Signal transducers and activators of transcription). STATs are latent cytoplasmic transcription factors that upon phosphorylation, typically by Janus-family kinases (JAKs), are activated. They then translocate to the nucleus, where they drive the transcription of their target genes. The signal transduction pathways downstream of many cytokine receptors depend on STAT protein activation.

TAM regulation of phagocytosis

In addition to a failure in the inhibition of TLR-induced inflammation, the development of blindness, sterility and autoimmunity in the TAM mutant mice also reflects dysregulation of a second TAM-dependent process — the phagocytic clearance of apoptotic cells and membranes. TAM receptor signalling is not required for this process during development, as the large number of apoptotic-cell corpses that are generated during embryogenesis appear to be cleared normally. Instead, it is essential for the phagocytosis that occurs continuously in adult organs as part of normal tissue homeostasis (FIG. 3). These organs include, but are not restricted to, those of the immune system.

Indeed, results from studies of the retina and testis — the two organs in which TAM-mediated regulation of what might be called 'homeostatic phagocytosis' was first appreciated — highlight similarities with the roles of TAM receptors in the immune system. As noted above, male mice that lack all three TAM receptors are sterile owing to the postnatal death of essentially all germ cells, a degeneration that results from the compromised function of Sertoli cells[20] (FIG. 3a). These cells, but not germ cells, express all three TAM receptors and both ligands[20,32]. Sertoli cells are highly phagocytic, binding and ingesting both apoptotic germ cells and cellular contents that are extruded as these cells mature into sperm[33]. This phagocytic activity is particularly important, as in mammals more than half the population of differentiating spermatogenic cells dies by apoptosis[34]. The absence of phagocytic clearance in the TAM-deficient testis leads to the toxic accumulation of dead cells and debris. Phagocytosis by Sertoli cells increases and decreases as a function of stage in spermatogenesis, with ingestion of apoptotic germ cells being minimal between spermatogenic stages IV–VIII (REF. 35). The levels of GAS6 and protein S expression also cycle in Sertoli cells as a function of stage in spermatogenesis, with ligand levels being lowest at stage VIII (REF. 20). This fluctuation in ligand expression levels may therefore provide a mechanism for the cyclic regulation of phagocytosis. It is interesting to note that rhythmic or cyclic variation in the intensity of TAM signalling — in Sertoli cells during spermatogenesis[20], in DCs and macrophages during inflammation[25] and in cells of the retinal pigment epithelium (RPE) during the circadian phagocytosis of photoreceptor membranes[10] (see later) — is a general feature of TAM action.

In the retina, MER and TYRO3 are expressed by a specialized set of large epithelial cells that line the back of the eye[10]. These RPE cells have several functions, among the most important of which is the phagocytosis of the distal ends of photoreceptor outer segments[36], which are the opsin-containing membranous organelles in which visual input is first transduced. New membrane stacks are added at the proximal base of each photoreceptor outer segment each day, and to maintain a constant steady-state length of the organelle, the distal ends of the outer segments must be pinched off and internalized by RPEs (FIG. 3b). This process does not occur in the TAM-deficient mice. As noted above, degeneration of photoreceptors was originally noted in mice lacking all three TAM receptors[20]. However, this was subsequently found — in rats, mice and humans — to be due to mutations of the *Mer* gene alone[37–41]. A long-studied inherited form of retinitis pigmentosa in rats[37–39], and a more recently described inherited form of the disease in humans[40,41], are both due to *Mer* gene mutations. MER and TYRO3 have been localized to the apical tips of the RPE cell processes that penetrate the photoreceptor outer segment layer and pinch off the distal ends of the outer segments[10]. The failure of RPE cells to carry out

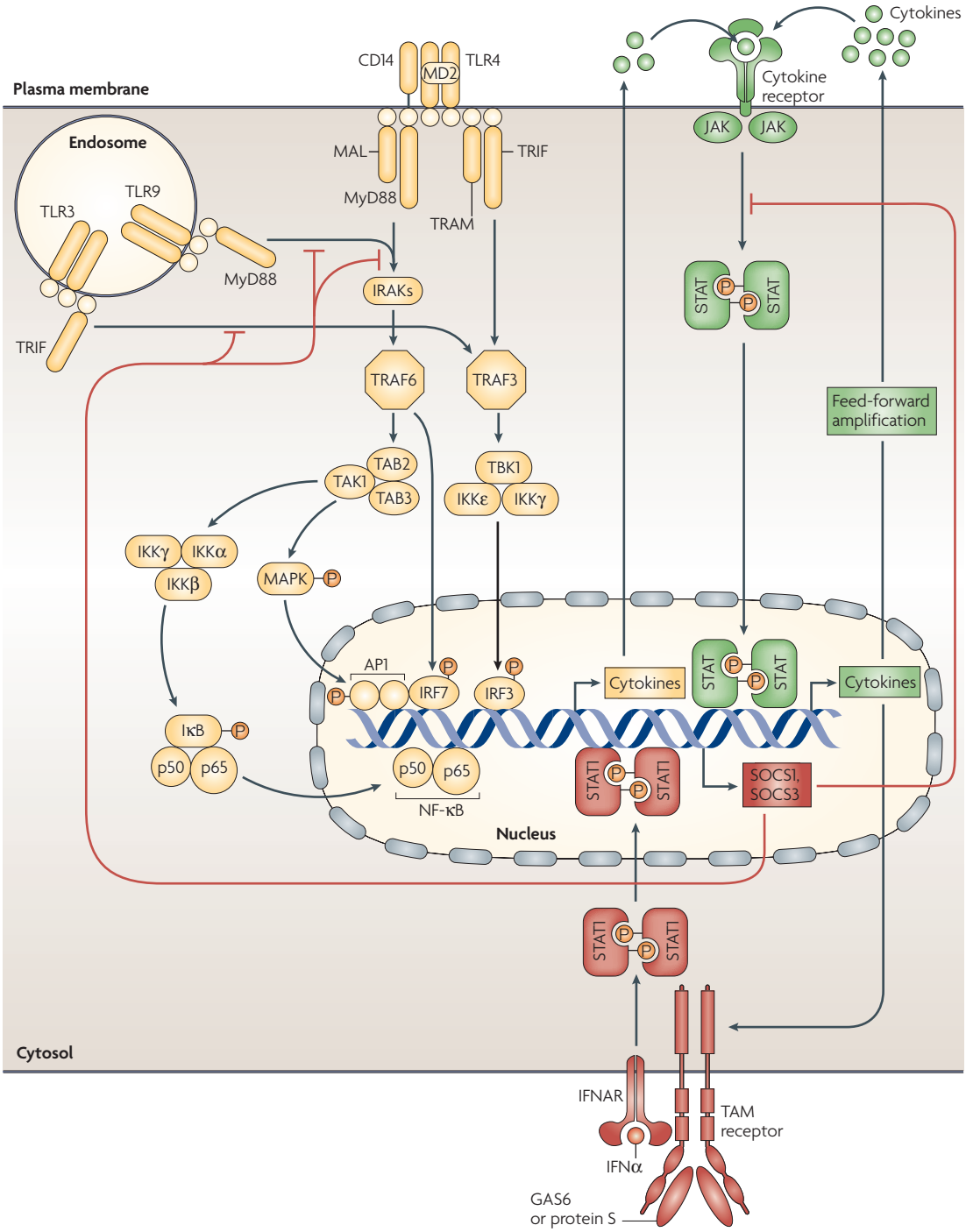


Figure 2 | **An inflammation cycle regulated by TAM signalling.** Quiescent macrophages and dendritic cells (DCs) are stimulated by pathogen encounter, which activates Toll-like receptor (TLR) signalling pathways (yellow). This results in an initial burst of pro-inflammatory cytokines, the levels of which are then greatly amplified in a feed-forward loop through cytokine receptor signalling pathways (green). Cytokine signalling also drives the upregulation of expression of the TAM receptor AXL, which engages TAM receptor signalling pathways (red). These result in the induction of expression of suppressor of cytokine signalling 1 (SOCS1) and SOCS3, which broadly inhibit both TLR and cytokine receptor cascades, thereby ending the inflammatory response. TAM receptor signalling requires coordinate interaction with both the type I interferon receptor (IFNAR) and the transcription factor STAT1 (signal transducer and activator of transcription 1), which is also used for both cytokine amplification and the upregulation of AXL. AP1, activator protein 1; GAS6, growth-arrest-specific 6; IKK, inhibitor of NF-κB kinase; IFNα, interferon-α; IRAK, interleukin-1-receptor-associated kinase; IRF, interferon-regulatory factor; JAK, Janus kinase; MAL, MyD88-adaptor-like protein; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; TAB, TAK1-binding protein; TAK1, transforming-growth-factor-β-activated kinase 1; TBK1, TANK-binding kinase 1; TRAF, tumour-necrosis-factor-receptor-associated factor; TRAM, TRIF-related adaptor molecule; TRIF, Toll/interleukin-1-receptor-domain-containing adaptor protein inducing interferon-β.

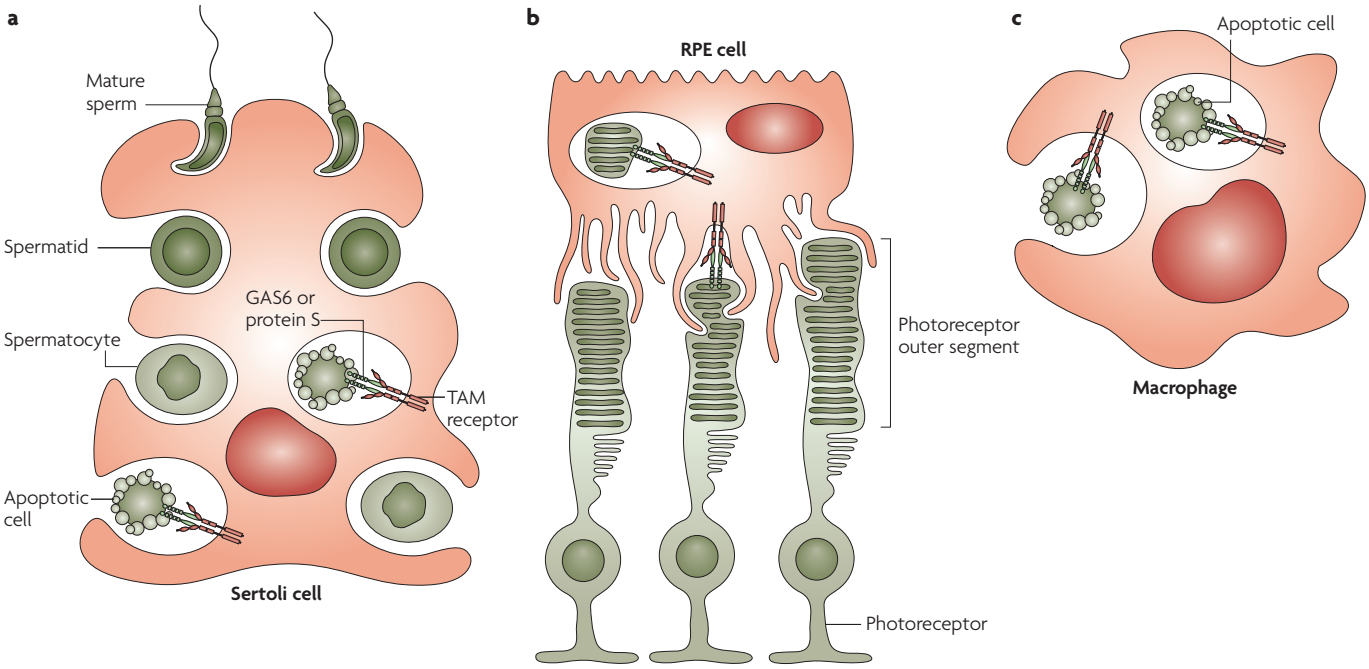


Figure 3 | **TAM signalling and the 'homeostatic phagocytosis' of apoptotic cells and membranes.** TAM receptor signalling is required for the clearance of apoptotic cells in fully differentiated adult tissues and organs that undergo constant challenge, renewal and remodelling. These include the testis, where Sertoli cells clear the large number of apoptotic cells that are generated during spermatogenesis (**a**); the retina, where retinal pigment epithelial (RPE) cells pinch off the distal ends of photoreceptor outer segments (**b**); and the lymphoid organs, where macrophages and dendritic cells remove apoptotic cells generated by infection (**c**). Each of these events is dramatically impaired in mice that lack TAM receptors. A significant fraction of TAM receptor signalling in macrophages, RPE cells and Sertoli cells appears to be autocrine and/or paracrine, in that each of these TAM$^+$ cells also express the TAM receptor ligands growth-arrest-specific 6 (GAS6) and/or protein S.

this process in the absence of TAM signalling results in the death of essentially all photoreceptors by 3 months of age, thereby resulting in blindness.

These defects in the clearance of apoptotic cells in TAM-deficient testes and eyes have direct parallels in the mutant immune system. Bacterial and viral infections generate a large number of apoptotic-cell corpses, which must be cleared by macrophages and DCs, and this form of homeostatic phagocytosis is also impaired in the TAM-deficient mice[42–44] (FIG. 3c). The presence of an elevated steady-state number of apoptotic cells in these mutant mice is likely to contribute to the development of autoimmune disease[45], as unremoved apoptotic-cell debris presents danger signals and autoantigens[46,47], and elevated numbers of apoptotic cells are often seen in the germinal centres of lymph nodes and other lymphoid organs in human autoimmune syndromes[48,49]. At the same time, apoptotic cells are known to have multiple immunomodulatory — but generally immunosuppressive — effects on APCs[50–52].

Most of the analyses of the role of TAM receptors in the phagocytosis of apoptotic cells, and of the ability of apoptotic cells to engage TAM receptor signalling, have been carried out with the MER receptor and with macrophages and DCs isolated from *Mer*$^{-/-}$ mice[42,43]. Recent studies, however, suggest that all three receptors contribute to these events, albeit to different degrees in different APC populations[44]. When challenged with a large number of experimentally induced apoptotic thymocytes, *Mer*$^{-/-}$ macrophages are inefficient in clearing the dead cells[42]. This defect is specific to the phagocytosis and clearance of apoptotic cells, and does not reflect diminished general phagocytic activity, as assayed by the uptake of labelled bacteria, yeast or latex spheres[42]. Particularly intriguing, with regard to the action of TAM receptors in macrophages, is the demonstration that the long-recognized ability of serum to stimulate cultured human macrophages to phagocytose apoptotic cells is due to the presence of protein S, and more specifically to its ability to bind phosphatidylserine[17]. An interaction between TAM and cytokine receptors during homeostatic phagocytosis has not been clearly demonstrated. However, IL-10 has been found to stimulate the phagocytosis of apoptotic cells by monocytes and macrophages[53,54], and *GAS6* and *MER* are among the monocyte genes that are upregulated by IL-10 (REF. 55).

Apoptotic cells appear to exert their immunosuppressive effect on DCs through a TAM signal transduction pathway that is similar to that outlined earlier for the inhibition of inflammation. The prior addition of apoptotic cells to cultured DCs, for example, has been shown to inhibit both LPS-induced NF-κB activation and secretion of TNF and IL-12, and this inhibition has been found to depend on and be transduced through MER[56,57]. Although apoptotic cells are immunosuppressive and can induce tolerogenic populations of DCs,

how they actually do this biochemically remains to be determined. Whereas some apoptotic cells might carry GAS6 and/or protein S, most cells in the body that die by apoptosis do not express these TAM ligands. We suggest an alternative possibility: that the primary, and perhaps only, stimulant that apoptotic cells provide to activate TAM receptors on macrophages and DCs is phosphatidylserine[58]. In this model, the relevant TAM ligands are the GAS6 and protein S that are produced by macrophages and DCs themselves. That is, TAM signalling induced by apoptotic cells is autocrine, similar to TAM signalling during the inhibition of inflammation. Phosphatidylserine, tethered to a large structure (the extracellular membrane face of the apoptotic cell (FIG. 1)) would serve to stabilize the interaction between TAM receptors and APC-produced TAM ligands, through binding to the Gla domains of the ligands. This would in theory greatly increase the binding affinity and effective potency of these ligands for the TAM receptors by slowing the rate at which GAS6 and protein S dissociate from the receptors. This form of TAM activation by phosphatidylserine is supported by recent findings on the evolution of the TAM receptors (BOX 2).

Given the dependence on phosphatidylserine of TAM receptor action during phagocytosis, it will be interesting to examine potential physical interaction between TAM receptors and the recently described direct phosphatidylserine receptors TIM4 (T-cell immunoglobulin domain and mucin domain protein 4)[59] and BAI1 (brain-specific angiogenesis factor 1)[60], particularly as a clear physiological interaction between MER and $\alpha_v\beta_5$-integrin, which is also required for the phagocytosis of both apoptotic cells[58] and photoreceptor outer segment membranes[61], has been demonstrated. Similarly, it will be important to assess downstream TAM signalling pathways that mediate phagocytosis[62] versus those that are required for immunosuppression[25,27,56]. A recent report suggests that these pathways may be dissociable[27].

TAM control of natural-killer-cell differentiation

In addition to recognizing pathogens directly, the innate immune system must also recognize and destroy cells that are infected with pathogens. The effectors of this arm of the innate response are NK cells[63], and the TAM signalling system also has an important role in regulating the activity of these cells[22]. This regulation again appears to be carried out in concert with cytokine receptors.

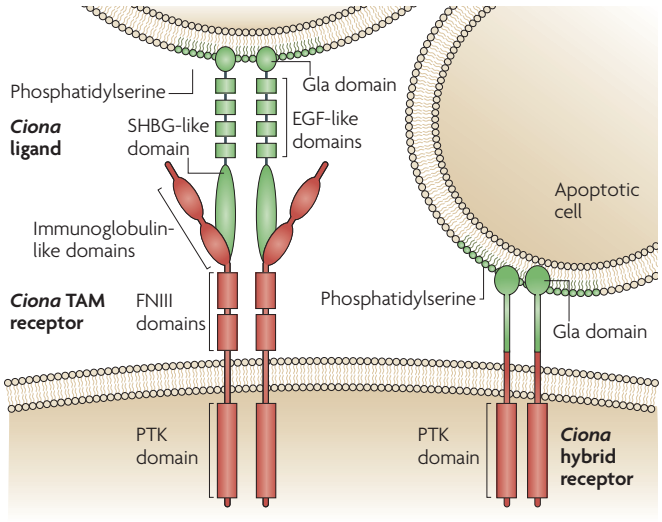
On activation, NK cells kill their target cells through the exocytosis of perforin- and granzyme-containing granules, and the secretion of CD95 ligand and TNF-related apoptosis-inducing ligand (TRAIL)[64–68]. NK cells also produce a variety of cytokines, including IFNγ, TNF and granulocyte/macrophage colony-stimulating factor (GM-CSF)[69–71]. NK-cell target recognition and killing activity rely on the expression of a set of so-called inhibitory receptors (such as members of the Ly49 and CD94 families) and activating receptors (such as NK1.1, DX5 and CD69), which recognize ligands on target cells[72–74]. The acquisition of expression of these receptors has been found to require TAM signalling in the bone marrow[22] (FIG. 4).

The molecular mechanics of this process have been recently reviewed[75]. The basic finding is that NK cells isolated from TAM-deficient mice have very poor cytotoxic activity. As for the TAM-deficient phenotypes outlined above, the impairment of killing activity increases as more TAM genes are inactivated, consistent with the fact that all three receptors are expressed by immature

Box 2 | **Primordial TAM receptors and ligands**

A single TAM-like receptor and a single GAS6 (growth-arrest-specific 6)- and protein-S-like ligand first appeared in evolution in the genomes of urochordates such as *Ciona intestinalis* and *Ciona savignyi*[89]. This was also the case for class 1 and class 2 cytokines and their receptors[90,91]. Given their physiological and physical interaction, it will be interesting to assess whether cytokine and TAM receptors may have co-evolved.

In addition to a vertebrate-like TAM receptor–ligand pair, the genomes of *Ciona* also encode a hybrid receptor molecule in which a TAM-like protein tyrosine kinase (PTK) and transmembrane domain are fused directly to a TAM-ligand-like Gla domain[89]. The *Ciona* TAM agonists GAS6 and protein S are able to physically bridge two cells: their C-terminal SHBG (sex hormone binding globulin) domains bind TAM receptors on one cell, and their N-terminal Gla domains bind to phosphatidylserine on the plasma membrane of an apposed apoptotic cell (see figure). However, the *Ciona* receptor–ligand hybrid fusion streamlines this signalling arrangement and may function as a direct phosphatidylserine receptor. This domain-shuffling evolutionary experiment was apparently abandoned with the emergence of true vertebrates — so far, it has not been observed in other genomes.



EGF, epidermal growth factor; FNIII, fibronectin type III.

NK cells in the bone marrow[22,76]. The acquisition of expression of inhibiting and activating receptors by these immature cells is driven by the stromal cells of the bone marrow, and these cells have been found to express both GAS6 and protein S[22] (FIG. 4). Furthermore, the ability of stromal cells to drive NK-cell maturation *in vitro* can be fully recapitulated if immature cells are grown on NIH3T3 fibroblasts that express either recombinant GAS6 or protein S. Although NK cells from mice lacking all three TAM receptors have normal levels of perforin and granzyme B, these cells lack the full complement of activating and inhibitory receptors that are expressed by cytotoxic NK cells, are unable to secrete IFNγ in response to stimulation, and most importantly, exhibit a 10-fold lower killing ability against target cells than wild-type NK cells[22]. Therefore, TAM signalling drives the end-stage differentiation of NK cells (FIG. 4).

Another factor that has a critical role in NK-cell maturation is IL-15. Analyses of both IL-15-deficient and IL-15 receptor (IL-15R)-deficient mice have demonstrated that this cytokine promotes the differentiation of NK-cell precursors ($CD122^+NK1.1^-$ cells) into immature NK cells ($CD122^+NK1.1^+$ cells), and have additionally suggested that IL-15 also operates at the slightly later stages of NK-cell maturation during which TAM signalling is required[77–81]. It is therefore interesting that a physical and physiological association between the TAM receptor AXL and the α-subunit of the IL-15R has been demonstrated[82]. This demonstration has been made in L929 fibroblasts and DCs rather than NK cells, but the AXL–IL-15Rα association is of clear functional consequence to these cells. IL-15 protects L929 cells from TNF-induced cell death, and this IL-15 effect is entirely dependent on the presence of AXL. IL-15Rα and AXL co-localize on the L929 cell plasma membrane and can be co-immunoprecipitated. (This is also the case in DCs.) In addition, treatment of L929 cells with IL-15 mimics treatment with GAS6, and results in the rapid tyrosine phosphorylation of both IL-15Rα and AXL. Thus, an intimate interaction between a TAM receptor and a cytokine receptor, which

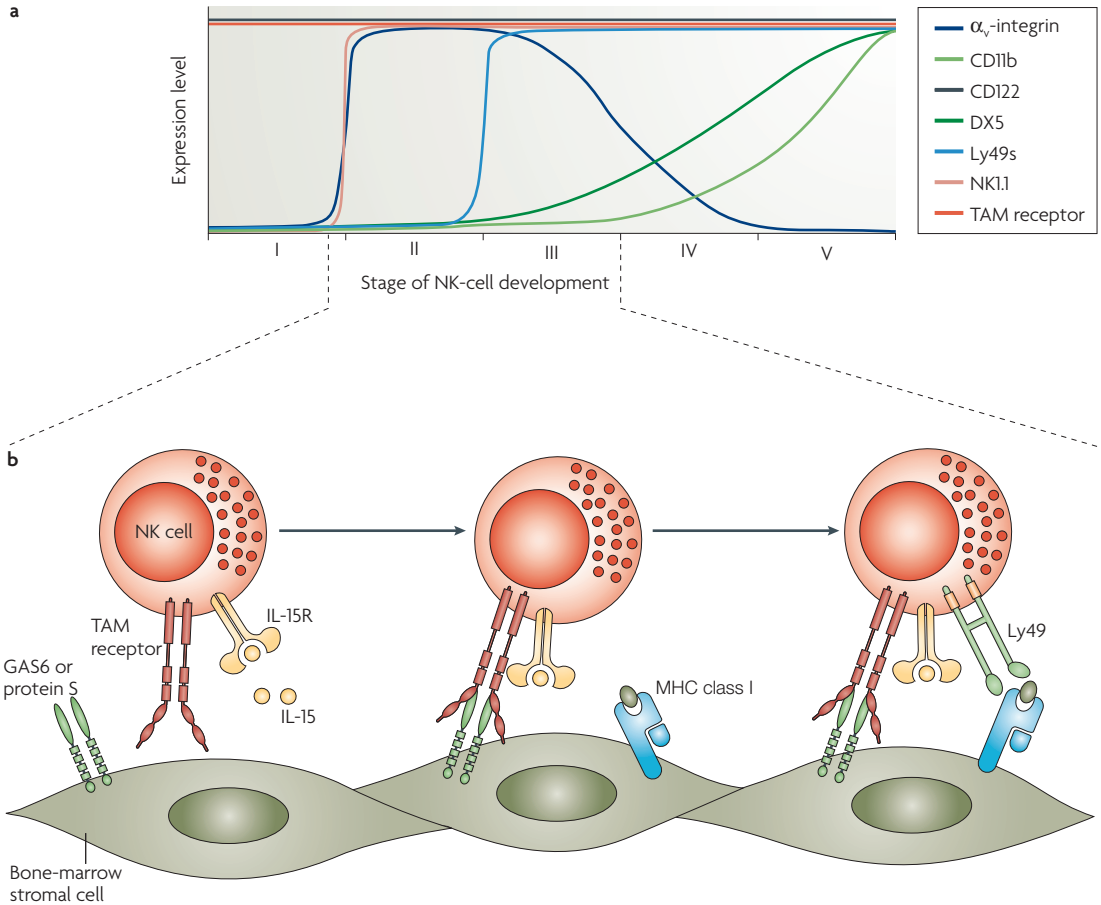


Figure 4 | **TAM signalling and the maturation of NK cells.** Natural killer (NK)-cell differentiation proceeds through five stages, which are delimited by the expression of markers such as CD122, NK1.1 and Ly49s (**a**). TAM receptor signalling is required for NK-cell maturation during stage III. In this instance, growth-arrest-specific 6 (GAS6) and protein S are produced by stromal cells of the bone-marrow niche, and activate the TAM receptors, TYRO3, AXL and MER, that are expressed by immature NK cells (**b**). In the absence of TAM receptor signalling, these immature NK cells do not acquire expression of the inhibitory and activating receptors (for example, Ly49) required for target-cell recognition and killing. TAM receptor signalling may require interaction with a cytokine receptor signalling system — in this case, the interleukin-15 receptor (IL-15R), as physical and physiological interactions between the α-subunit of the IL-15R and AXL have been demonstrated.

is structurally distinct from IFNAR, is again required for both TAM receptor and cytokine receptor function. In this example, and also with respect to the interaction of TAM receptors and the IFNAR signalling complex in DCs, it will be of interest to determine if the demonstrated physical association between cytokine receptors and AXL extends to TYRO3 and MER.

Prospects and implications

Recent insights into TAM receptor function have many implications for our understanding of innate immune regulation and the treatment of immune system disorders. These include a possible role for TAM-receptor-mediated inhibition of endotoxin tolerance and immune suppression — phenomena in which hypo-responsiveness to TLR engagement is induced by prior TLR activation[83,84]. In macrophages, components of the TAM pathway that are upregulated during inflammation must turn over with a half-life that allows responding cells to return to their baseline levels. If a secondary encounter with pathogen occurs before this happens, then the TLR response to the secondary pathogen will be blunted. Immunosuppression also occurs in end-stage sepsis. This compromises the ability of patients to eradicate their primary infection and also predisposes them to nosocomial infections. In this context, it is interesting to note that a recent clinical study found that the levels of circulating GAS6 are consistently elevated in patients with severe sepsis, and are correlated with the occurrence of septic shock[85]. These observations suggest that small molecule inhibitors of the TAM receptors may be reasonable candidates both for potential therapies in acute sepsis and for new-generation vaccine adjuvants for immunization. On the flip side, diminished or interrupted TAM receptor signalling may lead to the development of chronic inflammatory and autoimmune diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis. This clearly happens in TAM-deficient mice[21,25]; and in humans, reduced levels of free circulating protein S, which should result in reduced TAM receptor signalling, are evident in patients with SLE[86–88]. Therefore, the analysis of TAM receptor signalling is likely to have an increasingly prominent role in our understanding of both the normal innate immune response and its perturbation in disease.

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
AXL | GAS6 | IFNAR | IL-15R | MER | protein S | STAT1 | TYRO3

FURTHER INFORMATION
Greg Lemke's homepage: http://www.salk.edu/faculty/faculty_details.php?id=35
The human kinome: http://www.kinase.com/human/kinome

SUPPLEMENTARY INFORMATION
See online article: S1 (table)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

Supplementary Table 1 | TAM receptor and ligand expression

Cell or tissue	TAM receptor			TAM ligand	
	TYRO3	**AXL**	**MER**	**GAS6**	**Protein S**
Haematopoietic cells					
Dendritic cells					
Resting BMDCs (GM-CSF/IL-4)	FACS[1], WB[2]	FACS[1,3], WB[2]	FACS[1,3,4], WB[2]		RT-PCR[1]
Resting BMDCs (FLT3 ligand)		WB and FACS [5]	WB[5]	Q-PCR[5]	Q-PCR[5]
Activated BMDCs (FLT3 ligand)		IFNα inducible Q-PCR, WB and FACS[5]			
Macrophages					
Peritoneal macrophages	FACS[1]	FACS[1]	FACS[1,4], WB[6,7]	RT-PCR[1]	RT-PCR[1]
Activated BM-derived macrophages (M-CSF)		IFNα-inducible, Q-PCR[8]			
Kupffer cells				IHC[9]	
Alveolar macrophages			FACS[10]		
Osteoclasts	RT-PCR[11,12], WB[11] and IHC[12]			RT-PCR[11]	
Lymphocytes					
T cells			n.d. by FACS[4]		IL-4-inducible, NB[13], WB[13]
B cells			n.d. by FACS[4]		
NKT cells			FACS[4]		
Natural killer cells	RT-PCR[14]	RT-PCR[14]	RT-PCR[14], FACS[4]	n.d by RT-PCR[14]	RT-PCR[14]
Mast cells/basophils					
$CD34^+$ derived basophil/mast cells (SCF/IL-3)		IFNα-inducible, RT-PCR[15]			
Platelets	RT-PCR[16], FACS[17]	RT-PCR[16], FACS[17]	RT-PCR[16,18], FACS[17],	RT-PCR[16],	

			WB[6]	WB[16], FACS[16]	
Non-haematopoietic cells					
Bone-marrow stromal cells				RT-PCR[14]	RT-PCR[14]
Visual system					
RPE	IHC and WB[19]	n.d. by IHC and WB[19]	IHC and WB[19]		ISH and WB[19]
Retina				NB[20]	NB[20]
Reproductive system					
Sertoli cells	IHC[21,22] (+++), ISH[22]	IHC[21](+++), ISH[22]	IHC[21](+), ISH[22]	IHC[21](+), ISH[22]	ISH[22]
Leydig cells			IHC[21](+++), ISH[22]	IHC[21](+++), ISH[22]	ISH[22]
Nervous system					
Cerebellum					
Purkinje cells	ISH[23] IHC[23]	ISH[23]	ISH[23]	ISH[24]	
Granule cells	ISH[23]				
Bergmann glia	IHC[23]				
Neocortex	ISH[23]			ISH and IHC[24]	
Hippocampus					
CA1	ISH[23]			ISH[24]	
CA3	ISH[23]			ISH[24]	
Dentate gyrus	n.d. by ISH[23]	ISH[23]	ISH[23]		
Olfactory bulb	ISH[23]			ISH[24]	
Locus coeruleus				ISH[24]	ISH[24]
Choroid plexus				ISH[24]	ISH[24]
Dorsal root ganglion	NB[25]			ISH[26]	
Spinal cord				ISH (ventral horn)[26]	
Schwann cells	NB[25], WB[26]	WB[26]			NB[25]
Oligodendrocytes	RT-PCR[27]	RT-PCR[27], IHC[27]	RT-PCR[27]		

Astrocytes	NB[25] (see Bergmann glia)				NB[25]
Vascular system					
Endothelial cells		Rheumatoid arthritis synovium vessels IHC[28]			
Smooth muscle		Induced by injury IHC[29], rheumatoid arthritis synovium vessels IHC[28]		Induced by injury IHC[30]	

FACS: fluorescent activated cell sorting; IHC, immunohistochemistry; ISH, *in situ* hybridization, NB, Northern blot; n.d., non detected; WB, Western blot

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