NEURO-SIGNALS

Neurosignals 2009;17:277–287
DOI: 10.1159/000231894

Received: May 14, 2009
Accepted after revision: June 7, 2009
Published online: September 30, 2009

TAM Receptor Signalling and Demyelination

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Key Words
TAM receptor signalling · Demyelination · Tyrosine kinase · Multiple sclerosis

Abstract
The TAM family (Tyro3, Axl and Mer) of receptor protein tyrosine kinases play pivotal roles in a number of major cellular processes: cell survival and proliferation, immunomodulation and phagocytosis. These processes are central to both the initial development and pathological course of human multiple sclerosis. All three receptors and their ligands, Gas6 (growth arrest-specific gene 6) and protein S, are expressed in the central nervous system (CNS), including in oligodendrocytes, the myelin-producing cell of the CNS. Recent studies have shown that Gas6-dependent TAM receptor signalling is an important modulator of oligodendrocyte survival and microglial phenotype both in vitro and in vivo. Multiple lines of evidence allow us to hypothesise that, during a demyelinating challenge, dysfunctional TAM receptor signalling could lead to a 'vicious cycle' of cell death, reduced phagocytosis and deleterious immune hyper-activation. A current challenge in this field is to expand our understanding of TAM receptor signalling from rodent models of central demyelination to human disease.

Multiple sclerosis (MS) is a complex demyelinating disease of the central nervous system (CNS). The initiating insult in MS remains unknown. However, it is clear that the pathology of MS involves complex interactions between many systems and cell types, including both the adaptive and innate immune systems, nerve cells and glial cells. Although the majority of research in MS has focussed on the role of adaptive immunity in the development and progress of the disease, it has recently become clear that, at least in some cases, the primary insult could be directed at the oligodendrocyte. In a seminal study in 2004, Barnett and Prineas [1] examined newly forming lesions from MS patients. They found that prior to the onset of extensive demyelination, and in the absence of cells of the adaptive immune system, oligodendrocyte apoptosis could be detected, generally in the presence of increased numbers of microglia. This work points to oligodendrocyte apoptosis as an early event in the formation of a lesion, and also indicates that microglia could have an important role to play in the initial development of an MS lesion.

Our laboratory has principally focussed on the neurobiological component of MS. In particular, it is clear that one potential strategy for preventing or ameliorating demyelination is to inhibit the death of damaged oligodendrocytes. We have previously shown this strategy to be successful in mouse models of central demyelination whereby provision of exogenous leukaemia inhibitory

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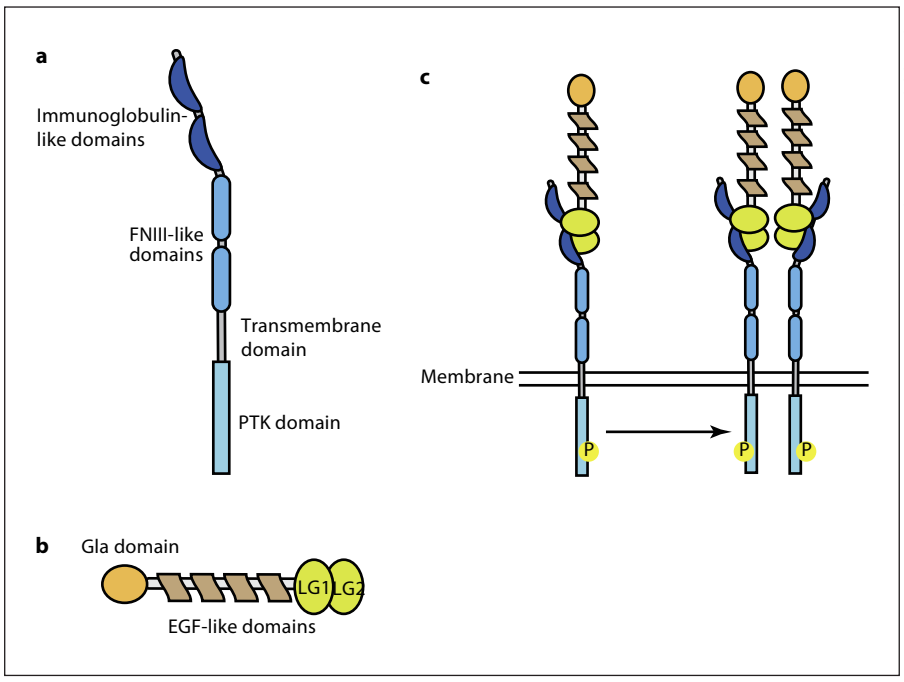

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Fig. 1. Schematic of TAM receptors and their ligands protein S and Gas6. **a** The three members of the TAM receptor family (Tyro3, Axl and Mer) have a common domain structure, with two N-terminal immunoglobulin-like domains, two fibronectin III (FNIII)-like domains, a single-pass transmembrane domain and a cytoplasmic protein tyrosine kinase (PTK) domain containing a number of phosphorylation sites. **b** The two TAM receptor ligands, protein S and Gas6, also share a common domain structure, with an N-terminal Gla domain (a region which is γ-carboxylated in a vitamin-D-dependent post-translational modification), four epidermal growth factor (EGF)-like domains and two laminin-G (LG) domains. **c** Binding of the ligand to the two immunoglobulin domains of the receptor is mediated via the LG1 region of the ligand. Formation of a 1:1 ligand:receptor complex initiates formation of a heterotetrameric complex with a 2:2 ligand:receptor ratio.

factor can prevent loss of oligodendrocytes and improve clinical outcome in experimental allergic encephalomyelitis [2] and in cuprizone-mediated demyelination [3].

In order to identify novel factors potentially important in oligodendrocyte survival, our laboratory employed a microarray strategy, investigating the expression of genes in mice during experimentally induced demyelination compared to control mice. Using this strategy, we identified the TAM receptor family as potentially important, with all members of the family regulated during demyelination.

Structure of the TAM Receptors and Ligands

The TAM receptors are three related protein receptor tyrosine kinases that were first cloned in 1991 as orphan receptors [4]. The three structurally related receptors, Tyro3, Axl and Mer, are collectively known as the TAM receptors. These receptors were reported to be widely expressed in the vertebrate nervous system [4].

The three receptors have the defining structure of two N-terminal immunoglobulin (IG) domains, followed by two fibronectin-III-like domains attached by a single-pass α-helical transmembrane domain to an intracellular tyrosine kinase domain (fig. 1a) [5–7]. In common with other receptor tyrosine kinases, the functional form of the receptor is a dimer, and the receptors have been reported to form both hetero- and homodimers [8]. Analysis of the structure of receptor-ligand complexes has determined that the two N-terminal immunoglobulin domains mediate binding to the ligand (fig. 1c) [9].

The growth arrest-specific gene 6 (Gas6) was first identified as a gene upregulated in response to growth arrest in serum-starved NIH 3T3 cells [10]. The mouse and the human genes were subsequently cloned and fully characterised, with the human Gas6 showing a similar pattern of expression in serum-starved human fibroblasts [11]. The protein was identified as structurally related to protein S, an important component of the anticoagulation cascade [11–13]. It was subsequently identified that both Gas6 and protein S were ligands for the TAM receptors [14–16]. The assignment of protein S as a ligand for the TAM receptors was initially controversial, as original observations found that Gas6, but not protein S, could be co-immunoprecipitated with TAM-Fc fusion proteins [14]. Further, other authors had previously shown that whilst human protein S was a potent activator of mouse Tyro3, they could not identify that human protein S was able to activate human Tyro3 [17]. However, more recent results have unequivocally shown that protein S functions as a ligand for both the Mer and Tyro3 receptors, although its status as a ligand for the Axl receptor remains unproven [18, 19].

The two ligands, protein S and Gas6, have a unique structure not found elsewhere in the vertebrate proteome. At the N-terminus of the protein is the Gla domain (a region containing 11 γ-carboxyglutamic acid residues), followed by 4 epidermal growth factor (EGF)-like domains and 2 laminin-G (LG) domains which together form the sex-hormone-like binding domain (fig. 1b) [11, 20, 21]. Both ligands are dependent upon vitamin K for post-translational modification of the Gla domain, as when warfarin is used to block vitamin-K-dependent γ-carboxylation, at least some of the observed functions of the ligands are abolished, including protection of fibroblasts from apoptosis during serum starvation [for review, see 22].

The domains within Gas6 and protein S have been shown to exert particular functions. The Gla domain, in common with other members of the vitamin-K-dependent family of proteins, has been shown to bind negatively charged phospholipids on the surface of cell membranes. Gas6 in particular has been shown to be specific for phosphatidylserine (PS), in comparison to other phospholipids tested [23]. Protein S has also been shown to bind to vesicles with PS bound to the surface, and to apoptotic cells [24].

Numerous studies have shown that the LG domains mediate binding to the TAM receptors [9, 25, 26]. In particular, examination of the crystal structure of the Gas6/Axl complex revealed that the functional signalling complex is a heterotetramer formed from 2 receptor molecules and 2 ligand molecules in a circular structure [9]. The major binding site within the ligand was fully contained within the LG1 domain which interacts with both the IG1 and IG2 domains of the receptor, with no direct interaction in the dimer between the 2 receptor molecules or the 2 ligand molecules [9]. It is also likely that formation of the heterotetrameric complex is initiated by formation of a 1:1 receptor:ligand complex (fig. 1c).

Recently, Pierce et al. [27] provided direct evidence that at least 2 of the TAM receptors, Axl and Tyro3, can form heterodimers. Further, unpublished observations from Lemke and Rothlin [8] indicate that the ability to form both hetero- and homodimers is not restricted to Axl and Tyro3, but extends to both ligands and all 3 receptors. This ability clearly adds another potential level of complexity to the regulation of TAM receptor signalling. It has also recently been shown that in mice lacking the Mer receptor, Tyro3 expression is also markedly downregulated, indicating that disruption of signalling through 1 TAM receptor can affect expression and presumably therefore signalling through another TAM receptor [19].

Expression of the TAM Receptors and Ligands

As noted above, the TAM receptors and their ligands are expressed in a wide variety of tissues. These tissues include cells of the immune, vascular, reproductive and nervous systems. An excellent summary of our current knowledge of the spatial and temporal expression of the TAM receptors and ligands can be found in a recent review by Lemke and Rothlin [8]. A notable common finding has been that, unlike other tyrosine kinase receptors, the TAM receptors are not widely or highly expressed during development, although expression is still detectable, but their expression is markedly upregulated postnatally, extending into adulthood, with the profile of ligand expression following a similar pattern. This expression pattern reflects the central role TAM receptor signalling plays in regulating homeostasis in a number of different tissue types (discussed below).

In the nervous system, TAMs and their ligands have been observed to be expressed by both neurons and glial cells. Within the rodent nervous system, Tyro3 appears to be most highly expressed, with maximal levels detected from late post-natal periods (P15 in the rat) and extending into adulthood [4, 28, 29]. Tyro3 is also highly expressed in human brain tissue [30]. The expression of both Axl and Mer within the CNS is more restricted than that of Tyro3, and overall expression is lower [29].

Of particular interest is the observation that expression for all 3 receptors can be localised specifically to oligodendrocytes. This expression has been reported for both human and rodent oligodendrocytes in vitro and in vivo [29, 31–33]. In the rodent brain, all 3 TAM receptors have been observed to be upregulated in white matter areas before and during myelination, although only expression of Tyro3 persists at detectable levels in the adult [29].

Protein S and Gas6, as indicated above, are both highly upregulated after birth, and expression of both persists at high levels in the adult [34]. However, both proteins can be detected during development. At early stages of development, Gas6 expression in the rat embryo is mostly confined to non-neuronal tissues [34]. In the rat brain, Gas6 was observed to be initially expressed at E17, although expression is weak, but from the day of birth onwards Gas6 is widely expressed in numerous brain regions, including a number of cortical regions, the hippocampus, midbrain and the cerebellum [34]. The expression of Gas6 has also been described in the ventral spinal cord [35]. In contrast, protein S is expressed only at low levels in the CNS, although expression has been observed in the

locus coeruleus, choroid plexus [34], as well as in astrocytes [16] and in retinal pigment epithelial cells [19]. Protein S expression has also been observed in the rabbit brain, with mRNA and protein detected in pyramidal neurons of the deep layer of the cortex and the hippocampus, in addition to the dentate fascia neurons of the hippocampus [36]. Interestingly, a significant upregulation of the concentration of Gas6 was found in human cerebrospinal fluid (CSF) in patients with chronic inflammatory demyelinating polyneuropathy, compared to either patients with non-inflammatory/non-autoimmune disease or those with acute inflammatory demyelinating neuropathy (Guillain-Barré disease).

These observations indicate that all components of the TAM receptor-signalling pathway are expressed both at the correct developmental stage and the correct place to affect myelination in the CNS. Further, levels of Gas6 have been observed to be upregulated in at least one chronic demyelinating disease, providing a strong basis for the investigation of a potential link between TAM receptor signalling and other chronic demyelinating diseases such as MS.

Function and Signalling of the TAM Receptors

Cell Survival and Proliferation

As mentioned above, the Gas6 gene was cloned in a screen for genes upregulated during serum starvation and the earliest studies on Gas6 concentrated on its function in cell survival and proliferation. Gas6 has been shown to positively influence survival in numerous cell types of many lineages. In the kidney, Gas6 increases the survival of mesangial and glomerular cells, both in vitro and in vivo [37, 38]. Gas6 has also been shown to be anti-apoptotic for various primary cell types and cell lines in culture, including fibroblasts [39, 40], vascular smooth muscle cells [41–43], endothelial cells [44–46], testicular cells [47], lens epithelial cells [41] and peripheral macrophages [48].

In the nervous system, Gas6 has been shown to have anti-apoptotic effects on a number of cell types, including neurons [27, 49, 50], Schwann cells [35], and human and rodent oligodendrocytes [31, 51, 52]. Of particular note, Shankar et al. [52] demonstrated that Gas6 could protect oligodendrocytes from tumour necrosis factor α (TNFα)-mediated toxicity, as TNFα is an inflammatory cytokine secreted by microglia during demyelination. Protein S has been shown to function to protect neurons in an animal model of stroke [53].

In addition to exhibiting anti-apoptotic effects, Gas6 has additionally been shown to stimulate mitogenesis for a limited number of cell types including cardiac fibroblasts [54] and Schwann cells [35]. Protein S can also function as a potent mitogen for vascular smooth muscle cells [55], although it has been shown to have an anti-proliferative effect on rat astrocytes after injury [56].

Many of these studies, however, are limited to an in vitro setting, and although they clearly show that both Gas6 and protein S can rescue many cells from apoptotic cell death, there remains no clear evidence of the relative importance of TAM receptor signalling on the prevention of apoptosis under pathophysiological challenge in vivo. Insights into the potential biological relevance of TAM receptor signalling have come primarily from studies utilising mice in which either the various TAM receptors or their ligands have been knocked out. Konishi et al. [57] studied the outcome of a mouse vascular injury model in wild-type and Axl knockout mice. In this model the activation of Axl signalling, which was at least partially dependent upon Gas6, increased the vascular remodelling which is a detrimental outcome of vascular injury, and this remodelling was likely to be a consequence of increased vascular smooth muscle cell survival. Thus, although Gas6/Axl signalling has been shown to be a biologically relevant signalling pathway in injury, the engagement of this pathway does not necessarily lead to positive outcomes.

In contrast to the above study highlighting a negative role for TAM receptor-dependent survival, more recent studies focussing on the nervous system have shown a positive role for TAM receptor signalling and the accompanying potentiation of neural cell survival. Pierce et al. [27] studied the migration and survival of gonadotropin-releasing hormone (GnRH) neurons in Tyro3 and Axl single- and double-knockout mice. The authors found that in the absence of appropriate signalling from Tyro3 and Axl, apoptosis amongst migrating neurons was increased, with a resulting deficit in the number of GnRH neurons compared to wild-type mice. This deficit resulted in disruption of the normal oestrous cycle in the mutant mice. Studies from our own laboratory (see below) have also shown a positive effect of TAM receptor signalling during demyelination in vivo [31].

Many lines of evidence point to a critical role for the phosphatidylinositol-3-kinase (PI3K) signalling pathway in transducing the survival signal initiated by TAM receptor activation (fig. 2a) [46, 57–61]. The p85 subunit of PI3K directly binds phosphorylated tyrosine in the Axl receptor [61, 62], leading to phosphorylation and activa-

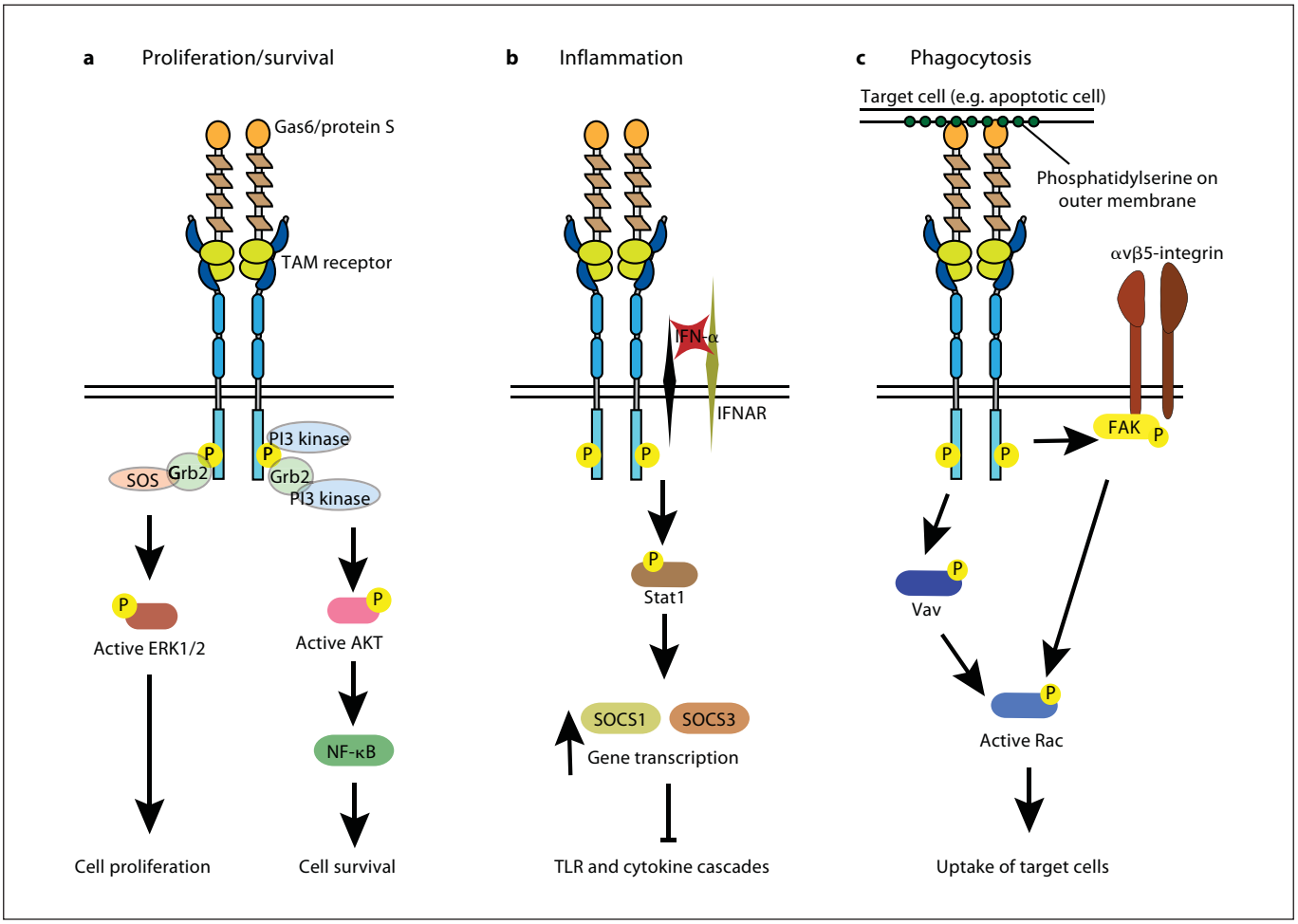

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Fig. 2. Simplified TAM receptor signalling pathways. TAM receptors are phosphorylated and activated in response to ligand binding. **a** The phosphatidylinositol-3-kinase (PI3K) can be engaged either by direct binding of the p85 subunit of PI3K or indirectly by binding of growth factor receptor-bound 2 (Grb2) adaptor protein, leading to phosphorylated Akt and nuclear translocation of NF-κB (nuclear factor κ-light-chain enhancer of activated B cells), ultimately leading to promotion of cell survival. Alternatively, binding of the Grb2 adaptor protein and recruitment of son of sevenless (SOS) can engage the extracellular signal-related kinase (Erk)-signalling pathway to promote cell proliferation. **b** Activation of the TAM receptors recruits the interferon-α receptor (IFNAR), leading to subsequent activation and nuclear translocation of signal transducer and activator of transcription 1 (Stat1). As a result, transcription of both suppressor of cytokine signalling (SOCS) 1 and 3 is increased, ultimately resulting in suppression of Toll-like receptor (TLR) and cytokine inflammatory cascades. **c** Mer can signal to induce phagocytosis via either an alpha(v) beta(5) integrin (αvβ5-integrin)-dependent mechanism involving focal adhesion kinase (FAK) or via an alternative pathway involving Vav. Both pathways converge on Ras-related C3 botulinum toxin substrate (Rac), initiating cytoskeletal reorganisation and eventually leading to phagocytosis.

tion of the Akt survival pathway, including nuclear translocation of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and upregulation of transcription of NF-κB-regulated genes [46, 58]. Alternatively, this pathway can be engaged indirectly by binding of the growth factor receptor-bound 2 (Grb2) adaptor protein, which can then bind PI3K [61]. It is of note that direct binding of PI3K sterically inhibits binding of Grb2. This is of particular importance in that it has been shown that Grb2 can engage the extracellular signal-related kinase (Erk) signalling pathway, which is the pathway by which TAMs signal proliferation (fig. 2a) [40, 60, 63, 64]. It has been shown that in human oligodendrocytes phosphorylation of Axl results in the recruitment of p85 and Grb2 to the receptor [61], although Gas6 does not appear to be a mitogen for oligodendrocytes.

Immunomodulation

Although the earliest studies on the role of TAM receptor signalling concentrated on the role of this family in cell survival and proliferation, the generation of knockout mice lacking the individual receptors, along with double- and triple-receptor knockouts, led to the discovery of the central role for the TAM receptors in immune regulation. Although even triple-knockout mice are viable and fertile, and develop apparently normally for the first few postnatal weeks, they develop a number of apparently degenerative phenotypes, the most dramatic of which is broad-spectrum autoimmunity [65]. In triple-mutant animals there is hyper-proliferation of B and T cells from about 4 weeks of age, and the proliferation is such that ectopic colonies of these cells can be detected in almost every tissue [65]. The B and T cells of the triple-mutant mice are also constitutively active, leading to the triple-mutant mice developing symptoms similar to a wide range of autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus [65]. Interestingly, the authors found that the dysregulation of B and T cells was not a cell-intrinsic phenomenon, but due primarily to the loss of TAM receptors in antigen-presenting cells such as macrophages and dendritic cells. These cell types are also constitutively active in TAM triple-knockout mice, and a loss of these receptors has been shown to lead to dysregulation of the innate immune system [66].

Unlike the signalling mechanisms involving PI3K to induce cell survival, the transduction of the anti-inflammatory effects of TAM receptors appears to involve the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway [66]. It has recently become clear that the inhibition of inflammatory cytokine expression observed in a number of cell types is transduced through the recruitment of the type 1 interferon receptor (IFNAR), a receptor known to otherwise stimulate inflammation (fig. 2b) [66]. Further, it has been shown that the suppression of inflammation after induction of IFNAR stimulation is dependent upon TAM signalling, at least in dendritic cells [66]. Interestingly, a recent study by Prinz et al. [67] assessing mice devoid of IFNAR found an exacerbation of clinical symptoms in the experimental allergic encephalomyelitis model of demyelination, and this exacerbation was determined to be due to a loss of IFNAR signalling on cells of the innate immune system. Given the number and variety of cell types in which TAMs exert an immunomodulatory effect, TAM-dependent IFNAR signalling is not likely to be the only signalling pathway via which TAMs control immune activity, but at present other pathways remain to be elucidated.

Whilst much of the work examining the regulation of immune responses has focussed on antigen-presenting cells of the periphery, recent work has shown that TAM receptor signalling can also regulate the activation of microglia within the CNS. Work from our own laboratory has shown that pre-treatment of primary rat microglia in vitro with exogenous Gas6 can reduce the subsequent expression of the inflammatory cytokine TNFα by these microglia when activated by lipopolysaccharide challenge [31]. This work has been supported by work from Grommes et al. [68] who demonstrated that treatment of a mouse microglial cell line with Gas6 reduced expression of both inducible nitric oxide synthase and interleukin-1β after lipopolysaccharide challenge. Interestingly, the reduction in the inflammatory phenotype of the microglia observed by Grommes et al. [68] was accompanied by an increase in the phagocytic response (see below).

Phagocytosis

The structure of both Gas6 and protein S suggested that, in common with other Gla-domain containing proteins, both ligands would be able to bind negatively charged phospholipids such as PS. As discussed above, it was shown that Gas6 can specifically bind PS [23]. However, the biological relevance of this interaction was unclear until mice lacking all three TAM receptors were generated and assessed, with these mice being observed to have deficits in spermatogenesis and to develop blindness [65, 69]. These apparently unrelated phenomena are in fact manifestations of the same underlying pathology – the failure of clearance of apoptotic cells as part of normal tissue homeostasis. The loss of germ cells in the testis is a result of the dysfunction of Sertoli cells, which fail to perform their normal function of clearance of the many apoptotic cells produced during normal spermatogenesis [69]. The blindness observed in TAM triple knockouts is also related to the failure of retinal pigment epithelial cells to phagocytose the distal ends of photoreceptors [19, 65, 69]. This phagocytosis is critical to the normal functioning of the retina. Indeed, it was found that Mer single knockout mice also showed this degenerative phenotype [19, 65], and that mutations in the Mer gene were responsible for retinal degeneration both in the rat and in humans [70–73].

The involvement of TAM receptor signalling, particularly that of Mer, has also been observed in other cell types. Of particular interest have been studies involving cells of the innate immune system such as macrophages and dendritic cells. Whilst many of these reports have

focussed on Mer, it should be noted that a recent study has identified the involvement of all three TAM receptors in the clearance of apoptotic cells by peripheral macrophages and dendritic cells, with different cell types requiring different combinations of TAM receptors [74]. Further, a recent study has also shown that mice deficient in Gas6 have dysfunctional peripheral macrophages which have a reduced ability to clear apoptotic red blood cells [75].

Unlike signalling via TAM receptors for cell survival and inflammatory regulation, comparatively little is known about the intracellular signalling pathways involved in TAM-initiated phagocytosis. It is hypothesised that the binding of Gas6/protein S to PS on the surface of an apoptotic cell can form a bridge between the bound ligand and the receptor on a phagocyte (fig. 2c) [23, 76]. Some studies have demonstrated a connection between Mer signalling and αvβ5-integrin, in which the Mer receptor and the αvβ5-integrin receptor co-operate to amplify internalisation signals: this amplification occurs via Mer-dependent phosphorylation of focal adhesion kinase (FAK), binding of activated FAK to the αvβ5-integrin receptor, and subsequent recruitment of a protein complex for Rac1 (Ras-related C3 botulinum toxin substrate 1) activation (fig. 2c) [77]. Whilst in some cells the presence of the αvβ5-integrin receptor appears to be required for Mer-dependent phagocytosis [77], it has nevertheless also been identified that in other cells the presence of the αvβ5-integrin receptor does not appear to be necessary for Mer-dependent phagocytosis to occur [78]. In the study by Grommes et al. [68] described above, the Gas6-induced phagocytosis by microglia was mediated by Vav phosphorylation, although this signalling mechanism also appeared to converge on the Rac pathway, initiating cytoskeletal reorganisation and eventually leading to phagocytosis (fig. 2c).

TAM Signalling Can Regulate Myelination, Microglial Activation and Phagocytosis

The above generic effects have specific relevance to the study of the molecular determinants of demyelination and remyelination within the CNS. This has been illustrated by recent work from our laboratory, which has recently studied the effect of the absence of Gas6 on cuprizone-mediated demyelination. Cuprizone is a copper chelator that, when fed to mice, initiates a reproducible and stereotyped focal demyelination, particularly of the corpus callosum [79, 80]. This demyelination takes places in the absence of infiltration of the adaptive immune system and has been shown to be T-cell-independent [81]. In the cuprizone model of demyelination, there is a large recruitment of microglia/macrophages both before and during demyelination [79, 82]. There is activation and proliferation of microglia and some infiltration of peripheral macrophages [82]. In addition to the effects on the innate immune system, there is loss of and damage to oligodendrocytes [83, 84]. As discussed above, studies of the newly forming MS lesions have shown that activation of microglia and oligodendrocyte apoptosis are some of the earliest events in the development of an MS lesion. Thus cuprizone-induced demyelination provides an excellent model system for studying these early events.

When Gas6 knockout mice are subjected to cuprizone-induced demyelination for 3 weeks, both myelination and microglial activation are affected. In the absence of Gas6, oligodendrocyte survival is compromised, with the result that, in comparison to wild-type mice, fewer oligodendrocytes can be detected both in the rostral and caudal corpus callosum. This loss of oligodendrocytes is accompanied by a reduction in overall myelination and fewer myelinated axons. In contrast, a study by Hoehn et al. [85], in which Axl knockout mice were challenged with cuprizone, did not observe any significant loss of oligodendrocytes in comparison with wild-type mice at any time point examined. However, the authors of this study did observe a reduction in overall myelination after 6 weeks of cuprizone challenge in Axl knockout mice. This apparent conflict between the relative effect on oligodendrocyte survival in the absence of Gas6 compared to the absence of the Axl receptor suggests that a loss of signalling through a single receptor (Axl) has a less profound effect than a reduction in signalling through all three receptors, such as in the absence of the ligand Gas6. Alternatively, it may be that either Tyro3 or Mer is the main TAM receptor responsible for transducing the anti-apoptotic effect of Gas6 in the context of cuprizone challenge.

In addition to a loss of oligodendrocytes and reduction in myelination, when Gas6 knockout mice are subjected to cuprizone challenge, there is a profound increase in microglial activation. The number of microglia detected in the corpus callosum of Gas6 knockout mice is significantly greater than in wild-type mice after 3 weeks of cuprizone challenge [31]. However, this study was limited to an examination of the effects of 3 weeks of cuprizone challenge. This study therefore provides only a single 'snapshot' of oligodendrocyte survival and microglial activation, and it is not possible to formally resolve which effects are the direct result of a loss of Gas6 and which, if any, are

secondary. In the study described above by Hoehn et al. [85] of Axl knockout mice, the authors examined a number of time points both during and after cuprizone-mediated demyelination. Again, in contrast to the findings in Gas6 knockout mice, the authors did not observe an increase in the number of microglia, but rather a significant decrease in microglia in Axl knockout mice after 4 weeks of cuprizone challenge. The authors also found that in the absence of Axl, there appeared to be a delay in phagocytosis and therefore the removal of myelin debris. Again, this apparent disparity in results between the 2 studies could reflect the ability of alternate members of the TAM receptor family to compensate for the loss of a single receptor, whereas the overall reduction in signalling that accompanies the loss of the ligand for these receptors could outstrip the ability of, for example, protein S to compensate for this loss. Additionally, it will be important to determine the phagocytic ability of microglia in the absence of Gas6 signalling. Whilst many more microglia are observed in the corpus callosum in the absence of Gas6, the phenotype of these microglia remains undetermined. It could be that, in the context of Gas6 deficiency, these cells have a pro-inflammatory phenotype, secreting cytotoxic cytokines and reactive oxygen species, rather than a more anti-inflammatory and phagocytic and immunomodulatory phenotype, which in other contexts would be responsible for the removal of myelin debris and apoptotic cell bodies, as well as contributing to repair.

Conclusions

The TAM receptors are unique amongst the larger family of receptor tyrosine kinases in that their expression is upregulated during postnatal development. Consistent with the expression data are the observations that knockout of either the ligand Gas6, or of all three receptors, still leads to viable mice, indicating that signalling via TAM receptors is not essential for embryonic development. However, studies of these knockout animals have also shown that TAM receptor signalling is central to many ongoing processes in the postnatal and adult animal.

Of particular interest is the important role that TAM receptor signalling plays in controlling three major processes: cell survival and proliferation, immunomodulation and phagocytosis. Of relevance to this review, it is apparent that these three processes are of central importance in the initiation and development of MS. We and others have shown that Gas6 is an important modulator of both oligodendrocyte survival and microglial phenotype both in vitro and in vivo. Further, the expression pattern of the TAM receptors hint at a potential role for TAM receptor signalling in the direct control of myelination, not just as a secondary outcome of its anti-apoptotic effects on oligodendrocytes.

As discussed previously, until very recently confusion has reigned concerning the status of protein S as a genuine ligand for the TAM receptors. In consequence, examination of the effects of protein S in various biological systems lags well behind that of Gas6, with the notable exception of its effects on retinal pigment epithelial cells. There are tantalising hints of the potential role that protein S could play in limiting injury in CNS disorders such as MS. For example, the findings that exogenous protein S can limit astrocyte proliferation and, by extension, potentially limit glial scar development, the formation of which can impede remyelination. It is therefore of critical importance that the biological effects of protein S on TAM receptor signalling are more closely investigated.

In demyelinating diseases as MS, which have a clear immune component, there is an important interplay between cell survival, phagocytosis and immune regulation. Efficient phagocytosis of apoptotic cells and myelin debris is an essential first step in remyelination, as myelin debris has been shown to inhibit remyelination [86, 87]. Further, it is very likely that the broad-spectrum autoimmunity observed in TAM receptor triple-mutant mice results not only from the failure of immune regulation, but at least in part from the potentiated availability and presentation of autoantigens as a consequence of the failure of removal of apoptotic cells [8]. Thus, it could be hypothesised that dysfunctional or reduced TAM receptor signalling could create a 'vicious cycle' of cell death, failure of phagocytosis, inhibition of remyelination, and immune hyperactivation.

Much of the work discussed above has been performed in rodents or in vitro models of disease. Human work has been limited, but promising. Gas6 has been shown to be a pro-survival factor for human oligodendrocytes, and Gas6 protein has been shown to be upregulated in the CSF of patients with a chronic inflammatory demyelinating disease. Extending these studies to the CSF of patients with MS would be of interest and potentially revealing, but is hampered by the fact that collection of CSF is currently not a routine procedure in the management of MS. Nevertheless, it is clear that an important first step in determining whether TAM signalling also plays a biologically significant role in MS pathology will be to establish the expression profiles of TAM receptors and the ligands in human MS lesions.

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