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REVIEW

TAM receptors in apoptotic cell clearance, autoimmunity, and cancer

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Abstract
Receptor tyrosine kinases, Tyro-3, Axl and Mer, collectively designated as TAM, are involved in the clearance of apoptotic cells. TAM ligands, Gas6 and Protein S, bind to the surfaces of apoptotic cells, and at the same time, interact directly with TAM expressed on phagocytes, impacting the engulfment and clearance of apoptotic cells and debris. The well-tuned and balanced actions of TAM may affect a variety of human pathologies including autoimmunity, retinal degeneration, and cancer. This article emphasizes some of the emerging findings and mechanistic insights into TAM functions that are clinically relevant and possibly therapeutically targeted.

Keywords: *TAM receptors, phosphatidylserine, Gas6, efferocytosis, immune tolerance*

Introduction

Efficient phagocytosis of apoptotic cells (efferocytosis) is critical for maintenance of tissue homeostasis and self-tolerance in metazoans [1], but also important during the resolution phase of inflammation. Apoptotic cells express plasma-membrane "eat-me" signals that ensure swift removal without the release of potentially immunogenic self-antigens [2]. Engulfment also promotes the release of the anti-inflammatory cytokines IL-10 and TGF-β, inducing macrophages and dendritic cells (DC) to be refractory to further stimulation. In genetic mouse models, deficiencies in phagocytic recognition and in the proper clearance of apoptotic cells can potentiate several chronic diseases, such as atherosclerosis [3], autoimmune diabetes [4], and systemic lupus erythematosus (SLE) [5]. In humans, several clearance factors, most prominently C1q and pulmonary surfactant D, are genetically strongly associated with the development of SLE [6].

However, while macrophages isolated from SLE patients frequently show defective clearance [7], the identification of the genetic risk factors underscoring these defects is a daunting challenge, as over forty receptors and soluble bridging proteins and even more intracellular adaptor and signaling proteins are implicated in clearance pathways in higher mammals [8]. Indeed, although homozygous twins display a high concordance rate to develop SLE (30-70%), a plethora of genetic studies did not pinpoint a general SLE gene in unrelated patients. There are many roads leading to Rome.

At the molecular level, one of the best-understood receptor-mediated clearance pathways involves the TAM receptor family (Tyro-3, Axl, and Mer). These homologous type I receptors with intrinsic tyrosine kinase (RTKs) activities in turn bind the phosphatidylserine (PS) opsonins Gas6 and Protein S [9]. Either Mer^{KO} single knockout [10] or $Tyro3^{KO}/Axl^{KO}/Mer^{KO}$ triple knockout mice [11], while unremarkable in their development, show age-dependent autoimmunity in adulthood. Mer^{KO} mice gradually develop SLE-like autoimmunity whose phenotypes include accumulation of apoptotic and secondary necrotic cells in peripheral tissues, defective clearance in vitro, chronically elevated TNF-α and IL-1 in serum, dysregulation of lymphocyte activation, and the production of autoantibodies towards dsDNA [5,10,12].

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Detailed mechanistic studies using genetic models have shown that Mer regulates immune functions in various cells in vivo. Efferocytosis by DC resulted in Mer-dependent inhibition of NF-κB, suppression of pro-inflammatory cytokine production, DC maturation and antigen cross-presentation [13]. Furthermore, studies employing the NOD model of type 1-diabetes showed that Mer^{KO} exasperated T cell-mediated β-cell autoimmunity which could be adoptively transferred between mice [4]. Mer^{KO} mice have increased numbers of splenic and bone marrow derived DC that secrete B cell pro survival factor (BAFF) to crosstalk at the level of B cell hyperactivity [14]. In addition, tingible body macrophages (TBM) localized in splenic germinal centers (GC) are severely affected in Mer^{KO} mice [15,16]. Typically, self-reactive B cells in the GC that undergo apoptosis during B cell clonal selection are engulfed by TBM [15,17]. Mer^{KO} TBM have impaired apoptotic B cell clearance [18] and promote the development of antibody-forming cells that contribute to autoimmunity [17].

Taken together, the functional integrity of Mer in myeloid cells is key for the maintenance of both central and peripheral self-tolerance through two inter-related mechanisms that involve the (i) prevention of the release of apoptotic cell-derived autoantigens, and (ii) inhibition of stress-mediated production and/or action of pro-inflammatory cytokines, that impinge on suppression of auto-reactive T and B cell expansion. Despite the important biological and conceptual advances in Mer biology, there are several challenges in the field awaiting clarification.

Translation of mouse biology to human biology

Although the loss of Mer function was associated with failed apoptotic clearance in mouse genetic models, no genetic analysis has yet to definitively link mutations or allelic variations in Mer to the risk of developing human autoimmune diseases. However, in a small study of Korean patients, some association was noted with reduced risk of leucopenia [19]. These studies do not rule out epigenetic regulation of TAM expression or signaling. Indeed, increased plasma levels of soluble Mer (which probably acts as a dominant negative decoy receptor) have been noted to correlate with disease activity and nephritis in SLE [20].

Studies by Cohen and colleagues noted that in a study of more than 100 SLE patients, some positive correlations were observed with decreased Protein S expression; Gas6 was unaffected [21]. Adding complexity, three additional ligands for TAM have been identified (tubby, tubby-like protein 1, and galectin-3), but their role in clearance has not yet been investigated [22,23]. One may predict that the number of TAM ligands identified may further increase in the near future.

Plasticity of TAM: Post-transcriptional and post-translational mechanisms that regulate TAM expression

All TAM are highly dynamic and subject to regulation by extracellular stress and cytokines. The promoter region of Axl and Mer contains cis-acting elements for various transcription factors, including AP-1, Sp1, Sp3 and E2F [24,25]. More relevant to this discussion, Mer is transcriptionally regulated by glucocorticoids which are established to display therapeutic benefit in SLE especially in advanced disease [26,27]. Also, a recent study has shown that C1q, the apoptotic cell opsonin most strongly linked as a genetic susceptibility factor to SLE, promotes the up-regulation of both Mer and Gas6, and utilizes a Mer-dependent pathway for efferocytosis [28]. This suggests that Mer expression is controlled indirectly by other endogenous factors that segregate with disease frequencies. Further studies that identify transcriptional cascades and miRNAs targeting TAM need to be better explored in their relationship to autoimmunity. Finally, ectodomains of Mer and Axl are routinely shed by proteases at the cells' surfaces [20,29] and appear as soluble decoy proteins in the extracellular space. Pathways that regulate these post-translational events also require further attention in relationship to autoimmune diseases.

Compensatory or specialized functions of individual TAM in apoptotic cell clearance and immunosuppression?

Although TAM are defined by sequence conservation in their ligand binding and kinase domains, it is unclear whether each has unique ligand binding preferences (only the Ig1-Ig2/Gas6 structure of Axl is solved), and whether each has specific post-receptor signaling functions. Consistent with this idea, it is proposed that different cell types use different TAM for clearance [30]. Although both DC and macrophages express all three TAM, Mer deficiency is mainly linked to defective efferocytosis in the latter; Axl and/or Tyro3 deficiency primarily alters phagocytosis by DC [30]. In a similar scenario, both retinal pigmented epithelial cells [31] and involuting mammary epithelial cells [32] show defective clearance in Mer-deficient mice, even though Axl is co-expressed in these cells.

One attractive possibility is that there are differences in the effector functions of family members, with Mer–dependent engulfment in the absence of inflammation, while Axl may function in the resolution phase, being induced by LPS and interferon-alpha [26,33,34]. Also, Axl activation has been shown to inhibit TLR signaling in DC preventing chronic inflammation [35]. According to Rothlin et al., this inhibition requires the cooperation of TAM and type I interferon receptor, leading to

STAT1 activation and the selective induction of suppressor of cytokine signaling (SOCS1/3) which promotes immune tolerance [35]. A similar pathway has not been explored for Mer.

TAM in cancer: The opposite spectrum of autoimmunity?

The preceding discussion has focused on the loss of TAM function in relation to autoimmunity whereas the converse appears to be the case in human cancers. While TAM deficiency profoundly contributes to autoimmune diseases, all three TAM (particularly Axl and Mer) are frequently overexpressed in human cancers and clinically associated with aggressive disease and poor survival outcome [9]. The loss of expression of microRNAs targeting TAM, such as miR-126, miR-199a/b and miR-34a, is associated with poor distal metastasis-free survival [36,37].

Although evidence indicates that Axl and Mer can activate classic oncogenic networks, such as PI3-kinase and Erk [9], the influence of TAM on tumor phagocytosis and immune regulation also suggests their role in tumor tolerance. In this capacity, TAM could counteract signals for immunogenic cell death (ICD), a form of tumor cell death that activates anti-tumor immune responses [38]. ICD is accompanied by the relocalization and exposure of specific intracellular danger signal proteins which activate DCs to cross-present tumor antigens. In an immune stimulatory context (i.e. when immature DC are recruited to the tumor site), ICD primes effective anti-tumor responses in immune competent hosts, whereas anti-ICD leads to tumor tolerance. Although the goal of chemotherapy is to induce tumor cell death, the idea that tumor phagocytes perform tolerogenic TAM-mediated efferocytosis provides a conundrum to the field. This way, tumor cells might compete with professional phagocytes and prevent them from recognizing dying tumor cells as dangerous.

In support of the previous discussion, small molecule inhibitors of Axl kinase (R428) reduce metastatic burden and extend survival in MDA-MB-231 and 4T1 immune competent orthotopic models of breast cancer metastasis [39]. Interestingly, R428 also synergizes with cisplatin to suppress liver micrometastases suggesting that the combination of TAM inhibition to block PS with the induction of ICD may be attractive to achieve tumor immunity. This idea is also supported from studies by Bondanza et al, who showed that for tumor vaccination, masking PS on irradiated lymphoma cells impairs PS-mediated efferocytosis and enhances tumor immunity *in vi*vo [40]. Finally, recent clinical studies show that antibody-mediated blockade of PS function in the tumor micro-environment (Bavituximab) is sufficient to induce ICD and durable tumor immunity, consistent with a role for TAM in tumor tolerance and should be explored as a mechanistic target of Bavituximab [41,42].

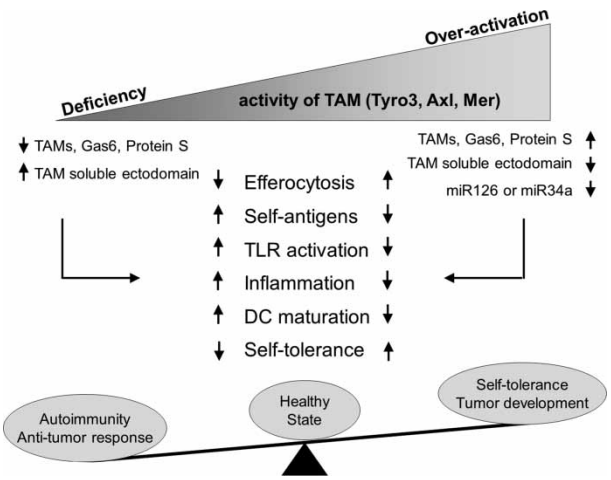

Figure 1. Dynamic regulation of TAM receptors in physiology and pathology: The activation state of TAM is controlled by both transcriptional and post-translational mechanisms to fine-tune immunogenic and tolerogenic signals. Catastrophic loss of TAM function in mouse knockouts results in loss of central tolerance and severe autoimmunity reminiscent of SLE, yet overexpression in cancers may lead to tumor tolerance. In vivo, the activity of TAM is regulated by specific transcription factors, microRNAs, availability of soluble ligands, and receptor shedding and soluble receptors. Elucidation of the controlling elements that determine TAM activity will lead to new insight and therapeutic strategies for both autoimmunity and cancer.

In recent years, there has been a surge in papers on TAM receptors, and a strong link to both auto-immunity (loss of function) and cancer (gain in function), suggesting that TAM function on two sides of a common coin (Figure 1). It is attractive to speculate that strategies positively or negatively targeting the expression and/or activity of TAM, and therefore altering immune responses against the dying self, will have multiple applications in the clinic.

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