arthritis research&therapy

EDITORIAL

Rethinking the red wolf disease: does Protein S suppress systemic lupus erythematosus clinical activity?

Gregg J Silverman*

See related research by Suh *et al.*, http://arthritis-research.com/content/12/4/R146

Abstract

In systemic lupus erythematosus, the forces responsible for disease initiation and self-perpetuation in these clinically heterogeneous populations remain poorly understood. Recent studies of the TAM (Tyro3, Axl and MerTK) family of receptor tyrosine kinases may lead to a better understanding of the fundamental control system responsible for the clearance of apoptotic cells and the regulation of inflammation. In a recent report, serum levels of the TAM ligand, Protein S, was found to correlate with certain disease manifestations and with C3 and C4 levels. Protein S levels could provide a quantitative clinical biomarker but it remains to be determined whether this factor directly affects disease activity.

Introduction

Systemic lupus erythematosus (SLE) is the prototypic chronic inflammatory systemic autoimmune disease, but many aspects of its pathogenesis remain poorly understood. As described in the following sections, the recent report from Suh and colleagues [1] may help us to integrate an understanding of how innate immune pathways affect autoimmune pathogenesis.

One of the most fundamental challenges to the immune system is the efficient recognition and clearance of the body's own cells when senescence, injury or other causes lead to their entry into programmed death pathways, which are a normal outcome of cell and tissue turnover. Apoptotic cell (AC) clearance is therefore important for resolving the cellular consequences of normal development during embryogenesis, and for cellular proliferation and differentiation that continues throughout life.

The homeostatic pathways that regulate apoptotic clearance are also involved in the resolution of inflammation. Yet inflammation is a beneficial host response to foreign challenge or tissue injury, representing a tightly choreographed sequence of changes in tissue and blood factors and cellular recruitment and subsequent clearance that ultimately restores tissue structure and function. Both exposure to ACs and the clearance of ACs have been recognized as important mechanisms for the resolution of inflammation *in vivo* (reviewed in [2]), while an inability to control inflammatory responses is at the root of many chronic diseases.

Conditions associated with defects in phagocytic clearance of dead and dying host cells, and especially C1q and IgM deficiency states, may lead to lupus-like disease [2]. These associated clearance defects may also result in cellular progression to secondary necrosis and the release of self-ligands (such as High-mobility group protein B1 (HMGB-1) and heat shock protein (HSP)) for inflammatory innate receptors and of self-antigens that drive stimulation and selection of autoreactive lymphocytes.

The TAM family and the GAS6 and Protein S ligands

Discovered in 1991, the TAM family of receptor tyrosine kinases (RTKs) may be amongst the most recent class of protein phosphatases to appear in evolution (reviewed in [3]). The three family members, TYRO3 (also termed SKY, BRT, ETK, TIF, DTK, and RSE), the prototypic member AXL (ARK, UFO, and TYRO7), and MERTK (c-EYK, NYK, and TYRO12), share a conserved structure of two immunoglobulin-like motifs and two fibronectin type III repeats in the extracellular domain, and a cytoplasmic domain with a conserved catalytic kinase region. TAM members play fundamental roles in diverse cell functions of proliferation, differentiation, survival, migration, and metabolism, and are variably expressed in

*Correspondence: gsilverman@ucsd.edu
Laboratory for B cell Immunobiology, Department of Medicine-0663, UC San Diego School of Medicine, 9500 Gilman Drive, La Jolla, CA 92093-0663, USA

neural, vascular, and reproductive tissues [3]. TAM members are also prominently expressed in the immune system, especially in professional phagocytic cells, macrophages (Mφs) and dendritic cells (DCs).

Ligand interactions are essential for TAM triggering. Best studied is the product of *growth-arrest-specific gene 6* (GAS6), a vitamin K-dependent protein widely secreted by most tissues [4]. GAS6 can bind and activate all three receptors via tyrosine autophosphorylation but with markedly different affinities (AXL ≥ TYRO3 >> MER) [4]. GAS6 may be primarily locally produced in tissues, with only limited levels in the circulation. Many cells express GAS6, which may provide autocrine functions for TAM triggering, and levels can increase during apoptosis death or in an inflammatory milieu [5].

The second ligand for the TAM system, Protein S, shares domain organization and approximately 44% sequence identity with GAS6. Both GAS6 and Protein S include a specific GLA domain that undergoes post-translational modification by vitamin K-dependent gamma-carboxylation to provide positively charged residues for binding of phosphatidylserine residues exposed on ACs [3]. Through GLA domains, GAS6 and Protein S serve as bridging molecules to TAM receptors on Mφs and DCs [6], enhancing AC uptake and engulfment [5].

Protein S is also a negative regulator of blood coagulation as it is a cofactor for activated Protein C-mediated inactivation of factors Va and VIIIa, which may suggest there are interconnections between the TAM system and anti-phospholipid syndrome. The specificities of these two ligands differ; Protein S was reported to be a specific agonist for TYRO3, while in cells that co-express TYRO3, Protein S is also a potent MERTK agonist [7]. Protein S is produced and secreted in liver and approximately 60% of circulating Protein S is in complex with C4-binding protein. Nonetheless, circulating levels are still 1,000-fold higher than those of GAS6, with great variation even amongst healthy individuals.

TAM dysregulation and systemic autoimmunity

In murine systems, the most serious consequences of mutations of the TAM genes involve the immune system. TAM-deficient mice have severe defects in AC clearance, impaired control of inflammatory responses, and develop frank autoimmune disease associated with sustained antigen presenting cell activation [8-11]. This likely results from the loss of TAM regulation of two related phenomena: the phagocytosis of ACs by Mφs and DCs, and control of innate inflammatory response to pathogens and self-ligands.

TAM receptor interactions may lead to downstream inhibition of the mitogen-activated protein kinase and NF-κB cascades, which dominate inflammatory signaling [5,6,10]. However, there remain controversies regarding downstream TAM signaling events, and different pathways may also be triggered in different cell types. Notably, in murine FLT3-L generated DCs, inhibition of inflammatory signaling has been reported to be dependent on SOCS (Suppressor of cytokine signaling) [10].

Low plasma Protein S but not GAS6 levels correlate with SLE disease activity

Suh and colleagues [1] reported results from surveys of serum GAS6 and Protein S levels in well-characterized SLE patients. They found that free Protein S levels were highly directly correlated with C3 and C4 levels, and that free Protein S levels were lower in SLE patients with a history of serositis, neurologic disorder, hematologic disorder and immunologic disorder. Levels of Protein S were also lower in patients with anti-cardiolipin antibodies, although there was no correlation with lupus anticoagulant or anti-β2 glycoprotein I antibody. Overall, there were no significant differences between levels in SLE patients and healthy adults, and there was also no relationship between GAS6 and Protein S levels within individual SLE patients. Findings for GAS6 levels were limited to evidence of elevation in patients with a history of neurologic involvement.

Conclusion

The studies from Cohen and coworkers [1] highlight a new perspective on how breaches in regulatory control of a fundamental homeostatic anti-inflammatory pathway, which first arose far before the mammalian adaptive immune system, may be a major determinant of SLE clinical manifestations and/or local activity. It may not be surprising that the involvement of some but not all organ systems was associated with lower free Protein S levels, as the contributions of infiltrating Mφ and DCs, or other pathways affected by the TAM system, may vary greatly in different affected anatomic sites. This likely also explains why there was no significant correlation with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) or the British Isles Lupus Assessment Group (BILAG) disease index [1].

The study did not examine the functional roles of Protein S and GAS6 in human disease, and also did not evaluate for fluctuations over time in an individual or with regard to changes in disease activity. Therefore, it remains to be determined whether changes in free Protein S levels primarily reflect differences in systemic production, or rates of consumption at inflammatory sites or via other degradation pathways, or rates of recruitment by activated Protein C in the microvasculature. The current findings therefore underscore the need for further investigation of how Protein S may directly (or indirectly) affect lupus pathogenesis.

Abbreviations
A, apoptotic cell; DC, dendritic cell; GAS6, Growth-arrest-specific gene 6; Mφ, macrophage; RTK, receptor tyrosine kinase; SLE, systemic lupus erythematosus.

Competing interests
The author declares that he has no competing interests.

Acknowledgements
Financial support: grants from the NIH; R01 AI068063 and ARRA supplement, R01AI090118, and from the ACR REF Within Our Reach campaign, the Alliance for Lupus Research and the Arthritis Foundation.

Published: 27 October 2010

References

1. Suh CH, Hilliard BA, Li S, Merrill JT, Cohen PL: **TAM receptor ligands in lupus: Protein S but not Gas6 levels reflect disease activity in systemic lupus erythematosus.** *Arthritis Res Ther* 2010, **12:**R146.
2. Savill J, Fadok V: **Corpse clearance defines the meaning of cell death.** *Nature* 2000, **407:**784-788.
3. Lemke G, Rothlin CV: **Immunobiology of the TAM receptors.** *Nat Rev Immunol* 2008, **8:**327-336.
4. Crosier KE, Crosier PS: **New insights into the control of cell growth; the role of the Axl family.** *Pathology* 1997, **29:**131-135.
5. Sen P, Wallet MA, Yi Z, Huang Y, Henderson M, Mathews CE, Earp HS, Matsushima G, Baldwin AS Jr, Tisch RM: **Apoptotic cells induce Mer tyrosine kinase-dependent blockade of NF-kappaB activation in dendritic cells.** *Blood* 2007, **109:**653-660.
6. Seitz HM, Camenisch TD, Lemke G, Earp HS, Matsushima GK: **Macrophages and dendritic cells use different Axl/Mertk/Tyro3 receptors in clearance of apoptotic cells.** *J Immunol* 2007, **178:**5635-5642.
7. Prasad D, Rothlin CV, Burrola P, Burstyn-Cohen T, Lu Q, Garcia de Frutos P, Lemke G: **TAM receptor function in the retinal pigment epithelium.** *Mol Cell Neurosci* 2006, **33:**96-108.
8. Lu Q, Lemke G: **Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family.** *Science* 2001, **293:**306-311.
9. Cohen PL, Caricchio R, Abraham V, Camenisch TD, Jennette JC, Roubey RA, Earp HS, Matsushima, G, Reap EA: **Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase.** *J Exp Med* 2002, **196:**135-140.
10. Rothlin CV, Ghosh S, Zuniga EI, Oldstone MB, Lemke G: **TAM receptors are pleiotropic inhibitors of the innate immune response.** *Cell* 2007, **131:**1124-1136.
11. Scott RS, McMahon EJ, Pop SM, Reap EA, Caricchio R, Cohen PL, Earp HS, Matsushima GK: **Phagocytosis and clearance of apoptotic cells is mediated by MER.** *Nature* 2001, **411:**207-211.

doi:10.1186/ar3162
Cite this article as: Silverman GJ: **Rethinking the red wolf disease: does Protein S suppress systemic lupus erythematosus clinical activity?** *Arthritis Research & Therapy* 2010, **12:**144.