Clinical Immunology (2015) 158, 231–241

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Monocyte and plasma expression of TAM ligand and receptor in renal failure: Links to unregulated immunity and chronic inflammation

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Received 7 October 2014; accepted with revision 31 January 2015
Available online 24 March 2015

KEYWORDS
Chronic kidney disease;
Inflammation;
Monocytes;
TAM receptors;
Axl;
Mer

Abstract Chronic inflammation is increased in patients with chronic kidney disease (CKD) and contributes to cardiovascular morbidity and mortality. Specific immune mechanisms and pathways that drive and maintain chronic inflammation in CKD are not well described. The TAM ligands (Gas6 and protein S) and receptors (Axl and Mer) have been recently recognized as playing a prominent role in immune regulation. The receptors exist in both soluble and cell-bound forms; the soluble receptors (sAxl and sMer) are believed to compete with the bound receptors and thus inhibit their function. In this study, we determined the expression of cell-bound and soluble TAM proteins in patients with CKD. CKD patients had significantly lower expression of Mer in monocytes, yet increased expression of soluble TAM receptors sAxl and sMer in plasma compared to controls. The metalloproteinase ADAM 17, responsible for cleavage of Mer to its soluble form, was increased in patient monocytes. Elevated levels of soluble TAM receptors were more evident in patients with progressive renal failure. These observations suggest that functional deficiency of TAM receptor-mediated regulation of inflammation may contribute to chronic inflammation in patients with CKD.

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http://dx.doi.org/10.1016/j.clim.2015.01.012
1521-6616/

1. Introduction

Patients with chronic kidney disease (CKD) have exceedingly high morbidity and mortality from cardiovascular disease compared with the general population [1–3]. Chronic inflammation accelerates vascular disease in patients with renal failure; however, limited knowledge exists on specific pathways and mechanisms leading to unregulated inflammation in human CKD. Indeed, specific therapies addressing or reducing chronic inflammation in CKD are lacking, and no specific treatment strategy has improved survival benefit in patients with renal failure.

Innate immunity is intensified in CKD. Key cytokines, such as IL-6, IL-1β and TNF-α, are elevated in hemodialysis patients (HD) and are linked to mortality and cardiovascular outcomes [4–7]. Increased exposure to foreign materials such as dialysate fluid and hemodialysis filters contributes to chronic exposure to lipopolysaccharide (LPS) and chronic TLR stimulation. Cytokine dysregulation, however, is also observed in early stages of CKD, before the need for dialysis. Elevated IL-6 levels are linked to inflammatory markers and mortality in early CKD, even before glomerular filtration rate (GFR) is significantly impaired [8]. Given the role of TAM ligands and receptors in modulating innate inflammation, we set out to test whether the TAM ligand–receptor pathway is regulated aberrantly in CKD.

TAM ligands and receptors are expressed by cells of the innate immune system and are important regulators of inflammation [9]. A subfamily of tyrosine kinases, Tyro3, Axl and Mer, or TAM, serves as receptors for the TAM ligand, Gas6 and protein S. In macrophages and dendritic cells, TAM receptor signaling limits the toll like receptor (TLR)-induced production of proinflammatory cytokines through the induction of the inhibitory protein suppressor of cytokine signaling (SOCS) [10,11]. Basic studies show that ligation of TAM receptor results in decreased production of cytokines IL-6, IL-1β and TNF-α in vitro [12]. In vivo, Mer signaling is key in preventing lipopolysaccharide (LPS)-induced injury and death [13,14]. Gas6 also serves as a bridge for mediating cell–cell interactions, by binding both to TAM receptors and to phosphatidylserine residues exposed on activated platelets, injured endothelial cells, and apoptotic bodies [15–18]. These interactions are crucial for phagocytosis and clearance of apoptotic cells, generating tissue repair and favoring an anti-inflammatory milieu [15–18]. Conversely, the total absence of TAM receptors results in widespread accumulation of apoptotic cells, lymphoproliferative disease, and autoantibody production [18–20]. Finally, studies have established a crucial role for Mer on a subset of macrophages, (M2c) known to possess immunoregulatory and anti-inflammatory function [21].

Gas6 is also upregulated by stimulated vascular smooth muscle cells and injured endothelial cells, and has been implicated in neointima formation after vascular injury [22,23]. Mer expression is upregulated by glucocorticoids and is crucial for phagocytosis of apoptotic cells by M2c macrophages [24]. Upregulation of Mer is also driven by intercellular cholesterol accumulation as well as proteins important in cholesterol regulation [25]. By efficient removal of dying cells and apoptotic debris, Mer on M2c macrophages is important in regulating and resolving inflammation [17,21]. The TAM ligand–receptor pathway therefore, plays an important role in regulating atherogenesis, plaque complications, thrombosis, and adaptive responses to vessel injury [26–28].

Emerging reports in humans show altered expression of TAM ligand and receptors in inflammation and autoimmunity. Studies have demonstrated links between plasma levels of TAM ligand and soluble TAM receptor with disease activity in systemic lupus erythematous [29,30]. In patients with septic shock, increased plasma concentrations of Gas6 correlated with disease severity and increased mortality [31]. Other studies show an association between Gas6 and C-reactive protein in patients with documented vascular disease [35]. Likewise, our study was the first to demonstrate increased TAM ligand Gas6 in CKD compared to controls [32]. Here we report findings of dysregulated expression of TAM receptors Axl and Mer in CKD, which may lead to defective TAM receptor mediated regulation of inflammation in patients with CKD.

2. Materials and methods

2.1. Subjects

This study was approved by the Temple University School of Medicine IRB. After written informed consent, blood samples were obtained from 64 patients with end-stage renal disease on chronic HD, 83 patients with non-dialysis CKD, and 23 healthy volunteers with no known medical history. Both CKD and HD groups were recruited from within the Temple University Nephrology practice. Inclusion criteria for CKD were evidence of proteinuria or renal dysfunction and no history of hemodialysis. Dialysis (HD) patients were CKD patients on HD for >6 months. Excluded from both CKD and HD groups were patients on warfarin therapy. In the CKD group, a single venous blood sample was obtained in the outpatient clinic. In HD, blood was collected prior to routine hemodialysis sessions. Blood samples were centrifuged at 3000 rpm and plasma aliquoted and stored at −20 °C or −80 °C.

2.2. Isolation of peripheral blood mononuclear cells and monocytes

Venous blood was drawn from patients and normal subjects into sodium heparin-containing vacutainers (Becton Dickinson). Peripheral blood mononuclear cells (PBMC) were purified using Ficoll/Hypaque sedimentation to remove red blood cells and granulocytes. Monocytes were isolated by negative magnetic bead sorting using the EasySep human monocyte enrichment kit without CD16 depletion (Stemcell, Vancouver, BC, Canada), following the manufacturer's instructions.

2.3. Flow cytometry (Mer)

0.5×10^6 peripheral blood mononuclear cells were stained with fluorescent antibodies for analysis by flow cytometry. Antibodies used in the study and specific for Mer (clone 125518) was obtained from R&D (Minneapolis, MN); Anti-CD14 (clone M5E2), -CD42b (clone HIP1), -CD56 (clone HCD56), -CD 123 (clone 6H6), and -FcERI (clone AER-37) were obtained from Biolegend, San Diego, CA; Anti-CD4 (clone RPA-T4) was