Serum amyloid P: a systemic regulator of the innate immune response

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ABSTRACT

The pentraxin SAP reduces neutrophil adhesion to ECM proteins, inhibits the differentiation of monocytes into fibrocytes, attenuates profibrotic macrophages, activates the complement pathway, and promotes phagocytosis of cell debris. Together, these effects of SAP regulate key aspects of inflammation and set a threshold for immune cell activation. Here, we present a review of SAP biology with an emphasis on SAP receptor interactions and how the effect of SAP on monocytes and macrophages has been explored to develop this protein as a therapeutic for renal and lung injuries. We also discuss how there remain many unanswered questions about the role of SAP in innate immunity.


Introduction

The mammalian immune system is organized into two arms: innate and adaptive immunity. Innate immunity is evolutionary more ancient and constitutes the first line of defense against foreign pathogens [1]. In vertebrates, adaptive immunity complements innate immunity and provides immunological memory [2]. Pathogen recognition molecules, such as pentraxins, are at the core of innate immunity [3, 4]. Pentraxins recognize evolutionarily conserved pathogen molecules, such as C-polysaccharide, regulate complement activation, and bind apoptotic cells to initiate and synchronize the immune response [4–7].

Pentraxins are a family of conserved proteins that appeared early on during the evolution of innate immunity [8] and have a 200-aa long pentraxin domain with a conserved pentraxin signature (HxCxS/TWxS, where x = any amino acid) [9]. Pentraxins are organized into two groups: the short and the long pentraxins. The short pentraxins are identified by their pentameric structure consisting of 25 kDa monomers and include CRP and SAP (for a review on CRP, see ref. [10]). The long pentraxins are an N-terminal domain attached to a pentraxin domain and include PTX3, PTX4, guinea pig apoxin, NPTX1, NPTX2, and NPTXR (for reviews, see refs. [4, 9, 11, 12]).

The short pentraxins CRP and SAP are pattern recognition molecules secreted by the liver that interact with pathogens and cell debris to promote their removal by macrophages and neutrophils [13]. SAP binds to rough LPS, and lack of SAP causes hypersensitivity to laboratory strains of Escherichia coli [14]. In addition, CRP and SAP interact with components of the complement pathway to regulate complement activation [15, 16]. However, the regulation of the innate immune system by SAP is not limited to its effects on the complement pathway and phagocytosis. SAP binds directly to monocytes, neutrophils, and macrophages to modify their activation and alter their differentiation to modulate the immune response.

REGULATION OF NEUTROPHIL FUNCTION BY SAP

At the onset of inflammation, neutrophils are recruited to the damaged tissue, where they release ROS and promote clearance of pathogens and cell debris. This recruitment is mediated by cytokines, tissue damage, complement activation, and changes in adhesion receptors on the surface of endothelial cells [17–19]. The migration and activation of neutrophils are tightly regulated by factors expressed and secreted by endothelial cells and macrophages [17]. However, factors present in plasma also affect neutrophils [17].

SAP binds to neutrophils to regulate their function

One circulating factor that regulates neutrophil accumulation in tissues is SAP [20]. SAP binds to human and murine neutrophils and decreases TNF-α- and IL-8-induced neutrophil binding to ECM components [20, 21]. SAP also reduces TNF-α-induced human neutrophil adhesion to endothelial cells [22]. One possible mechanism underlying the effect of SAP on neutrophils involves SAP binding to, and thus, potentially blocking, the adhesion receptor L-selectin on neutrophils [22]. This is supported by the observation that adding anti-L-selectin antibodies to human neutrophils decreases their binding to umbilical vein endothelial cells [22]. A second possible mechanism involves SAP binding to FcγRs on neutrophils.
SAP inhibits neutrophil spreading

In addition to decreasing neutrophil adhesion, SAP decreases human neutrophil spreading, a necessary step for cell polarization and migration [20, 30–33]. Paradoxically, SAP does not influence human neutrophil migration in response to IMLP in a Boyden chamber [20]. This inconsistency may be a result of the differences in the adhesion receptors used during neutrophil migration on matrix proteins and after stimulation with chemotactic stimuli in a Boyden chamber. On fibronecetin, neutrophils use the β1 (VLA-4 and VLA-5) integrins to migrate, whereas in an uncoated Boyden chamber, β2 (CD11b/CD18) integrins are the key adhesion receptors [34, 35]. SAP may act as a chemoattractant of human neutrophils, although this finding has not been replicated [21]. Alternatively, it is possible that the timing of stimuli (i.e., SAP) could determine how neutrophil spreading and migration are influenced.

Indirect effects of SAP on neutrophils

Neutrophils secrete proteases, such as elastase, to degrade the ECM and facilitate tissue infiltration (for a review, see ref. [36]). SAP but not CRP binds to neutrophil elastase and inhibits its enzymatic activity [37]. This can hinder neutrophil extravasation and the secondary damage caused by the proteolytic activity of elastase in tissues [36–40]. SAP also induces macrophages to produce the anti-inflammatory cytokine IL-10, which in turn, decreases TNF-α and CXCL8 production. This then results in decreased neutrophil recruitment [23, 41–43]. These observations suggest that SAP regulates many aspects of neutrophil biology to exert an anti-inflammatory effect and set a threshold for neutrophil recruitment and activation (Fig. 1). In agreement with this, we have observed that SAP injections can decrease neutrophil accumulation in a mouse model of acute respiratory distress syndrome [20].

SAP inhibits monocyte-to-fibrocyte differentiation

Monocytes present within the blood are attracted to sites of injury where they differentiate into macrophages, dendritic cells, or fibrocytes [44, 45]. Fibrocytes are spindle-shaped, fibroblast-like cells and at least, in part, mediate tissue repair and fibrosis (for a review, see ref. [45]). Fibrocytes have been detected in human pathological conditions, including pulmonary fibrosis, keloid scars, asthma, chronic kidney disease, and nephrogenic systemic fibrosis [45–49]. In addition to contributing to the mass of fibrotic lesions, fibrocytes promote angiogenesis, which can then promote the growth of the lesion, and secrete TGF-β, which activates resident fibroblasts [50]. Fibrocyte differentiation is regulated by several factors, including cytokines, TLR ligands, semaphorins, and hyaluronic acid [45, 51–53]. We found that when human, mouse, or rat PBMCs were cultured in serum-free media, some of the cells became fibrocytes after 3–5 days [54]. The fibrocytes did not appear during this timeframe when serum was present [54]. We purified the fibrocyte differentiation inhibitor from human serum and identified it as SAP [54]. When PBMCs were cultured in serum that was depleted of SAP, fibrocytes appeared rapidly, indicating that SAP is the main endogenous inhibitor of fibrocyte differentiation in the blood. In agreement with this, we observed that depleting SAP from dermal wounds in pigs can facilitate fibrocyte differentiation and scar-tissue formation [55]. We also tested whether SAP could inhibit fibrocyte differentiation and fibrosis in bleomycin-induced lung fibrosis [56]. We found that SAP injections led to reduced numbers of fibrocytes in the lungs and reduced fibrosis in rats and mice and that delaying SAP injections until inflammation and fibrosis were already apparent could also reduce symptoms [56].

SAP inhibits fibrocyte differentiation, in part, by binding to FcγRs [57]. In support of this, we have found that cross-linked but not monomeric IgG inhibits fibrocyte differentiation and that blocking the signal transduction pathway of the FcγRs with pharmacological inhibitors blocks the ability of SAP and cross-linked IgG to inhibit fibrocyte differentiation [58]. In mice, deletion of the FcγRI, which is necessary for FcγRI and FcγRIIa signaling, significantly reduces sensitivity to SAP [23, 57, 59]. However, deletions of FcγRIIb, FcγRIII, and FcγRIV
do not affect sensitivity to SAP [57]. We found similar results using small interfering RNA knockdowns of human receptors [57]. However, in all cases, SAP still caused some inhibition of fibrocyte differentiation [23, 57], indicating the presence of additional SAP receptors on monocytes. These observations suggest that SAP, in part, uses FcγRI and FcγRII to inhibit fibrocyte differentiation (Fig. 2).

SAP REGULATES MURINE MACROPHAGE POLARIZATION

Macrophages are considered one of the most important innate effector cells (for reviews, see refs. [60–62]). Macrophages can be classified into the classically activated macrophages (M1) and the alternatively activated macrophages (M2) [60, 63]. M1 macrophages are induced in response to TNF-α, IFN-γ, and specific TLR agonists [60, 64]. The classically activated M1 macrophages modulate host defense against intracellular pathogens, tumor cells, and tissue debris but are also responsible for tissue damage associated with their release of ROS [60, 63, 65–67]. M2 is a general term for several overlapping macrophage subsets, which are induced in response to IL-4, IL-10, IL-13, and SAP [23, 60, 64]. The role of M2 macrophages in the immune system is highly dependent on the activating stimuli (i.e., IL-10 vs. IL-4) and the environmental context. The alternatively activated M2 macrophages can be classified into three main groups: immunoregulatory macrophages, profibrotic/wound-healing macrophages, and tumor-associated macrophages [60, 63, 68, 69]. The hallmark of immunoregulatory macrophages in humans and mice is high levels of the anti-inflammatory cytokine IL-10 and low levels of the proinflammatory cytokine IL-12 [60]. Wound-healing macrophages express high levels of IL-10 and IL-12, whereas tumor-associated macrophages are identified by their secretion of a variety of angiogenic factors [60, 68].

SAP promotes immunoregulatory macrophages in mouse renal injuries

In a mouse model of systemic lupus erythematosus, macrophages in the kidneys have elevated expression of IL-10, iNOS, and TNF-α [64]. IL-10 is a marker for M2 macrophages, whereas iNOS and TNF-α are typically associated with M1 macrophages [60, 64]. When the mice were injected with SAP, the expression of the M2 markers IL-10 and arginase 1 in the kidney macrophages was increased, whereas the levels of the M1 markers iNOS and TNF-α decreased [64]. This change in gene expression involved the PI3K/Akt–ERK signaling pathway and indicates a shift toward an immunoregulatory phenotype in macrophages [64].

In mouse models of renal fibrosis, SAP injections decreased expression of the M1 markers Mip2a and IL-1β, and the profibrotic M2 markers CCL17 and CCL22 on renal macrophages [23, 60]. These changes were accompanied by a significant increase in the levels of IL-10 [23]. In IL-10 and FcγRI knockout mice, the effects of SAP on renal fibrosis were reduced [23]. Together, these observations suggest that SAP, in two different models of renal injuries, polarizes macrophages toward an immunoregulatory phenotype.

SAP attenuates profibrotic macrophages in mouse lungs

In TGF-β-driven mouse models of pulmonary fibrosis, SAP alleviates fibrosis, in part, through its effect on macrophages [70, 71]. In this model of pulmonary fibrosis, SAP injections decreased M2 markers, while increasing the M1 marker CXCL10 on pulmonary macrophages [60, 70]. This is in stark contrast to the role of SAP in renal injuries, where it promotes immunoregulatory macrophages and decreases M1 macrophages. This inconsistency may be attributed to differences that exist in the milieu of kidneys and lungs. In support of this, similar to TGF-β-driven pulmonary fibrosis, SAP attenuated M2 macrophage activation in the spore-induced allergic airway disease of mice [60, 72]. Furthermore, in spore-induced allergic airway disease, SAP injections increased expression of the M1 marker IFN-γ in lung macrophages, whereas not significantly altering levels of the immunoregulatory marker IL-10 [72]. Together, these observations suggest that SAP has a significant role in regulating macrophage polarization, but the outcome is tissue-dependent and at times quite different. (Fig. 3).

CONCLUDING REMARKS

SAP plays a significant role in the regulation of the innate immune system by binding to FcγRs. SAP decreases neutrophil adhesion, regulates macrophage activation, enhances phagocytosis of cell debris, activates the complement pathway, and inhibits fibrocyte differentiation. Together, these effects of SAP inhibit many aspects of innate immunity that contribute to inflammation and fibrosis. Depletion of SAP on dermal wounds in pigs and injections of SAP in animal models of acute respi-
Figure 3. SAP inhibits profibrotic macrophages in mice. SAP attenuates profibrotic macrophages in renal and pulmonary injuries of mice.

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N.C., D.P., and R.H.G. contributed to writing.

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DISCLOSURES

D.P. and R.H.G. are inventors on patents for the use of SAP as a therapeutic for fibrosing diseases and patents for the use of SAP-depleting materials to enhance wound healing. D.P. and R.H.G. are members of the Science Advisory Board of and have stock options from Promedior, a start-up company that is developing SAP as a therapeutic for fibrosing diseases, and receive a share of milestone payments made by Promedior to Rice University.

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