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A novel hypothesis for an alkaline phosphatase 'rescue' mechanism in the hepatic acute phase immune response

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ABSTRACT

The liver isoform of the enzyme alkaline phosphatase (AP) has been used classically as a serum biomarker for hepatic disease states such as hepatitis, steatosis, cirrhosis, drug-induced liver injury, and hepatocellular carcinoma. Recent studies have demonstrated a more general anti-inflammatory role for AP, as it is capable of dephosphorylating potentially deleterious molecules such as nucleotide phosphates, the pathogenic endotoxin lipopolysaccharide (LPS), and the contact clotting pathway activator polyphosphate (polyP), thereby reducing inflammation and coagulopathy systemically. Yet the mechanism underlying the observed increase in liver AP levels in circulation during inflammatory insults is largely unknown. This paper hypothesizes an immunological role for AP in the liver and the potential of this system for damping generalized inflammation along with a wide range of ancillary pathologies. Based on the provided framework, a mechanism is proposed in which AP undergoes transcytosis in hepatocytes from the canalicular membrane to the sinusoidal membrane during inflammation and the enzyme's expression is upregulated as a result. Through a tightly controlled, nucleotide-stimulated negative feedback process, AP is transported in this model as an immune complex with immunoglobulin G by the asialoglycoprotein receptor through the cell and secreted into the serum, likely using the receptor's State 1 pathway. The subsequent dephosphorylation of inflammatory stimuli by AP and uptake of the circulating immune complex by endothelial cells and macrophages may lead to decreased inflammation and coagulopathy while providing an early upstream signal for the induction of a number of anti-inflammatory gene products, including AP itself.

1. Introduction

According to the current 'danger model' for immune recognition and inflammatory responses, pathogenic and endogenous stimuli alike have the capability to induce an immune reaction in the body [1,2]. Pathogenic insults are referred to as 'pathogen-associated molecular patterns', or PAMPs, and endogenous insults as 'damage-associated molecular patterns', or DAMPs. Both types of molecules are able to stimulate receptors on multiple cell types known as pattern-recognition receptors, or PRRs, such as the Toll-like receptors (TLRs) [1–3]. PAMPs are usually encountered upon exposure to microorganisms, while DAMPs result from generalized tissue damage and do not necessarily correspond to infection. However, both signal types, which can be elicited by infection, trauma, hypoxia/ischemia, hemorrhagic shock, and numerous other insults, lead to the same sequences of non-specific protective and reparative processes in the body, known collectively as inflammation [3,4]. Inflammation thus may be pathogen-associated or sterile, and in either case it may occur locally or, as its severity increases, spread to become 'systemic inflammatory response syndrome' (SIRS) [4].

Inflammation and aberrant hemostasis are intimately related. For instance, SIRS induces heightened production of pro-inflammatory

Abbreviations: ADP, adenosine diphosphate; AE2, anion exchanger 2; Ala, alanine; AMP, adenosine monophosphate; AP, alkaline phosphatase; Arg, arginine; ATP, adenosine triphosphate; ASGP-R, asialoglycoprotein receptor; Asn, asparagine; ASOR, asialoorosomucoid; BDL, bile duct ligation; biAP, bovine intestinal AP; CABG, coronary artery bypass graft; cAMP, cyclic AMP; CFTR, cystic fibrosis transmembrane conductance regulator; CRD, carbohydrate recognition domain; CURL, compartment for uncoupling of receptor and ligand; Cys, cysteine; DAMP, damage-associated molecular pattern; DIC, disseminated intravascular coagulation; Fab, fragment, antigen-binding; Fc, fragment, crystallizable; FcγR, Fc gamma receptor; FcγRIIb, Fc gamma receptor IIb; FcRn, neonatal Fc receptor; FVIII, factor VIII; GCAP, germ-cell AP; Gly, glycine; GPI, glycosylphosphatidylinositol; IAP, intestinal AP; IFγ, interferon gamma; IgA, immunoglobulin A; IgG, immunoglobulin G; IL-1, interleukin-1; IL-2, interleukin-2; IL-6, interleukin-6; IMCD, inner medullary collecting duct cells; LPS, lipopolysaccharide; LRC, ligand–receptor complex; LSECs, liver sinusoidal endothelial cells; MDCK, Madin–Darby canine kidney cells; MHC, major histocompatibility complex; PAMP, pathogen-associated molecular pattern; Phe, phenylalanine; pIgA, polymeric IgA; pIgR, polymeric immunoglobulin receptor; PLAP, placental AP; PLC, phospholipase C; PLD, phospholipase D; polyP, polyphosphatase; PRR, pattern-recognition receptor; R-ASGP-R, rescue ASGP-R; Ser, serine; SIRS, systemic inflammatory response syndrome; TfR, transferrin receptor; TGN, trans-Golgi network; TLR, Toll-like receptor; TNAP, tissue non-specific AP; TNF, tissue necrosis factor; TNFα, tissue necrosis factor alpha; Tyr, tyrosine; vWF, von Willebrand factor

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cytokines which can promote the formation of microthrombi and thus systemic coagulation [4,5]. Moreover, inflammation due to septic bacterial infection can lead to disseminated intravascular coagulation, or DIC [6]. Not surprisingly, inflammatory liver disease often correlates with severe hemostatic abnormality which may be pro- or anti-coagulant [7,8]. Elevated serum levels of the liver isoform of the enzyme alkaline phosphatase (AP; EC 3.1.3.1) have been associated classically with inflammatory hepatic disease states including chronic hepatitis [9,10], primary sclerosing cholangitis [11], liver cirrhosis [10,12], cystic fibrosis [13], and hepatocellular carcinoma [10,14–18]. AP is a well-known glycosylphosphatidylinositol (GPI)-anchored ecto-phosphomonoesterase with differential tissue expression and multiple isozymes and isoforms. APs act to dephosphorylate a number of substrates which have been implicated specifically in the promotion of inflammation and coagulopathy through their PAMP or DAMP effects, including pathogenic lipopolysaccharide (LPS) and endogenous polyphosphate (polyP) and nucleotide phosphates, respectively [19,20].

Dephosphorylation of such molecules by AP has been linked to anti-inflammatory and anti-thrombotic effects. The bacterial endotoxin LPS, for example, can be released into circulation during infection or upon the disruption of intestinal barrier integrity that commonly occurs in both fibrotic liver disease and ischemia/reperfusion injury [21,22], triggering a host of potentially lethal inflammatory responses mediated by TLR4 signaling (reviewed in [23]). LPS is also able to trigger the contact pathway of clot formation, which leads to increased thrombus generation [19,24]. AP-catalyzed dephosphorylation of LPS in the lipid A region is known to reduce its toxic potency [25,26]. This detoxification promotes survival during a systemic LPS challenge in rats [26,27]. AP in the intestine has been shown to protect mice from luminal bacterial translocation across the gut mucosal barrier and systemic LPS toxicity after ischemia and reperfusion [28,22]. Also, extreme increases in plasma AP levels accompany *E. coli*-induced bacteremia in humans [29]. PolyP, an inorganic phosphate polymer, is found in many microorganisms and is also released into circulation from activated human platelets and mast cells [24,30]. PolyP powerfully induces coagulation, thrombosis, and inflammation as well as capillary leakage in a polymer length-dependent manner by acting at multiple points along the clotting cascade [30,31]. AP demonstrates strong exophosphatase activity on polyP, cleaving individual phosphates from each terminal of the linear molecule, thereby decreasing the length of the polymer over time. The end result of this is the inhibition of polyP-induced coagulation and inflammation [24,30]. Endogenous adenosine nucleotides such as ATP can be released lytically or non-lytically from hepatocytes and other cell types into the extracellular environment in response to various inflammatory stimuli, triggering anti-inflammatory signaling cascades through high-affinity P2Y receptors and, at higher concentrations, pro-inflammatory signaling cascades through low-affinity P2X receptors on the membranes of neighboring cells [32–37,111]. ATP and ADP-induced pro-inflammatory processes include vasodilation and prothrombotic signaling, and serum concentrations of ADP are key in the regulation of platelet activation [38], resulting in polyP release and subsequent triggering of the contact pathway [39]. AP has the unique ability to remove phosphates sequentially from ATP and ADP, thereby keeping the levels of these nucleotides in check and helping to promote anti-inflammatory P2Y signaling while simultaneously generating the purine adenosine [40], which induces a widespread anti-inflammatory effect via P1 receptor signaling [32].

It has been found recently that clinical bolus treatment followed by continuous infusion with exogenous bovine intestinal AP (biAP) decreases the incidence of post-operative systemic inflammation in coronary artery bypass graft (CABG) patients [41]. Similar decreases in levels of pro-inflammatory cytokines have been observed at a later stage in the inflammatory process in dialysis patients with severe sepsis-induced acute kidney injury [42,43]. Based on outcomes such as these, AP is attracting renewed attention for its potential as a therapeutic agent in the prevention and treatment of generalized inflammation.

Treatment of rat liver slices with biAP was shown to induce endogenous production of liver AP mRNA within 2 h [41]. Clinical administration of intravenous biAP in CABG patients induced an initial peak in plasma AP concentration followed by a sharp decrease due to liver uptake; this was counteracted unexpectedly by a subsequent increase in plasma concentrations of tissue non-specific AP (TNAP) from the liver, detected 4–6 h after surgery [44]. The exact mechanism underlying these observations of TNAP expression and secretion from the liver in response to exogenous biAP remains unknown. A sensitive negative feedback model for the expression of AP on apical membranes of polarized cells involving ecto-purinergic P2Y receptor activation by ATP and pH control by bicarbonate has been proposed for many polarized epithelia [45–47], including liver parenchymal cells [48]. It is known that endogenous AP is expressed at the hepatocyte canalicular membrane, and also that exogenous circulating asialo-AP is cleared by the liver through uptake by the asialoglycoprotein receptor (ASGP-R) from the sinusoidal membrane [41,49]. However, it is still unclear how newly synthesized AP on the canalicular membrane might be delivered along the opposite trajectory to the circulation in response to an inflammatory threat or to detection of AP in the serum, and whether or not this mechanism might involve transport of AP by the ASGP-R or another receptor.

The ASGP-R (reviewed in [50–54]) binds to an assortment of circulating asialoglycoproteins [52,55]. The receptor has two subpopulations, known as State 2 and State 1 receptors, which follow different pathways as they transport and process their ligands. While there are relatively equal numbers of receptors in each category, State 2 receptors are significantly more active in terms of ligand uptake and degradation than are State 1 receptors [52,56]. It remains unclear whether there are two distinct receptor populations or, if not, exactly which factors determine whether a given ASGP-R will utilize the State 2 or the State 1 pathway, although phosphorylation and palmitoylation of the receptor have been implicated in these terms [52,57]. The ASGP-R has been shown to play a vital role in host survival during a narrow range of severe inflammatory insults by clearing desialylated platelets and von Willebrand factor (vWF), which accumulate in the serum under conditions of sepsis induced by a neuraminidase expressing microorganism such as *Streptococcus pneumoniae* [6]. This is a major, high-turnover process for this receptor and, based on its mechanism, is presumably attributable to State 2 recycling endocytosis activity. However, little has been published to date regarding State 1 ASGP-R activity and function, and the likelihood of an auxiliary role for this route during inflammation has not yet been discussed.

The prevalent antibody immunoglobulin G (IgG) is known to associate in binding interactions with AP [200–203] and with the ASGP-R [58–61]. We propose a mechanism in which liver AP, generated at the hepatocyte canalicular membrane, is transported across the cell to the basolateral membrane by the ASGP-R as an antigen in an IgG-bound immune complex and secreted into the serum in a regulated manner as a result of inflammation. This model does not involve paracellular transit of AP or IgG, but rather requires the ASGP-R, through 'rescue' activity, to mirror the behavior of IgG receptors like the neonatal Fc receptor (FcRn) in other tissues by internalizing and transporting canalicular AP–IgG immune complexes in response to a sufficient inflammatory stimulus. Secretion from the liver of such complexes would make plasma-soluble AP available to react with circulating PAMPs and DAMPs and subsequently be encountered and processed by sinusoidal endothelial cells or macrophages. This processing by endothelial cells and macrophages may provide an upstream signal for the induction of a number of anti-inflammatory gene products, including AP itself. A hypothesis for such a system is supported by abundant evidence regarding the characteristics, individual binding and transport aspects, and relationships to inflammation and to one another of AP, the ASGP-R, and IgG, which we will present here.