Alkaline Phosphatase, an Unconventional Immune Protein

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Recent years have seen an increase in the number of studies focusing on alkaline phosphatases (APs), revealing an expanding complexity of function of these enzymes. Of the four human AP (hAP) proteins, most is known about tissue non-specific AP (TNAP) and intestinal AP (IAP). This review highlights current understanding of TNAP and IAP in relation to human health and disease. TNAP plays a role in multiple processes, including bone mineralization, vitamin B6 metabolism, and neurogenesis, is the genetic cause of hypophosphatasia, influences inflammation through regulation of purinergic signaling, and has been implicated in Alzheimer's disease. IAP regulates fatty acid absorption and has been implicated in the regulation of diet-induced obesity and metabolic syndrome. IAP and TNAP can dephosphorylate bacterial-derived lipopolysaccharide, and IAP has been identified as a potential regulator of the composition of the intestinal microbiome, an evolutionarily conserved function. Endogenous and recombinant bovine APs and recombinant hAPs are currently being explored for their potential as pharmacological agents to treat AP-associated diseases and mitigate multiple sources of inflammation. Continued research on these versatile proteins will undoubtedly provide insight into human pathophysiology, biochemistry, and the human holobiont.

Keywords: alkaline phosphatase, hypophosphatasia, tissue non-specific AP, intestinal AP, lipopolysaccharide, microbiome

INTRODUCTION

Alkaline phosphatases (APs) belong to a superfamily of proteins (EC 3.1.3.1) sharing conservation of metal binding sites, amino acids required for activity, and predicted fold structure (1). APs are used extensively in life sciences education, as a tool in molecular biology research and as a blood serum marker for liver and bone health, and yet we know surprisingly little about the potential these proteins have to influence our health. In general, APs are anchored to outside surface of the plasma membrane and catalyze the hydrolysis of phosphate groups from a variety of different substrates (dephosphorylation) in an alkaline environment, freeing inorganic phosphate (Pi) (2–4). APs are ubiquitous, with members of the AP super family of proteins extending from the archaea (5) to humans (2). Their ubiquity across life and their expansion and subsequent dynamic evolution in vertebrates implies both variety and conservation of function (6, 7). There are four genes encoding APs in humans. Three genes, *ALPI*, *ALPP*, and *ALPPL2*, display tissue-specific expression (TSAP proteins), whereas the fourth, *ALPL* is tissue non-specific in expression [tissue non-specific AP (TNAP) proteins] (**Table 1**). Unlike tissue distribution, surprisingly less is known about the function of these proteins, especially ALPP and ALPPL2 (**Table 1**). This mini-review will briefly highlight current knowledge of TNAP and intestinal AP (IAP) function in human health and disease (see **Figure 1** for summary).

TISSUE NON-SPECIFIC AP

The most direct link between APs and human disease is hypo-phosphatasia (HPP), a disease characterized by mutations in TNAP associated with decreased enzyme activity in specific organs (10, 11) (**Figure 1B**). This decrease in AP activity results in variable symptoms that range from perinatal HPP that can result in still birth from profound skeletal hypomineralization (11, 12), potentially lethal seizures in infantile HPP (13–15), to milder phenotypes such as bone fractures and periodontal disease in juvenile HPP and adult HPP (16, 17). A relatively recent mouse model for HPP, in conjunction with medical data and genetic analysis has provided insight into the mechanism of HPP pathophysiology regarding at least two TNAP substrates, extracellular pyrophosphate (PPi), and pyridoxal-5-phosphate (PLP) (7).
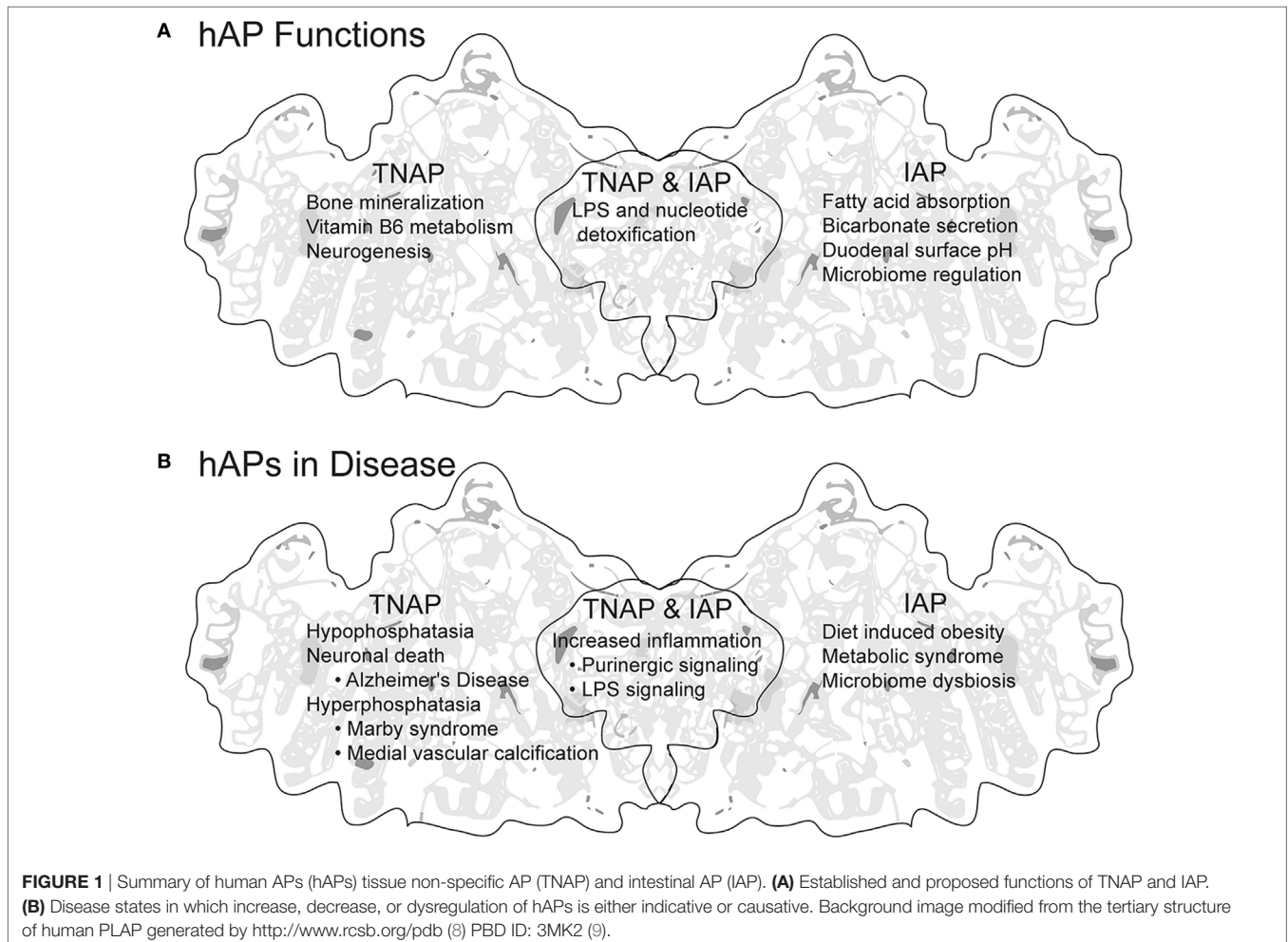
HYPOPHOSPHATASIA

Tissue non-specific AP is anchored to the cell membranes of osteoblasts and chondrocytes and to matrix vesicles released by those cells, where it degrades PPi to Pi. PPi is an inhibitor of mineralization (18) and regulation by TNAP controls propagation of extracellular mineralization of apatite crystals. TNAP deficiency increases the amount of inhibitory PPi thus decreasing extracellular mineralization, and humans with HPP show a loss of mineralization fronts (19). This has been recapitulated in a TNAP knockout mouse model for infantile HPP (20–22). The loss of mineralization results in various symptoms including softening of bone, bowing and spontaneous breakage of bones, rickets, and tooth (dentin/cementum/enamel) defects (23).

TABLE 1 | Description of human alkaline phosphatases (APs).[a]

AP gene	AP protein	Tissue distribution	Known function
ALPL	Tissue non-specific AP	Liver, kidney, skeletal tissue, nervous system	Bone and tooth deposition
ALPP	PLAP[b]	Syncytiotrophoblasts, reproductive tumors	Unknown
ALPPL2	GCAP[b]	Testis, reproductive tumors	Unknown
ALPI	IAP[b]	Intestine, enterocyte	Fatty acid absorption, lipopolysaccharide detoxification

[a]Information from Ref. (2, 7).
[b]TSAPs.

FIGURE 1 | Summary of human APs (hAPs) tissue non-specific AP (TNAP) and intestinal AP (IAP). **(A)** Established and proposed functions of TNAP and IAP. **(B)** Disease states in which increase, decrease, or dysregulation of hAPs is either indicative or causative. Background image modified from the tertiary structure of human PLAP generated by http://www.rcsb.org/pdb (8) PBD ID: 3MK2 (9).

Pyridoxal-5-phosphate, the active form of vitamin B6 (24), is elevated in the serum of HPP patients (25, 26). Hydrolysis of PLP to pyridoxal (PL) by TNAP facilitates diffusion of PL across cell membranes, where it is then re-phosphorylated into PLP. PLP is a versatile cofactor for an estimated 4% of enzymatic reactions and is used by over 110 enzymes to produce or metabolize various molecules (27). PLP-dependent enzymes in the brain are responsible for the production of important neurochemicals including serotonin, dopamine, and gamma-aminobutyric acid (28). The decrease in PLP and resulting decrease in PLP-dependent metabolism in the brain in perinatal HPP patients has been implicated as the cause of neonatal seizures (29, 30).

NON-HPP TNAP PATHOPHYSIOLOGY

Tissue non-specific AP has been implicated in non-HPP related medical conditions (**Figure 1B**). TNAP is expressed during embryonic neural and spinal chord development, and promotes axonal growth *in vitro* and neurogenesis in adults (31), suggesting an importance in proper neural function. Indeed, increased TNAP activity in the brain has been demonstrated in postmortem hippocampus and serum samples from Alzheimer's disease patients and has been implicated in neuronal death through increased dephosphorylation of tau (32). Increased serum levels of AP (TNAP and/or TSAPs) due to mutations in GPI anchor synthesis, termed hyperphosphatasia, results most notably in Marby syndrome characterized by seizures, intellectual disability, and facial dysmorphology (33). TNAP upregulation in the vasculature contributes to medial vascular calcification causing vascular stiffening and eventually heart failure (34, 35). An emerging function for TNAP is regulation of purinergic signaling. Extracellular ATP and ADP, through the binding of nucleotide receptors, act as signals inducing inflammation after an acute event such as necrosis induced by damage or infection that releases intracellular nucleotides. In contrast, degradation of extracellular ATP and ADP to AMP and adenine causes cessation of inflammatory signaling, and induction through adenine receptors of an anti-inflammation response (36, 37). TNAP has been implicated in protection against inflammation in multiple diseases and promotion of intestinal microbial populations through hydrolysis of extracellular ATP/ADP to AMP and adenosine (38–40).

INTESTINAL AP

Intestinal AP is expressed in villus-associated enterocytes where it regulates fatty acid absorption through secretion of vesicles at both the luminal and basolateral surfaces (41, 42), regulates bicarbonate secretion and duodenal surface pH (43), and has been implicated in the regulation of diet-induced obesity (44, 45) and metabolic syndrome (46, 47) (**Figure 1A**). But perhaps, the most remarkable function of IAP centers on its protective interactions with the bacterial symbionts that inhabit or invade our enteric system. IAP has been shown to dephosphorylate (detoxify) the lipid A moiety of lipopolysaccharide (LPS), the outer lipid layer of the outer membrane of Gram-negative

bacteria (48). In vertebrates, these phosphates are important for binding of LPS to the toll-like receptor 4/MD-2 innate immune receptor complex (49), initiation of NF-kB signaling, and immune response induction (50–52).

Intestinal AP deficiency has been associated with inflammation in the human intestine (53) and in the intestines of vertebrate models in which AP levels are decreased (54). Supplementation of IAP to animals where intestinal inflammation is induced directly or indirectly (with antibiotic use for example) reduces inflammation (53, 55, 56). In addition, a protective role has been ascribed to IAP in mouse models of necrotizing enterocolitis (57–59). This protective role may include IAP-dependent shaping (60) and homeostasis (61) of the microbiome. Along with direct regulation of intestinal homeostasis, IAPs and LPS detoxification have been implicated in other immune-related processes including prevention of bacterial translocation by endogenous or pharmacologically administered IAPs (62–64), and resolution of intestinal inflammation and tissue regeneration (65–67). It should also be noted that in addition to vertebrate IAP, TNAP has been shown to dephosphorylate LPS when it is applied to tissue sections from rat livers (68) and in the mouse uterus (69). With the current and increasing interest in the microbiome, IAP function as it relates to interaction with the endogenous microbes and its influence on human health will undoubtedly be clarified in the coming years.

CLINICAL USE OF APs

Although there are a multitude of AP studies focusing on vertebrate models of disease, there are relatively few publications to date reporting pharmacological use of APs as a treatment in humans. At the time this article was written, a search of http://clinicaltrials.gov using AP as a search term produced over several hundred responses, however, the vast majority assay for AP levels in serum (a constant hazard when searching any science or medical database using "alkaline phosphatase" as a search term). However, there were at least 11 clinical trials concerning AP treatment of HPP, 3 concerning AP treatment of sepsis with renal injury or failure, 2 concerning AP treatment during or after cardiac surgery, and at least 1 each concerning AP treatment of rheumatoid arthritis, and ulcerative colitis (UC). Interestingly, these studies use several AP sources such as isolated bovine IAP (bIAP), recombinant bIAP, and recombinant human Aps (hAPs). AP enzyme replacement therapy is also currently available to treat HPP. A recombinant soluble human TNAP has been approved for use in perinatal, infantile, and juvenile-onset HPP (70, 71) and has proven successful in symptom improvement and survival in perinatal and infantile HPP (72, 73). In addition to HPP, use of AP as treatment increased renal function in sepsis-induced acute kidney injury (74, 75) and showed short-term improvement of severity of UC in patients with moderate-to-severe UC (76). These studies are a first glimpse into AP use as a treatment for disease, with very positive results. Given the jack of all trades nature of APs and the potential for APs as pharmacological agents in various diseases, studies like these should increase in the coming years.

PERSPECTIVE

The ability of APs to detoxify LPS appears to be an evolutionarily conserved function as it was recently implicated in symbiont recognition and homeostasis in the invertebrate squid-*Vibrio* symbiosis model (77). As it is becoming clear that metazoans developed in a microbial world (78), it seems likely that APs have been and may continue to be an evolutionary force shaping the diversity and function of our endogenous microbial populations. Indeed, alterations in IAP have been shown to influence the composition of the intestinal microbiome (60). We can even expand this thinking—if hAPs evolved from an ancient ancestral bacterial AP, then APs may have had a prominent role in shaping basic human biochemistry in addition to our interactions with microbes, and thus exerted a profound influence on human health.

The reader of this review will notice that many of the articles cited might be considered old, with contributions from the 1960s, 1970s, and 1980s. In fact, the study of APs goes back close to 100 years when a bone enzyme freeing phosphate was first mentioned by Robison and Soames (79). That begs the question: how is it, after 90+ years, we still know relatively little about the overall functions of APs? The recent resurgence of interest in APs, should it continue, will hopefully provide more insight into all aspects of AP biology, especially as it relates to health. The ubiquity and functions of AP distinguish them as unconventional immune proteins, and to this writer, APs are unendingly fascinating.

AUTHOR CONTRIBUTIONS

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