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**Interplay between intestinal alkaline phosphatase, diet, gut microbes and immunity**

DeCoffe D *et al*. Intestinal alkaline phosphatase role in the gut

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**Abstract**

Intestinal alkaline phosphatase (IAP) plays an essential role in intestinal homeostasis and health through interactions with the resident microbiota, diet and gut. IAP’s role in the intestine is to dephosphorylate toxic microbial ligands such as lipopolysaccharides, unmethylated cytosine-guanosine dinucleotides and flagellin as well as extracellular nucleotide such as uridine diphosphate. IAP’s ability to detoxify these ligands is essential in protecting the host from sepsis during acute inflammation and chronic inflammatory conditions such as inflammatory bowel disease. Also important in these complications is IAP’s ability to regulate the microbial ecosystem by forming a complex relationship between microbiota, diet and the intestinal mucosal surface. Evidence reveals that diet alters IAP expression and activity and this in turn can influence the gut microbiota and homeostasis. IAP’s ability to maintain a healthy gastrointestinal tract has accelerated research on its potential use as a therapeutic agent against a multitude of diseases. Exogenous IAP has been shown to have beneficial effects when administered during ulcerative colitis, coronary bypass surgery and sepsis. There are currently a handful of human clinical trials underway investigating the effects of exogenous IAP during sepsis, rheumatoid arthritis and heart surgery. In light of these findings IAP has been marked as a novel agent to help treat a variety of other inflammatory and infectious diseases. The purpose of this review is to highlight the essential characteristics of IAP in protection and maintenance of intestinal homeostasis while addressing the intricate interplay between IAP, diet, microbiota and the intestinal epithelium.

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**Key Words:** Intestinal alkaline phosphatase; Inflammation; Infection; Lipopolysaccharides; Inflammatory disease; Intestinal microbiota; Diet

**Core tip:** Intestinal alkaline phosphatase (IAP) is important for intestinal health. IAP’s role in the intestine encompasses both protection from systemic infections and chronic inflammatory diseases such as inflammatory bowel disease. There is a complex interplay occurring between IAP, diet, micobiota and the intestinal epithelium which has accelerated research on IAP as a potential therapeutic against these inflictions. The purpose of this review is to highlight the essential characteristics of IAP in maintain homeostasis in the intestine while addressing the complex interplay between IAP, diet, microbiota and the intestinal epithelium.

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**INTESTINAL ALKALINE PHOSPHATASE**

Alkaline phosphatases (AP) are hydrolase enzymes that catalyze the breakdown of monophosphate esters by removal of their phosphate groups. AP are grouped into two classes: the tissue non-specific alkaline phosphatases (TNAP), which are expressed in bone; liver; and kidney[[1](#_ENREF_1)] and tissue specific isozymes which include placental alkaline phosphatase (PLAP), germ cell alkaline phosphatase; and intestinal alkaline phosphatase (IAP).

 IAP is a homodimer with each subunit comprised of two Zn++ and one Mg++ ions[[2](#_ENREF_2)], which are essential in maintaining the structural integrity and catalytic activity of the enzyme. IAP functions predominantly in alkaline conditions with optimal activity occurring at a pH of 9.7[[3](#_ENREF_3)]. IAP is ubiquitously expressed by enterocytes in the duodenum[[1](#_ENREF_1)] and thereafter to a much lesser extend in the jejunum, ileum and colon[[4](#_ENREF_4),[5](#_ENREF_5)] and is largely absent in the stomach[[5](#_ENREF_5),[6](#_ENREF_6)]. IAP molecules exist in high concentrations within lumenal vesicles secreted by enterocytes on the brush border of the microvilli (Figure 1). Though largely membrane bound, small levels of IAP are also released bidirectionally into the blood as well as the lumen[[7](#_ENREF_7)] where they may travel throughout the intestinal tract. Interestingly, IAP levels in humans differ across blood types with types O and B showing the highest levels of IAP while type A shows the lowest[[8](#_ENREF_8)]. Preliminary work from our lab has shown that IAP is expressed within immune cells in the colonic lamina propria[[9](#_ENREF_9)]. Further investigation are ongoing identify the type of immune cells and the physiological relevance of this finding.

IAP is the most evolutionarily conserved of the APs sharing 89.5% of its amino acid sequences with PLAP[[10](#_ENREF_10)]; in humans these genes are located on chromosome 2[[11](#_ENREF_11)]. In contrast, TNAP types share only 50% of their amino acids with IAP[[10](#_ENREF_10)] and are located on chromosome 4[[11](#_ENREF_11)]. This indicates an evolutionary split between tissue specific and the tissue non-specific isotypes. The IAP enzyme in humans is encoded by the *ALPI* gene[[12](#_ENREF_12)] while two isozymes have been identified in mice: duodenal IAP (dIAP) and global IAP (gIAP). Mouse dIAP and gIAP share 79% genetic homology[[13](#_ENREF_13)] and are encoded by the A*kp*3 and A*kp*6 genes, respectively[[14](#_ENREF_14),[15](#_ENREF_15)]. There are also two known IAP isozymes in rats: IAP І and IAP ІІ encoded by the Alpi1 and Alpi2 genes, respectively. Phylogenetic comparisons show that mouse gIAP is similar to both mouse dIAP and rat IAP І but not to human IAP. In general, most murine studies focus on IAP as a whole and as a result the specific function of each isozyme is poorly understood. In this review the term IAP will be used to represent both isozymes in reference to murine models.

**IAP, LPS AND INFECTION**

Lipopolysaccharide (LPS) is a major component of the cell wall of Gram-negative bacteria. Its aberrant presence in the blood (endotoxemia) can stimulate strong inflammatory responses as seen during infection-induced sepsis but at lower levels can also stimulate low levels of chronic inflammatory responses like those seen during obesity, diabetes and heart disease. Circulating LPS binds to toll-like receptor 4 (TLR 4) on immunocytes activating the secretion of pro-inflammatory cytokines via the master regulatory nuclear factor-kappaB (NF-κB) pathway[[16](#_ENREF_16)]. During infection, if the pathogen can overwhelm the host defenses, increasing cytokine levels can ultimately lead to septic shock and death. IAP plays a crucial role in preventing sepsis against enteric infection by dephosphorylating the endotoxin harboring lipid A moiety of LPS[[17](#_ENREF_17)]. IAP is a mucosal defense factor that limits bacterial translocation across the mucosal barrier into the mesenteric lymph nodes[[16](#_ENREF_16),[18](#_ENREF_18)]. It has been shown that administration of bovine IAP prevents translocation of bacteria in mice infected with *Salmonella enterica* serovarTyphimurium[[19](#_ENREF_19)]. We recently demonstrated IAP’s role in survival during enteric infection in a model of high-fat feeding. We compared various high-fat diets (HFD) and found that while ω-6 polyunsaturated fatty acid (PUFA) exacerbated damage associated with colitis, increased IAP expression and activity protected the mice against sepsis during *Citrobacter rodentium* infection. In contrast, isocaloric and isonitrogenous diets supplemented with ω-3 PUFA led to impaired IAP activity resulting in increased sepsis-associated mortality during infection[[9](#_ENREF_9)]. Beyond IAP’s local activity at the gut mucosa, about 1%-2% of IAP is released into the bloodstream or the gastrointestinal lumen, which broadens the range of action against both local and systemic infections[[19](#_ENREF_19)]. IAP is present at high levels during infection and as a result can be found in its active form in the stool[[16](#_ENREF_16)], suggesting its potential clinical use as a marker of infection.

**IAP’S ROLE IN REGULATING INFLAMMATION**

In addition to LPS, IAP can also dephosphorylate other pro-inflammatory ligands such as uridine diphosphate (UDP) nucleotides released from damaged cells[[20](#_ENREF_20),[21](#_ENREF_21)], unmethylated cytosine-guanosine dinucleotides (CpG), a component of bacterial DNA[[22](#_ENREF_22)] and flagellin, a protein in bacterial flagellum[[23](#_ENREF_23)]. These ligands or microbe-associated molecular patterns (MAMPs) activate TLR 2, 3, 5 and 9 on immune and epithelial cells[[24](#_ENREF_24)] inducing downstream inflammatory responses. To mitigate these responses, IAP expression increases through activation of the inflammatory mediator Resolvin E1 (RvE1). RvE1 is a lipid mediator derived from eicosapentaenoic acid, a long chain ω-3 PUFA[[25](#_ENREF_25)]. RvE1 acts as an agonist of the G protein-coupled receptor Chemerin receptor 23 (Chem R23) found on neutrophils, macrophages and dendritic cells; which in turn can attenuate immune cell transmigration. Importantly, Chem R23 receptors are also present on intestinal epithelial cells, where their activation increases epithelial IAP expression and activity. This was demonstrated in a chemically induced model of mouse colitis, where administration of an IAP inhibitor increased disease severity while simultaneously depleting RvE1 function. Oral intervention with RvE1 during colitis in these mice restored IAP levels and reduced disease severity, indicating that RvE1 is important in IAP activation and its subsequent anti-inflammatory actions[[26](#_ENREF_26)]. However, as shown by Ghosh *et al*[[27](#_ENREF_27)] the consumption of an ω-6 PUFA rich diet supplemented with ω-3 PUFA before *Citrobacter rodentium-*induced infection resulted in impaired IAP expression and function. In contrast the consumption of a high ω-6 PUFA rich diet alone had the opposite effect. This suggests that the impairment of IAP may be a result of altered ratio of ω-6 to ω-3 PUFA and not simply ω-3 PUFA itself. This is important because a high ratio of ω-6 PUFA: ω-3 PUFA has been implicated in pro-inflammatory disease. Taken together these results suggest that IAP is important in regulating inflammation.

Inflammation itself however is also capable of regulating *IAP* gene expression and activity, though the mechanisms at play are not well understood. Malo *et al*[[28](#_ENREF_28)] showed that two inflammatory bowel disease (IBD) associated pro-inflammatory cytokines interleukin (IL)-1β and tumor necrosis factor (TNF)-α are involved in reducing *IAP* gene expression and their subsequent altering of the function and health of the intestinal epithelium. More research is required to define how inflammatory markers regulate IAP expression and activity.

**IAP AND CHRONIC INFLAMMATORY DISEASES**

Irregular IAP expression has been implicated in many chronic inflammatory states such as IBD, celiac disease and obesity. For example, IBD patients have reduced *IAP* gene expression with a 2.8 fold decrease in inflamed tissues, likely as a result of local epithelial cells damage. Oral administration of IAP during murine colitis significantly reduced colonic inflammation[[5](#_ENREF_5)] as demonstrated by decreases in mRNA levels of key pro-inflammatory cytokine TNF-α and nitric oxide producing inducible nitric oxide synthase (iNOS). It has been reported that patients with ulcerative colitis can have significantly reduced colonic pH levels[[29](#_ENREF_29)]. Despite these acidic conditions IAP remains functional though its mechanism is not currently understood. One report using chicken IAP showed that the enzyme is inactivated at pH 6; however the addition of Zn ions resulted in reactivation of IAP even at pH 4.5. This study indicates that under certain conditions IAP can function at even low pH levels[[30](#_ENREF_30)]. Another factor important in IBD is increased levels of UDP which has been directly associated with intestinal inflammation[[20](#_ENREF_20)]. These extracellular nucleotides are released during cellular damage and death[[21](#_ENREF_21)] such as seen during active IBD, and bind to receptors on macrophages, epithelial cells and infiltrating T-cells. By dephosphorylating UDP, IAP blocks the subsequent inflammatory responses induced by this nucleotide[[20](#_ENREF_20)].

Gut homeostasis is vital for maintaining a balanced metabolism, not surprisingly then endogenous IAP has been recently implicated in metabolic syndromes[[31](#_ENREF_31)]. In an elegant series of studies by Kaliannan *et al*[[31](#_ENREF_31)], it was shown that IAP knock-out (KO) mice display features of metabolic syndrome such as: obesity, elevated blood glucose, endotoxemia, glucose intolerance and hyperinsulinemia. Supplementation with oral IAP was able to prevent and reverse these conditions in both the KO mice as well as in models of HFD-induced metabolic syndrome.

**IAP, MICROBIOTA AND INTESTINAL HOMEOSTASIS**

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It has been suggested that IAP can directly regulate the ecology of the gut microbiota. IAP-KO mice have been shown to display an altered ecosystem with reduced total fecal bacteria compared to their wild type (WT) counterparts[[32](#_ENREF_32)]. WT mice have higher levels of Lactobacillaceae group and *Escherichia coli* expression compared to KO mice. The absence of IAP prevented *E. coli* from colonizing the gut. This is important because the loss of commensal bacteria such as *E. coli* can create a vulnerable gut environment allowing colonization of potential pathobionts[[33](#_ENREF_33)]. Interestingly, oral administration of IAP resulted in increased colonization of *E.coli* in the IAP-KO mice supporting the idea that IAP is an important regulator of the commensal microbiota[[32](#_ENREF_32)]. Oral administration of IAP in mice showed a reduction in severity rates of *Salmonella enterica* serovar Typhimurium and *Clostridium difficile*, infections[[34](#_ENREF_34)]. These changes were associated with IAP’s ability to rapidly restore the commensal gut microbiota[[34](#_ENREF_34)] though the mechanism behind this remains to be elucidated. One plausible explanation provided by Alam *et al*[[34](#_ENREF_34)] is through IAP’s capacity to control pH, which in turn alters bacterial growth. IAP achieves this by hydrolyzing luminal phosphates such as adenosine triphosphate (ATP). This results in increased levels of bicarbonate ion secretion which is important for maintaining homeostasis and gut barrier function[[6](#_ENREF_6)]. It should be noted however, that *Salmonella enterica* and *Clostridium difficile* colonization in the gut in these models required prior antibiotic treatments in order to deplete the microbiota, potentially obscuring IAP’s true role in reducing inflammatory responses[[35](#_ENREF_35)]. Bates *et al*[[35](#_ENREF_35)] showed that IAP reduced inappropriate inflammatory responses in germ-free zebrafish larvae compared to control larvae incubated with a stereospecific IAP inhibitor, L-phenylalanine. IAP rich larvae had decreased neutrophils in their gut epithelium compared to the IAP inhibited larvae which were able to recruit neutrophils in response to LPS stimulation. IAP’s exact mechanism for regulating the microbiota is not well known, however the current research suggests that there is a complex connection between these two intestinal factors.

**INTERPLAY BETWEEN FOOD AND IAP**

Diet has been reported as an important factor in many pathological conditions such as IBD, metabolic syndrome, diabetes and infection. IAP is regulated by dietary macronutrients as well as fasting. Mice fasted for two days showed significant decreases in IAP expression and as a result decreased LPS-dephosphorylating activity compared to non-fasted mice[[16](#_ENREF_16)]. This is important in the context of trophic enteral feeding because as suggested by Goldberg *et al*[[16](#_ENREF_16)] starvation in critically ill patients may lead to IAP downregulation and therefore an increased susceptibility to pathogenic infection. The re-introduction of food in these mice resulted in the recovery of IAP expression, suggesting an adaptive response of IAP to food availability. IAP is important during HF feeding as it is involved in the rate-limiting step of fatty acids transport across the plasma membrane into the enterocytes[[15](#_ENREF_15),[36](#_ENREF_36)]. IAP secretion increases in response to HFD[[13](#_ENREF_13)], likely in a negative feedback manner to negate the resulting increases in intestinal LPS levels[[31](#_ENREF_31),[37](#_ENREF_37)]. Another study showed that gIAP, but not dIAP, protein expression significantly increased in response to an HFD with 45% fat content (77% from saturated fatty acids (SFA)[[14](#_ENREF_14)]. HFD increases the uptake of fatty acids by enterocytes through a gIAP-dependent mechanism. In contrast, another study using an HFD consisting of 36.3% SFA caused reduced IAP activity and increased TLR 4 activation in diet-induced obese prone rats[[37](#_ENREF_37)]. Therefore, increased consumption of HFD induces an inflammatory response and results in downregulation of IAP expression. The differences between these two studies suggest that the amount of fat in a diet can discordantly influence IAP regulation. Further studies are required to investigate the specific effects of SFA on IAP. Work from our lab has shown that mice fed diets high in ω-6 PUFA (20% w/w) have increased IAP expression within the lamina propria. In attempt to mitigate colitic responses, ω-3 PUFA was supplemented in these HFD which resulted in decreased IAP expression and activity[[9](#_ENREF_9)]. However, these mice unexpectedly suffered from higher incidences of sepsis-associated mortality. Taken together, these findings suggests that in addition to the amount of fat, the consumption of particular dietary fatty acids can also alter IAP expression and activity, a vital consequence to the host during infection. The effects of HFD on IAP expression may also be potentially linked to one particular IAP isozyme. dIAP knock out (A*kp*3-/-) mice showed increased weight gain HFD diet groups compared to WT[[15](#_ENREF_15)]. These phenotypic responses however, may be compensatory actions of intact gIAP (A*kp*6) in these mice. Therefore, A*kp*6-/- experiments are needed to provide more insight, as each isozyme may function differently during inflammation and infection.

Protein consumption may also play an important role in regulating IAP. Rats fed protein-free diets showed a 36%-38% reduction in IAP activity[[38](#_ENREF_38)]. In this experiment however, the rat diets were high in starch and therefore IAP levels may potentially be altered due to high levels of carbohydrates rather than from the lack of protein. These results taken together suggest that there is a complex interplay between diet and IAP activity, and that customized diets may be beneficial in regulating IAP levels.

**IAP AS A THERAPEUTIC AGENT**

There is growing evidence that IAP administration during infection and inflammation may be a novel therapeutic strategy in reducing disease complications. IAP supplementation may also be an effective tool in battling the rising problems of antibiotic related infections such as *Clostridium difficile* associated diseases (CDAD). In support of this, oral administration of IAP concurrently with antibiotics in mice was shown to completely prevent CDAD as well as other enteric pathogens like *Salmonella enterica* serovar Typhimurium[[34](#_ENREF_34)]. This is critical because administering antibiotics can induce a microbial imbalance in the gut, known as dysbiosis. This creates a favorable environment for pathogens and increases host susceptibility to infection.

Another therapeutic application of IAP for decreasing pro-inflammatory responses in neonates suffering from necrotizing enterocolitis (NEC)[[39](#_ENREF_39)]. Administration of IAP in rats reduced serum levels of pro-inflammatory cytokines TNF-α, IL-5 and IL-1β during NEC. These levels resemble that of the healthy controls indicating that exogenous IAP has the ability to downregulate the immune response and normalize inflammation in these infants, however this did not decrease the overall incidence of NEC. Tuin *et al*[[5](#_ENREF_5)] showed that supplementation with IAP during rat colitis also significantly reduced pro-inflammatory markers TNF-α, IL-1β and iNOS. As a result there was significant improvement in colonic morphology, though no improvement in clinical symptoms such as: weight loss, diarrhea or rectal bleeding. IAP was also shown to be beneficial in mice with cystic fibrosis (CF); a known chronic pulmonary disease associated with intestinal bacterial overgrowth[[40](#_ENREF_40)]. Exogenous IAP restored intestinal permeability and reduced 80% of intestinal bacteria overgrowth in mice with CF[[41](#_ENREF_41)]. Most clinical trials to date report administration of IAP through intravenous injections as to avoid major degradation and digestion in the stomach and upper intestinal tract. A method that has not been reported in humans is rectal enemas as this allows direct IAP delivery to the large intestine. Also to date, exogenous IAP in the form of enteric coated or delayed-release capsules has not been reported. IAP appears to have extensive therapeutic potential and has relatively safe pharmacokinetic properties, as a result several clinical human trials have begun. There are currently four completed IAP human trials[[42-45](#_ENREF_42)] and two ongoing studies which includes research on acute rheumatoid arthritis and safety and efficacy of IAP use during heart surgery (Table 1).

**CONCLUSION**

IAP’s role in catalyzing the breakdown of monophosphate esters is well established, however, only within the last decade has the role of IAP as a key regulator of inflammation, infection and gut microbiota has been thoroughly explored. IAP also plays a key role in protecting the host during chronic inflammatory diseases. IAP expression and activity is altered as a result of its complex interplay between dietary factors, the microbiota and the host. In light of these findings IAP has been marked as a potential therapeutic agent to help treat a variety of inflammatory and infectious diseases.

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**Figure 1 Intestinal alkaline phosphatase regulates gut homeostasis**. Intestinal alkaline phosphatase (IAP) secreted by the enterocytes plays a vital role in various physiological functions in and around the intestine. Though mainly membrane-bound, IAP can be found both in the lumen and blood. High concentration of IAP molecules are present in protein-rich lumenal vesicles on the lumenal and apical side of the epithelium. IAP dephosphorylates both lumenal and circulating lipopolysaccharides (LPS) derived from the cell wall of gram negative bacteria effectively eliminating their toxic constituent. Preliminary work from our lab and others have shown IAP expressed on infiltrated immunocytes in the lamina propria. IAP is also crucial in preventing the transmigration of bacteria across the epithelium layer, preventing downstream activation of immunocytes and the subsequent inflammatory responses. Through its complex relationship with food, commensal bacteria and immune cells, IAP plays an essential role in gut homeostasis.

**Table 1 Summary of completed and ongoing clinical human trials using exogenous bovine intestinal alkaline phosphatase**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Area of study** | **Study design** | **Route of administration** | **Study phase** | **Treatment effect** | **Sample size/ Estimated enrollment**2 | **Reference/Trial number**2 |
| **Completed trials** |
| Coronary artery bypass  | Randomized, placebo | Intravenous  | 2 | Increased endogenous alkaline phosphatase release. | 32 | Kats *et al* (2012)[[42](#_ENREF_42)] |
| Severe sepsis and septic shock on acute kidney injury  | Double-blind, randomized and placebo | Intravenous | 2 | Improved renal function. | 36 | [Heemskerk](http://www.ncbi.nlm.nih.gov/pubmed?term=Heemskerk%20S%5BAuthor%5D&cauthor=true&cauthor_uid=19114895) *et al* (2009)[[44](#_ENREF_44)] |
| Moderate to severe ulcerative colitis  | Uncontrolled | Oral | 2 | Short term improvement of moderate/severe ulcerative colitis  | 20 | Lukas *et al* (2010)[[45](#_ENREF_45)] |
| Sepsis in patients with acute kidney injury (AKI) | Double-blind, randomized and placebo | Intravenous  | 2 | Renal protection from sepsis in patients with AKI  | 36 | Pickers *et al* (2012)[[43](#_ENREF_43)] |
| **Ongoing trials** |
| Acute rheumatoid arthritis  | Non-randomized | Subcutaneous  | 1 and 2 | Ongoing study | 10 | NCT014164931 |
| Safety and efficacy during heart surgery | Randomized double-blind, placebo-controlled | Intravenous | 3 | Ongoing study | 228 | NCT011446111 |

1The trial numbers associated with ongoing clinical studies was obtained from clinicaltrials.gov; 2Refers to ongoing trials.