Intestinal alkaline phosphatase: novel functions and protective effects

Jean-Paul Lallès

Important protective roles of intestinal alkaline phosphatase (IAP) – including regulation of intestinal surface pH, absorption of lipids, detoxification of free nucleotides and bacterial lipopolysaccharide, attenuation of intestinal inflammation, and possible modulation of the gut microbiota – have been reviewed recently. IAP is modulated by numerous nutritional factors. The present review highlights new findings on the properties of IAP and extends the list of its protective functions. Critical assessment of data suggests that some IAP properties are a direct result of dephosphorylation of proinflammatory moieties, while others (e.g., gut barrier protection and microbiota shaping) may be secondary to IAP-mediated downregulation of inflammation. IAP and tissue-nonspecific alkaline phosphatase isoforms characterize the small intestine and the colon, respectively. Gastrointestinal administration of exogenous IAP ameliorates gut inflammation and favors gut tissue regeneration, whereas enteral and systemic IAP administration attenuates systemic inflammation only. Finally, the IAP gene family has a strong evolutionary link to food-driven changes in gastrointestinal tract anatomy and microbiota composition. Therefore, stimulation of IAP activity by dietary intervention is a goal for preserving gut homeostasis and health by minimizing low-grade inflammation.

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INTRODUCTION

Obesity and metabolic disorders, including insulin resistance, type-2 diabetes, hypertension, and cardiovascular disease, are becoming more prevalent worldwide due to lifestyle changes associated with overconsumption of Western diets. Bacterial lipopolysaccharide (LPS) has been proposed as a causal link in diet-induced obesity (DIO) because of its increased passage across the intestinal barrier following high-fat diet intake, its growth-promoting effects on adipose tissue, and its proinflammatory properties. More recently, a survey pointed to the possible involvement of intestinal alkaline phosphatase (IAP) in the development of obesity, and very recent experimental evidence demonstrates the ability of IAP to prevent fat-induced metabolic syndrome in mice. IAP has been recently shown to display anti-inflammatory properties that derive from its dephosphorylating activity, which results in detoxification of LPS and repression of the downstream Toll-like receptor (TLR)-4-dependent and MyD88-dependent inflammatory cascade. Given the large number of important reports on IAP function in recent decades, the first review on IAP and its nutritional modulation was published 3 years ago. Since then, many additional reports have emerged, justifying an update on this topic. Four major functions of IAP were described previously: 1) regulation of bicarbonate secretion and duodenal surface pH; 2) modulation of intestinal LCFA absorption; 3) detoxification of LPS, resulting in amelioration of intestinal and systemic inflammation; and 4) regulation of gut microbial communities and their translocation.

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across the gut barrier.\textsuperscript{6} Here, an update on these functions is provided, in addition to novel functions ascribed to IAP (Figure 1), and the entire literature is critically analyzed. All these data, including a few translational studies performed in humans, strongly suggest that IAP is a major regulatory enzyme of gut homeostasis and health. As diet is implicated as a causal determinant in metabolic syndrome and obesity,\textsuperscript{6} knowledge of IAP modulation by dietary factors is also updated.

\section*{NOVEL PROPERTIES OF IAP}

IAP is dynamically secreted apically and basolaterally by the enterocyte. IAP has been reported to modulate intestinal absorption of dietary LCFAs by phosphorylating-dephosphorylating the fatty acid (FA) transporter CD36. It also limits fat-induced inflammation, metabolic syndrome, and obesity in rodent models. The involvement of IAP in some of these properties, however, is probably secondary to the anti-inflammatory action of IAP.

\subsection*{Presence of IAP in apical lipid-rich cellular microdomains, and dynamic apical and basolateral secretion of IAP}

Lipid-rich microdomains, also called lipid rafts, are apical membrane structures of intestinal epithelial cells (IECs) that are rich in IAP and other functional proteins.\textsuperscript{7,8} IAP is selectively endocytosed at the apical side of enterocytes during intestinal fat absorption.\textsuperscript{9} Recent investigations suggest that luminal FAs are inserted specifically into these rafts,\textsuperscript{10} but the consequences of this insertion on raft IAP dynamics remains unclear. Importantly, IAP-rich lipid rafts have been recently linked to gut permeability and inflammation.\textsuperscript{11} A kinetic study of different mouse models of colitis revealed that lipid raft disruption precedes alteration in colonic permeability. Moreover, IAP distribution into raft-enriched membrane fractions tended to differ between ulcerative colitis patients in remission and controls.\textsuperscript{11} Therefore, additional studies to clarify the functional consequences of lipid-raft-associated IAP in inflammation are needed.

It was previously reported that IAP is secreted from the basolateral domain of IECs via so-called surfactant-like particles, e.g., during intestinal fat absorption.\textsuperscript{6} At the apical domain, however, IAP was thought to be released into the intestinal lumen via a mechanism involving the hydrolysis of its glycosyl-phosphatidyl-inositol linkage and lysophosphatidylcholine.\textsuperscript{12} Recently described new aspects of apical mechanisms of IAP release show that enterocyte microvilli secrete 90-nm-diameter luminal vesicles highly enriched in functional proteins, including IAP that preferentially locates in lipid rafts.\textsuperscript{13} These vesicles were shown to dephosphorylate LPS from various gram-negative bacteria; more importantly, they prevented the adhesion of both pathogens and commensal bacteria to IECs in vitro.\textsuperscript{14} Conversely, the presence of pathogens (e.g., enteropathogenic \textit{Escherichia coli}) stimulated apical secretion of microvillar vesicles.

Collectively, these data provide new insight into a unique defense mechanism: the secretion of IAP-rich vesicles from both luminal and basolateral domains of the enterocytes (Figure 2). Luminal vesicles influence gut concentrations of pathogen-associated microbial patterns (PAMPs), whereas basolateral-derived vesicles inactivate PAMPs as they translocate into the interior milieu. Finally, intracellular IAP blunts PAMP-stimulated nuclear factor
kappa B-mediated inflammatory responses. Whether IAP has other functions within these lipid rafts is presently unknown.

**Ability of IAP to limit LCFA-induced inflammation, metabolic syndrome, and obesity, and possible role of IAP in the control of intestinal absorption of LCFA**s

*Modulation of intestinal absorption of LCFA*s. A possible role of IAP in intestinal FA absorption was proposed when mice in which the *Akp3* gene coding for the IAP isoform was deleted were shown to absorb more fat and to gain more weight than wild-type controls.\(^{15,16}\) Although a link between IAP and FA transporter CD36 was suspected,\(^4\) the underlying mechanisms have, until recently, remained obscure. Global IAP (gIAP), the product of the *Akp6* gene expressed throughout the small intestine, would be responsible for the increased fat uptake in IAP-expressed knockout mice.\(^{17,18}\) Both gIAP (but not duodenal IAP, or dIAP) and CD36 were specifically increased in the jejunum of mice, and gIAP (but not dIAP) was shown to coprecipitate with CD36 when the fat content of the diet was increased from 15% to 45%. It was thus suggested that gIAP phosphorylates and dephosphorylates CD36, the latter being responsible for an enhanced capacity for fat absorption.\(^{17,18}\) However, this mechanism is likely to have only a marginal effect on fat absorption, since deletion of the CD36 gene in mice did not affect uptake of LCFA*s.\(^{19}\) An alternative explanation has been proposed recently, suggesting that increased FA absorption observed in *Akp3* knockout mice could result from lower luminal surface pH and high free ATP (adenosine triphosphate) concentrations.\(^{20}\) Thus, the direct role of IAP isoform (from *Akp3* gene) in the modulation of intestinal absorption of LCFA*s has been questioned.

*Control of fat-induced inflammation, metabolic syndrome, and obesity.* Consumption of high-fat diets is known to increase IAP, and this may be a mechanism of adaptation to the parallel increase in intestinal entry of LPS.\(^4\) Mice and rats prone to DIO were reported to display lower intestinal IAP activity after consuming a high-fat diet for a few weeks, while IAP activity remained unaltered in DIO-resistant animals.\(^{21,22}\) As this did not appear to be genetically driven, reduced IAP activity in DIO-prone rats was suggested to be a consequence of fat-induced inflammation.\(^{22}\) In other words, DIO may develop in individuals mounting higher inflammatory responses to fat, which in turn would inhibit IAP activity and gene expression, enhancing inflammation further. Inflammatory cytokines such as interleukin (IL) 1β and tumor necrosis factor-α (TNF-α) are known to downregulate IAP in IECs.\(^{23}\) Conversely, DIO-resistant rats are able to keep inflammation under control through mechanisms that limit IAP repression. The molecular basis for interindividual differences in the magnitude of DIO-induced innate immune responses and inflammation remains poorly understood.

*Intestinal resolution of inflammation.* Tissue regeneration following inflammation is regulated by a family of molecules called resolvins.*\(^{24}\) Resolvin E1, a metabolite of the omega-3 (n-3) FA eicosapentaenoic acid (EPA), interacts with the leukotriene B4 receptor on immune cells to downregulate inflammation.\(^{25}\) Resolvin E1 binds to the ChemR23 receptor that is highly expressed on neutrophils and oral epithelial cells.\(^{26}\) This receptor is also present in IECs at the apical membrane above tight junctions and in close vicinity to mucosal actin.\(^{27}\) Importantly, resolvin E1 stimulates IECs to express a mucosal protective factor that was shown to be an IAP isoform.\(^{27}\) Resolvin E1 also controlled chemically induced colitis in mice via the upregulation of colonic IAP.\(^{27}\) Therefore, resolution of inflammation at the gut level operates through two complementary protective mechanisms: LPS detoxification by IAP, and specific induction of cellular IAP expression by resolvin E1.

**Possible indirect participation of IAP in the control of intestinal barrier function**

Intestinal barrier function facilitates vectorial transport of nutrients, water, and ions while excluding potentially toxic substances. Barrier defects are implicated in many diseases of the gut and other organs.\(^{28}\) Indirect evidence suggests a protective role of IAP on gut barrier function by ameliorating inflammation.\(^{4,29,30}\) A direct role of IAP in the control of intestinal permeability has also been reported, e.g., in mice in which the *Akp3* gene coding for the IAP isoform in this animal species was deleted,\(^{15,16}\) but this conclusion has been questioned because the *Akp6* gene is upregulated in *Akp3* knockout mice.\(^{17,18}\)

Recently, a 15-fold increase in gut permeability to macromolecules was observed in rat pups with
necrotizing enterocolitis (NEC), confirming earlier findings of a barrier defect in this disease. Administration of a low dose of exogenous (bovine) IAP to pups with NEC resulted in complete restoration of gut barrier function. Exogenous IAP had no significant effects on tissue-relative concentrations of two major tight junction proteins (claudin-1 and claudin-3) that were increased by NEC. Unfortunately, data on cellular localization of these proteins were not reported, although protein localization is essential for barrier functionality.

A possible link between IAP and intestinal barrier function is also suggested in cystic fibrosis (CF). This disease afflicts Caucasians and involves a lack of functional CF transmembrane conductance regulator anion channel, with dramatic consequences on gut ion exchange physiology and barrier function. A recent report indicates that mice with CF possess lower IAP activity and lower Akp3 (but not Akp6) gene expression in the small intestine. As CF is also associated with bacterial overgrowth, antibiotic treatment of mice with CF was shown to restore both Akp3 gene mRNA levels and IAP activity. Oral administration of exogenous IAP restored barrier function and damped bacterial overgrowth. However, experiments utilizing the IAP-specific inhibitor L-phenylalanine failed to reduce gut permeability in wild-type or CF mice, suggesting an indirect involvement of IAP in the protective mechanism. Another possible explanation for CF-associated intestinal barrier alteration and reduced IAP could be the accumulation of free ATP in the intestinal lumen. Indeed, CF transmembrane conductance regulator anion channel is involved in the control of bicarbonate secretion and surface pH. Free ATP is a potent danger signal that induces strong inflammatory cytokine responses. These responses would in turn inhibit IAP activity. Finally, two pig lines divergently selected for residual feed intake (i.e., for higher body-weight-gain to feed-intake efficiency ratio) were shown to display different ideal IAP activities but similar gut permeability to the marker probe fluorescein dextran (molecular weight, 4,000 kDa) and to LPS.

Collectively, these data do not support a direct link between IAP activity and gut permeability. The beneficial effects of IAP on intestinal barrier function may reflect IAP-mediated inflammation downregulation.

**Colonic alkaline phosphatase: association with TNAP isoform, upregulation by oxidative stress, and usefulness as a marker of inflammation**

While many studies have focused on IAP in the small intestine, conflicting results about IAP activity in the colon have been reported, with both reduced and increased IAP activity observed during colonic inflammation. A consensual view has emerged, which indicates that IAP and tissue-nonspecific alkaline phosphatase (TNAP) isoforms are essentially expressed in the small intestine and the colon, respectively. In response to inflammation, colonic IAP is further depressed, while the TNAP isoform is upregulated. An increase in TNAP results from both colonic epithelial cells and neutrophils, the latter being known to express the TNAP isoform and to accumulate in the inflamed colon.

Importantly, the TNAP isoform is specifically upregulated by oxidative stressors, including monochloramine, hydrogen peroxide, saponin, and deoxycholate, but surprisingly, not by LPS in IECs. The major change seems to be a chemical switch in N-glycosylation of alkaline phosphatase (AP) isoforms, thus modulating AP enzyme activity. The IAP isoform is found in both the cell cytoplasm and the apical membrane, while TNAP is exclusively cytosolic. Thus, oxidative stress may (in rats) or may not (in mice) upregulate TNAP gene expression.

Collectively, these data indicate that the IAP isoform is downregulated during colonic inflammation, while the TNAP isoform is upregulated. Colonic upregulation of TNAP by inflammation may be a protective adaptation against oxidative stress and inflammation. Anti-inflammatory treatments, e.g., oral IAP administration, stimulate IAP expression and IAP-dependent resolvin-E1-mediated amelioration of inflammation while reducing oxidative stress and TNAP expression and activity.

**IAP AND GUT BACTERIA**

IAP plays an important role in the crosstalk between the gut microbiota and the host at the intestinal interface by detoxifying proinflammatory bacterial and endogenous components and by shaping the microbiota. This latter effect, however, could be direct or indirect, since IAP is anti-inflammatory and inflammation itself is known to impact the composition of the gut microbiota and the gut PAMP load.

**Detoxification of free nucleotides and various bacterial PAMPs by IAP**

Many nucleotides are proinflammatory, but adenosine is anti-inflammatory. Previous work indicated that IAP dephosphorylates ATP and other related adenosine nucleotides. Additionally, IAP was recently shown to dephosphorylate and, thus, detoxify, uridine diphosphate nucleotide. The IAP isoform detoxifies LPS through dephosphorylation of the lipid A moiety. Recent investigations showed that free IAP could also dephosphorylate two other PAMPs, flagellin and CpG DNA motifs, but not the Pam-3-Cys synthetic ligand in vitro (Table 1). These PAMPs stimulate inflammation through TLR-4, TLR-5,
**Role of IAP in shaping the gut microbiota**

Earlier work in zebrafish and mice introduced the notion that IAP participates in shaping the gut microbiota, and additional evidence on this role has now been provided. Free IAP dephosphorylated heat-killed bacteria of both gram-negative and gram-positive origins, but it did not seem to influence, by itself, the growth of cultured *Escherichia coli*, *Listeria monocytogenes*, or *Salmonella typhimurium* (Table 1). However, while IAP expressed in IECs delayed the growth of *E. coli*, it had no effect on *Clostridium difficile*, *S. typhimurium*, or *Enterococcus faecalis*. Importantly, when cellular production of IAP was promoted, IECs were able to downregulate IL-8 production induced by various gram-negative bacteria like *E. coli* or *S. typhimurium* but not that induced by gram-positive bacteria such as *E. faecalis*, *L. monocytogenes*, or *Staphylococcus aureus*. Mice in which the IAP *Akp3* gene product was deleted displayed a fecal microbiota that is very different from that in wild-type controls. IAP knockout mice had no detectable *E. coli* and reduced numbers of total aerobic and anaerobic bacteria. Conversely, they showed relative increases in the ratios of Clostridia and increases in lactobacilli and enterococci. Importantly, part of these effects may have reflected changes in inflammatory tone in *Akp3* knockout mice. Intestinal inflammation could not be detected by histology, but signs of chronic inflammation in the liver were reported in *Akp3* knockout mice. Among other possible reasons for these changes, intestinal surface pH might be proposed because bacterial growth is pH sensitive, and IAP controls surface pH via an ATP-dependent mechanism of bicarbonate secretion. Importantly, IAP expression on intestinal cells has also been shown to specifically inhibit the intestinal pathogen *S. typhimurium* in vivo. Finally, IAP-rich microvillar vesicles secreted into the lumen by IECs may limit bacterial growth, but not through a direct IAP-dependent mechanism.

**Prevention of bacterial translocation by IAP in the gut of mice**

IAP was shown earlier to limit intestinal bacterial translocation in mice, but little information was reported on the possible mechanisms. IAP-rich luminal vesicles secreted by enterocytes at the tips of microvilli were recently reported to prevent adhesion of both pathogenic and commensal bacteria to IECs in vitro, but this was apparently not related to vesicle-bound IAP. Additional data indicated that intrarectal administration of bovine IAP could prevent bacterial translocation in different models of colitis in mice. This may be indirectly linked to IAP-dependent downregulation of inflammation. Finally, increased bacterial translocation following *S. typhimurium* challenge was observed in IAP knockout mice and was not lethal, in contrast to results in wild-type mice. These data were interpreted as evidence of tolerance to LPS in IAP *Akp3*–deficient mice.

Collectively, these data support a role for cellular-induced IAP in regulating *E. coli* numbers and in inhibiting some gram-negative pathogens in mice. Conversely, the role of free IAP in influencing gut microbiota

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**Table 1**: Ability of bovine calf intestinal alkaline phosphatase to dephosphorylate bacterial pathogen-associated microbial patterns (PAMPs) and live or heat-killed bacteria in vitro and to downregulate interleukin-8 (IL-8) production by HT-29 cells.

<table>
<thead>
<tr>
<th>PAMP or bacteria</th>
<th>Toll-like receptor</th>
<th>Phosphate release in vitro</th>
<th>Reduction of IL-8 production (HT-29)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial PAMPs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pam-3-Cys</td>
<td>TLR-2</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LPS</td>
<td>TLR-4</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Flagellin</td>
<td>TLR-5</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>CpG DNA</td>
<td>TLR-9</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Gram-negative bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Various</td>
<td>Live</td>
<td>Dead</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>Various</td>
<td>Live</td>
<td>Dead</td>
</tr>
<tr>
<td><strong>Gram-positive bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Various</td>
<td>Live</td>
<td>Dead</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Various</td>
<td>Live</td>
<td>Dead</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>Various</td>
<td>Live</td>
<td>Dead</td>
</tr>
</tbody>
</table>

*Abbreviations: CPG DNA, cytosine nucleotide-phosphate-guanine nucleotide of bacterial DNA; LPS, lipopolysaccharide; NR, not reported; Pam-3-Cys, synthetic substrate.*

*Adapted from Chen et al. (2010).*
composition may be modest. The precise underlying mechanisms are not yet well understood, but they may be secondary to changes in gut inflammatory tone.\textsuperscript{6,47,54}

\textbf{IAP AND INFLAMMATORY DISEASES}

As already mentioned, IAP plays a key role in ameliorating cellular inflammatory responses. Many reports show that diverse inflammatory diseases are often associated with abnormally low IAP expression or activity. More precisely, a defect in the IAP isoform may be associated with small intestinal inflammation, while overexpression of the TNAP isoform, as seen in colonic diseases,\textsuperscript{44} merely reflects inflammation-driven neutrophil infiltration of tissue.

\textbf{Possible involvement of an IAP defect in diseases of the small intestine}

NEC is a multifactorial pathological condition affecting the ileum and colon of very-low-birthweight babies. This disease involves genetic predisposition, abnormal microbial colonization, ischemia, intestinal immaturity, and feeding with milk formula, the last two being consistent risk factors.\textsuperscript{56} Recently, Whitehouse et al.\textsuperscript{57} observed in a rat model that pups with NEC displayed lower IAP protein expression and activity levels than controls. They suggested that IAP reduction precedes the onset of the disease and may be involved causally. Although supported by data showing that exogenous (bovine) IAP could prevent NEC,\textsuperscript{31} this has not been demonstrated unequivocally. Celiac disease is a chronic inflammation of the small intestine caused by gluten from wheat and most other cereals.\textsuperscript{58} Depressed duodenal IAP protein expression and activity is especially marked in severe cases of celiac disease in young patients.\textsuperscript{59,60} Importantly, tissue co-localization between IAP and LPS-receptor TLR-4 was reported.\textsuperscript{60} The IAP defect was reversed after a gluten-free dietary regimen was initiated.\textsuperscript{60} In farm animals, the postweaning syndrome characterized by gut anatomical and functional alterations is still a problem when rearing pigs.\textsuperscript{61} A defect in IAP was reported recently and was interpreted as a possible reason for intestinal inflammation and associated sensitivity to enteric infections.\textsuperscript{62}

In summary, these data support the notion that IAP could play a role in these diseases. However, it is still unclear whether inhibition of IAP activity is a primary or secondary factor, as already mentioned for DIO.\textsuperscript{22}

\textbf{Possible opposing effects of two AP isoforms in IBD}

Chemicals such as dextran sulfate sodium (DSS) or trinitrobenzene sulfonic acid are often used for inducing colitis in mouse models of IBD. Importantly, some of these compounds (DSS) upregulate the IAP isoform, while others (trinitrobenzene sulfonic acid) induce the TNAP isoform in colonocytes and in rodent colon.\textsuperscript{43} Mice in which the IAP Akp3 gene product has been deleted were more sensitive to chronic colitis in two IBD models (one being DSS), thus suggesting a protective role for the endogenous IAP isoform during colonic inflammation.\textsuperscript{63} Defects in IAP isoform expression were also reported in other studies with DSS.\textsuperscript{44,46} Conversely, colonic expression of the TNAP isoform was reported to increase markedly in chemical (DSS and trinitrobenzene sulfonic acid) models of IBD.\textsuperscript{41–43} In humans, IAP protein levels were lower in colonic biopsies of inflamed versus noninflamed zones in young patients with Crohn’s disease or ulcerative colitis.\textsuperscript{64} Such IAP defects are also found in adults with Crohn’s disease or ulcerative colitis.\textsuperscript{40,65}

Therefore, two intestinal AP isoforms operate in IBD. IAP is reduced, probably as a direct consequence of inflammation. In contrast, TNAP is upregulated. Importantly, the TNAP isoform has two origins: colonocytes and neutrophils. While the former source is probably an adaptive response to counteract oxidative stress and inflammation, the latter source directly reflects the inflammatory response, as indicated by neutrophil infiltration of colonic tissues. Differences between studies suggest that genes, proteins, and activities of both isoforms should be investigated more systematically in order to obtain clearer answers.

\textbf{POTENT ANTI-INFLAMMATORY ACTIVITY OF IAP IN THE INTESTINE AND OTHER ORGANS}

Emerging data suggest that exogenous IAP (most often of bovine origin) is a potent anti-inflammatory agent for treating inflammatory diseases of the gut and other organs (Table 2).\textsuperscript{4,40,42,43,63,65–73} The activity of (human) IAP is highly pH sensitive and is reduced in acidic environments like the stomach, a result of changes in enzyme site structures and ion (e.g., zinc) movements.\textsuperscript{74} However, this reduction in IAP activity, which depends on the duration of exposure to extreme pH, is less than the reduction in liver AP activity and, more importantly, is partially reversible.\textsuperscript{74} Then, IAP can be proteolyzed in the small intestine. The recovery of polymerized inulin-protected bovine IAP was evaluated to be around 30\% in rat intestine.\textsuperscript{75} Increases in fecal concentrations of AP activity following oral exogenous IAP administration may reflect partial escape of the added IAP to bacterial fermentation in the colon and (or) stimulation of endogenous IAP production in the small intestine.\textsuperscript{5,6} In order to improve IAP resistance to alterations in the gastrointestinal (GI) tract and to avoid the use of heterologous (e.g., bovine) IAP, a
### Table 2  Summary of systemic effects of exogenous (bovine or placental) intestinal alkaline phosphatase (IAP) on survival and gut tissue, peritoneal fluid, or plasma cytokine profiles in disease states.

<table>
<thead>
<tr>
<th>Type of model or study</th>
<th>Species, IAP form, and route of administration</th>
<th>Effect on survival</th>
<th>Cytokine</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal models</td>
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<tr>
<td>NEC</td>
<td>Rat, bIAP, i.p.</td>
<td></td>
<td>IL-1β</td>
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<td>NEC</td>
<td>Rat, bIAP, oral</td>
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<td>IL-4</td>
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<td>DSS colitis (acute)</td>
<td>Rat, bIAP, oral</td>
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<td>IL-6</td>
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<tr>
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<td>IL-8</td>
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<tr>
<td>DSS colitis (chronic)</td>
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<td>IL-10</td>
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<td>TNBS colitis</td>
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<td>IFN-γ</td>
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<td>TNF-α</td>
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<td>WASP colitis</td>
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<tr>
<td>Peritonitis</td>
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<tr>
<td>Sepsis</td>
<td>Mouse, placental, IAP</td>
<td>Improved</td>
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<td>IL-10</td>
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</table>

**Abbreviations:** bIAP, bovine intestinal alkaline phosphatase; c.i., continuous infusion; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; i.p., intraperitoneal; i.v., intravenous; NEC, necrotizing enterocolitis; TNF, tumor necrosis factor; TNBS, trinitrobenzene sulfonic acid; WASP, Wiskott-Aldrich syndrome protein; =, no change; -, decreased.

- *Cytokine tissue gene expression. Gene expression of IL-10 receptor was decreased.
- *Cytokine tissue concentration.
- *Cytokine levels measured in peritoneal lavage fluid.
- IL-1β measured in plasma and tumor necrosis factor-α measured in both colonic digesta and plasma.
- IL-1β measured in plasma and tumor necrosis factor-α measured in both colonic digesta and plasma.
- IL-1β measured in plasma and tumor necrosis factor-α measured in both colonic digesta and plasma.
- *Cytokine levels measured after 7 days of treatment. Plasma C-reactive protein and fecal calprotectin decreased at day 21 and day 63 after treatment initiation.
- Renal failure associated with sepsis or septic shock. Bovine IAP administered as intravenous bolus plus intravenous continuous infusion for 48 h.
recombinant human chimeric AP that combines IAP enzyme domain and placental AP stability domain is being developed.76

**Intestinal and colonic inflammation and peritonitis**

To date, all of the published data report beneficial, anti-inflammatory effects exerted by exogenous IAP, although the magnitude of protection depends on the route of administration. These effects include intestinal tissue protection in rodent models of NEC,69–71 and CF,72 and colonic tissue protection in animal models of IBD.40,42,63 Exogenous IAP also reduces inflammation in patients with ulcerative colitis65 and in peritonitis in a mouse model.68

**Systemic inflammation**

Administration of exogenous IAP has been shown to reduce systemic inflammation in various animal models69–71,77 and in several diseases in humans78 (Table 2), as demonstrated in NEC,66,67 peritonitis,68 sepsis,69–71,78 and even brain disease.77

Sepsis is caused by infection and LPS-induced systemic inflammation, which rapidly can lead to acute kidney injury. Importantly, the TNAP (or kidney) isoform is present in renal proximal tubules as a defense system against LPS. Two double-blind, randomized, placebo-controlled studies in septic patients reported improvements following intravenous administration of IAP.77,78 The first study showed that IAP was able to downregulate inducible nitric oxide synthase activity and to reduce urinary excretion of nitric oxide metabolites.79 In the second study, renal function was also improved and systemic inflammation reduced.73 The beneficial effects of IAP treatment were attributed to dephosphorylation-dependent detoxification of both LPS and extracellular ATP.78

Administration of exogenous IAP was also found to be protective in patients undergoing coronary artery bypass surgery,72 and exogenous IAP reduced neurological alterations in a model of autoimmune encephalomyelitis in mice.77

As described above, while an increase in IAP in the intestine is generally protective due to direct inhibition of inflammation, high levels of TNAP expression and activity in the colon most probably reflect inflammation-driven neutrophil infiltration of colonic tissues. Because most serum AP is of bone and liver origin, the gut and kidney contribute relatively little to this activity. Thus, serum AP activity is considered a biomarker of inflammation and cardiometabolic risk and has been positively correlated with other systemic markers of inflammation (e.g., C-reactive protein) in the general US population as well as in patients with renal disease and Alzheimer's disease.81,82 Higher levels of plasma AP activity are also indicative of elevated risk of mortality in the general US population.83

Collectively, these results indicate that, although all increases in AP activity may be an adaptive response, the purpose of which is to control PAMPs (e.g., LPS), increases in colonic (but not intestinal) and serum total AP activities are clear signs of inflammation.

**Influence of route of administration on effects of exogenous IAP**

Oral or enteral administration of exogenous IAP was able to reduce the development of NEC, while systemic administration of IAP strongly downregulated systemic inflammation without reducing intestinal tissue injury caused by NEC.66,67 Intrarectal administration of IAP was more effective than oral administration in reducing colitis and bacterial translocation, although both routes conferred efficacy.43 A plausible reason for this difference is that IAP given by the oral route may have been partially digested in the intestine.57,75 Importantly, oral IAP administration was able to stimulate endogenous IAP in the small intestine but had no effect on the colonic TNAP isoform.43 Administration of exogenous IAP stimulated the release of endogenous TNAP isoform into the systemic circulation in patients undergoing cardiac vascular surgery,62 but not in the brain of mice.77 However, the pathophysiological significance of TNAP isoform induction in these studies is unclear.

**DIETARY MODULATION OF IAP: AN UPDATE**

It was previously reported that dietary fat, protein, carbohydrates, vitamins, and minerals are all important dietary modulators of host IAP gene expression and enzyme activity.9 Since then, additional information has been published.

**Stimulation of IAP by free calcium, bound phosphorus, and vitamins**

Dietary calcium was recently shown to stimulate the activity of IAP in vivo, which in turn limited intestinal absorption of calcium as a percentage of total calcium intake in rats.84 This is important because increased calcium intake can be easily achieved, e.g., by eating dairy products. Beneficial effects of dietary calcium are well documented in models of colonic inflammation.85,86 However, the role of IAP activity was not considered in these studies, and it is unclear whether this activity may have also contributed to the protective effects. Of note,
calcium-induced protection against colonic inflammation requires a sufficiently high concentration of phosphorus in the colon. Earlier work concluded that free dietary phosphorus had an inhibitory effect on IAP. By contrast, bound phosphate (e.g., esterified to glucose residues of some potato starch varieties) was able to stimulate IAP activity dose-dependently throughout the small intestine of rats.

Vitamin K (phylloquinone) and vitamin K2 (menaquinone-4) were already mentioned as IAP stimulants in a previous review. Subsequent reports suggest that these vitamins stimulate both the expression of Akp3 and Akp6 IAP genes and the activity of AP protein in the mouse jejunum. Underlying mechanisms were suggested to involve the nuclear steroid and xenobiotic receptor SXR, but direct evidence is lacking.

**Differential regulation of IAP by dietary FAs**

As already mentioned, fat intake stimulates gut IAP in rodents, and triglycerides with saturated LCFA or medium-chain FAs increase IAP expression and/or activity. Conversely, polyunsaturated LCFA were shown to decrease IAP expression. Recently, an important study revealed that, while a diet enriched in (omega-6 [n-6]) polyunsaturated FAs promoted inflammation in mice, the addition of (n-3) polyunsaturated FAs provided by fish oil reduced inflammation but was also associated with higher rates of sepsis-induced mortality. The authors concluded that added fish oil depressed both gut IAP activity and associated LPS dephosphorylation capacity, thus favoring circulating LPS. In a rat model of chemically induced colonic inflammation, however, the total activity of AP (isoforms not specified) in the colon was shown to decrease following partial replacement of linoleic acid (C18:2 n-6) with α-linolenic acid (C18:3 n-3). Therefore, these studies indicate that the role of dietary FAs is critical and that clinical outcomes may depend on their combination.

**Stimulation of IAP by various plant bioactive compounds**

Different medicinal plants are able to modulate IAP activity, and various spices (e.g., black pepper, red pepper, ginger, and bioactive compounds piperine and capsaicin) have been shown to increase IAP activity in the small intestine. Elevated IAP activity was associated with elongation of intestinal microvilli, higher membrane fluidity, and a reduced cholesterol-to-phospholipid ratio. Caraway (Carum carvi L.) decreased the level of colonic tissue AP in a rat model of chemically induced colorectal cancer. An anti-inflammatory compound prepared from fungi was shown to reduce colonic inflammation and alterations and to decrease colonic AP activity.

More recently, narrow-leaved cattail (Typha angustifolia L.) rhizome flour and green dwarf banana (Musa sp. AAA) flour both were found to decrease colonic AP activity in a rat model of colitis. Coumestrol, a phytoestrogen known to regulate intestinal calcium absorption, was found to inhibit IAP gene expression and enzyme activity, although differential regulation was evident in the duodenum and the jejunum in neonatal pups and lasted until 10 days postparturition. It was suggested that estrogen receptor-alpha (ER-α) may be involved in this transient inhibition.

**Stimulation of IAP by some probiotic bacteria**

In a chronic model of chemically induced colorectal carcinoma in rats, the probiotic mixture VSL#3 prevented carcinoma development and limited colonic dysplasia. Probiotic-treated rats displayed a 50% reduction in fecal AP activity, an observation interpreted as reflecting a probiotic-mediated reduction in small intestine epithelial cell death. An alternative explanation may be that this treatment normalized colonic TNAP activity, which is elevated during colonic inflammation. Lactobacillus plantarum AS1 isolated from fermented food inhibited chemically induced colorectal cancer in rats, and this was associated with a reduction in colonic (TN)AP activity. Collectively, available data suggest that various plant bioactive compounds and specific probiotics can contribute to gut protection, either directly, by influencing IAP-mediated mechanisms, or indirectly, by ameliorating inflammation, especially in the colon. However, effects of FAs on IAP appear more complex and warrant further investigation.

**EARLY PROGRAMMING OF IAP**

Nutrition and environment are increasingly suspected to contribute to the development of various diseases of adulthood, including obesity and associated metabolic diseases. For example, intrauterine growth retardation is a risk factor for metabolic syndrome and obesity. However, little is known about the early programming of the GI tract in this context. Recently, it was hypothesized that perinatal malnutrition programs key intestinal functions, including IAP activity. In a rat model of intrauterine growth retardation induced by maternal protein deficiency during the perinatal period, it was shown that adult offspring fed a high-fat diet failed to adapt to this diet by increasing AP activity in jejunal tissue and cecal content. Interestingly, mRNA levels of the transcription factors Klf4 and Cdx1 were lower in jejunal epithelial cells of intrauterine-growth-retarded rats fed the high-fat diet.
Different AP isoforms are encoded by multiple genes that display either ubiquitous or tissue-specific expression. In a search to decipher the origins of two AP genes newly discovered in zebrafish, Yang et al. published an outstanding study on AP gene evolution across animal species. They found that AP genes are organized in three clades, except in mammals, which display only two clades (AP-1 and AP-2). They also found that two major genome duplications occurred in vertebrates during evolution, generating these three clades. According to this phylogenetic analysis, AP genes of the intestines have been duplicated and lost several times over different vertebrate lineages. Yang et al. interpreted their findings as a reflection of the dynamic evolutionary changes in the dietary regimens that would have driven both adaptation of GI tract anatomy and microbial symbiont composition. Although their analysis was based on fecal microbiota data, which are not representative of bacterial diversity in upstream compartments of the GI tract, Yang et al. made some interesting observations. For example, they found that domestic cats and dogs have only one copy of the AP gene, matching a GI tract anatomy associated with a monotonous dietary regimen. Mice and rats appear more closely related to humans in terms of AP gene content, belonging to clade 1. AP clade 2 displays the most dynamic evolution. Indeed, artiodactyls with more complex GI tract systems display a higher intestinal AP gene diversity. Bovine ruminants with anatomically complex forestomachs possess six AP gene copies. The porcine species, an omnivorous monogastric animal, has three copies, two of them being closely related. Interestingly, one porcine AP gene closely resembles two bovine AP genes, leading the researchers to suggest that swine and bovine lineages had diverged after this tandem AP gene duplication occurred.

Importantly, intestinal AP-dependent control of LPS (and PAMP) bioactivity in these farm animal species is still of major concern. Postweaning in livestock is a period of high susceptibility to gut disorders and enteric infections, and was recently shown to be associated with the down regulation of IAP gene expression and activity. Furthermore, a pig line divergently selected for low residual feed intake (or for higher body-weight-gain to feed-intake ratio) showed higher ileal IAP and lower intestinal (and systemic) inflammation associated with lower plasma LPS than the line with high residual feed intake. In dairy cattle, subclinical rumen acidosis is characterized by ruminal and colonic blooms of gram-negative bacteria and LPS, resulting in increased concentrations of circulating LPS; this leads to inflammation, metabolic disorders, and a drop in milk fat production that inversely correlates with plasma levels of LPS. However, little has been reported on AP gene expression or activities in the GI tracts of ruminants.
Collectively, these data support a dynamic phylogenetic evolution of AP gene family members involved in the control of gut microbiota-host symbiosis and inflammation, with evolution occurring across animal species.

CONCLUSION

Intestinal IAP is at the crossroads between diet, fat absorption, the microbiota, LPS, and inflammation, factors that have all been implicated as causal in obesity and metabolic disorders. This review gathers important new findings on IAP production, modulation, and circulation in and around intestinal cells; on absorption of calcium, fat, and minerals; on detoxification of novel nucleotides and bacterial PAMPs; on amelioration of intestinal inflammation; and, finally, on modulation of the composition of gut microbiota (Figure 3). However, the effects of IAP on some targets, such as gut permeability and bacteria, might be indirect, through downregulation of inflammation and, possibly, through modulation of intestinal epithelial surface pH. Members of the intestinal AP gene family appear to reflect evolutionary changes in dietary regimens across animal species and over time, as well as changes in associated gut microbial symbionts. This makes intestinal AP a crucial component of a detoxification system for the maintenance of both host and microbiota homeostasis. Diet appears to be a major modulator of the compositional and functional diversity of the microbiota and may also be considered a regulator of intestinal AP expression and activity in health and disease. In that regard, many components of our diet, including minerals, vitamins and micronutrients play a beneficial role. Unbalanced diets, typified by the low fiber intake of Western diets, promote gut dysbiosis, thereby altering both intestinal barrier function and translocation of bacterial PAMPs and thus contributing to inflammation, obesity, and metabolic disorders. Diets rich in nondigestible but fermentable fiber components may thus help prevent a number of clinical disorders. Intestinal AP will continue to play a central role in this equilibrium and, perhaps, in apparently unrelated diseases. Finally, due to poor correlation between mouse models of inflammation and human inflammatory diseases and the limited data available from human studies, much more evidence on the roles of IAP in humans is needed.

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Figure 3 Relationships between intestinal alkaline phosphatase, proinflammatory compounds, gut microbiota, inflammation, and functional outcomes. Abbreviations: LPS, lipopolysaccharide; PAMPs, pathogen-associated microbial patterns.
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