Alkaline phosphatase treatment of acute kidney injury

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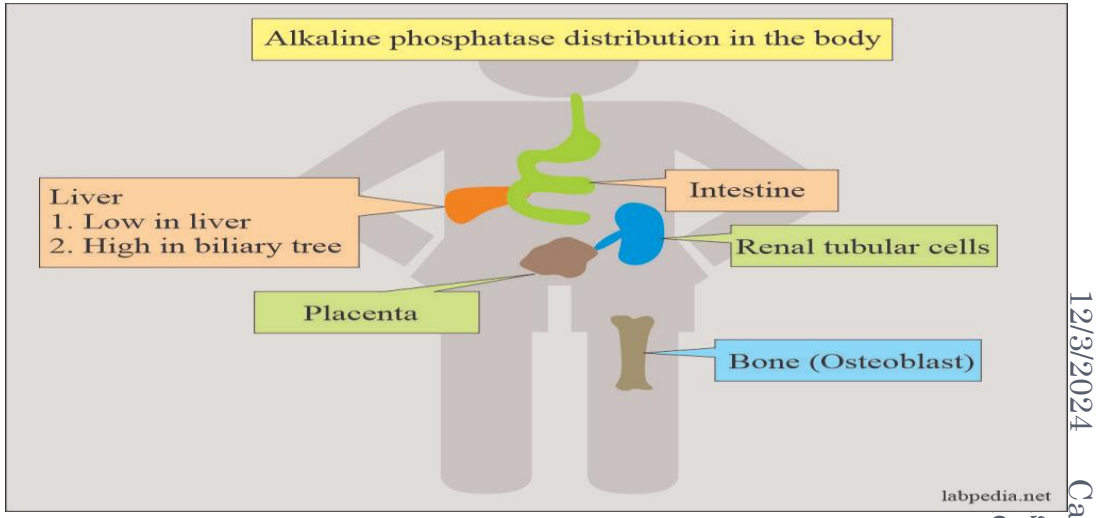
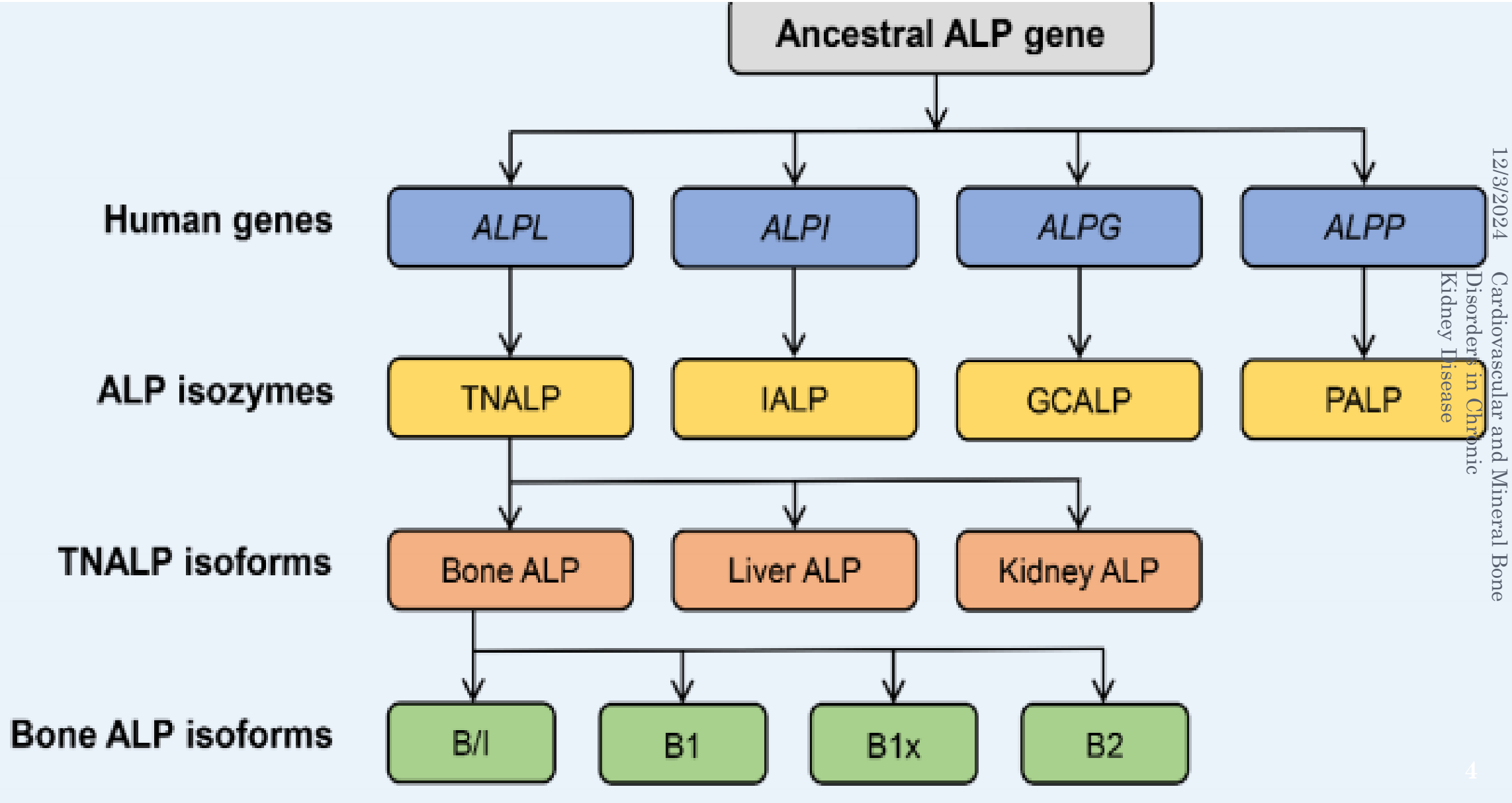
Introduction

- ALP is an endogenous enzyme that is expressed in bone and physiological barrier tissues such as the intestinal tract and kidney tubules
- The main function of all ALP iso-enzymes is the dephosphorylation of other proteins, which alters the function of these proteins

Introduction

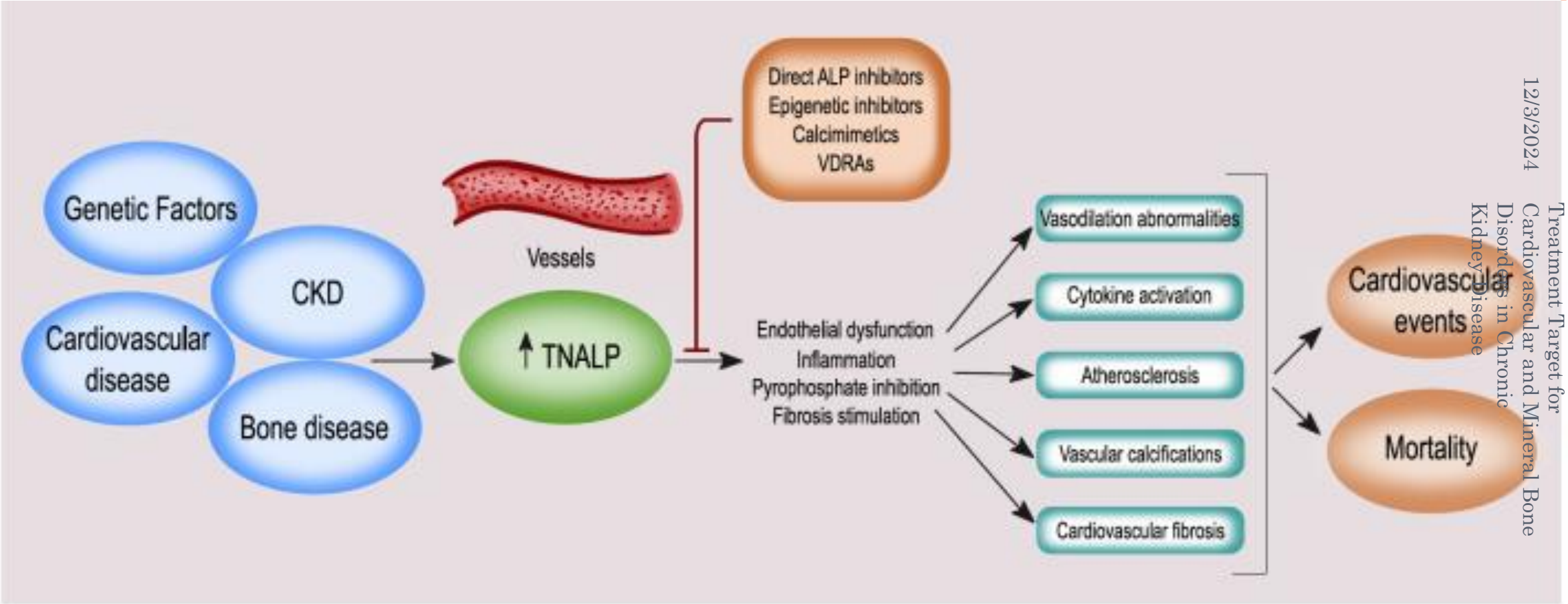


- Four ALP isozymes
  - ✓ Tissue-nonspecific ALP (TNALP ⇒ liver/bone/kidney ALP),
  - ✓ Intestinal ALP (IALP)
  - ✓ Placental ALP (PALP)
  - ✓ Germ cell ALP (GCALP ⇒ placental-like ALP).


Ancestral ALP gene
Human genes
ALPL
ALPI
ALPG
ALPP
ALP isozymes
TNALP
IALP
GCALP
PALP
TNALP isoforms
Bone ALP
Liver ALP
Kidney ALP
Bone ALP isoforms
B/I
B1
B1x
B2

TNALP

- Tissue non-specific ALP (TNALP) is expressed in bone, liver and kidney tissue and makes up practically all circulating ALP
- Normal TNALP function is essential for bone mineralization, however, it also contributes to vascular calcification and possibly to the genesis of Alzheimer's disease
- Administration of exogenous TNALP has been approved for the treatment of bone demineralization in severe hypophosphatasia


Genetic Factors
CKD
Cardiovascular disease
Bone disease
Vessels
TNALP
Direct ALP inhibitors
Epigenetic inhibitors
Calcimimetics
VDRAs
Endothelial dysfunction
Inflammation
Pyrophosphate inhibition
Fibrosis stimulation
Vasodilation abnormalities
Cytokine activation
Atherosclerosis
Vascular calcifications
Cardiovascular fibrosis
Cardiovascular events
Mortality

TNALP

- ALP has been suggested as a targetable promotor of organ fibrosis.
- Cardiac TNALP is highly upregulated after a myocardial infarction and is associated with increased myocardial fibrosis .
- Inhibition of ALP can effectively attenuate the myocardial fibrosis induced by myocardial infarction

PALP

- During pregnancy, circulating PALP activity increases several fold and play a role in maternal to fetal nutrient transport and may be protective against offspring obesity
- Germ cell ALP's functional role is still unclear, circulating germ cell ALP is thought to be an early tumor marker for seminomas

IALP

- About the establishment of the intestinal flora in early childhood, there is an increase in the IALP activity during this time
- It is essential to know that IALP also makes part of the gut microbiota modulation since the born
- A study using an IALP knockout model in SpragueDawley pups increased dysbiosis with bacterial translocation, and inflammation was reported

IALP

- IALP plays a vital role in the intestinal barrier function, affect bicarbonate secretion, duodenal surface pH, nutrient resorption, local intestinal inflammation, and gut microbiota
- Disturbances of IALP functions are associated with persistent inflammatory diseases associated with aging , IBD, type 2 DM, obesity, metabolic syndrome, and CKD

IALP

- IALP decreased total body weight, serum lipids, glucose levels, and liver lipids, and changed the composition of gut microbiota.
- Oral supplementation of IALP in aging mice regulates the intestinal barrier function, microbial homeostasis, decrease age-related intestinal permeability and systemic inflammation, potentially leading to less fragility and prevention of chronic age-related diseases

IALP

- IALP plays a role in maintaining gut homeostasis by regulating gut microbiome tolerance and lipid absorption
- Because of extensive evidence for its role in the immune response to pathogens in the gut and its superior enzymatic activity, it has been proposed that IALP can be used as a treatment for inflammatory disorders ,ulcerative colitis and AKI
- It is available as a bovine-sourced preparation (bIALP) and in a human recombinant form (recALP)

Hypothes

- It has been hypothesized that supplementation of ALP can alter certain aspects of the host-response to pathogens and injury
- To understand how this could be used to treat AKI, we must understand the physiologic functions of ALP, its interaction with the host-response and the role of the host response in AKI pathophysiology

THE HOST-RESPONSE

- The kidney injury–inducing host-response is mediated by signalling molecules that are known as pathogen- and damage-associated molecular patterns (PAMPs and DAMPs)
- PAMPs and DAMPs induce kidney damage by activation of toll like receptors (TLRs) and purinergic or pyrimidinergic receptors.
- In rodent models of sepsis and kidney IRI, inhibition of either TLR4 or the purinergic P2X7 receptor protects against AKI

PAMPs

- PAMPs are exogenous or endogenous microorganism-derived molecules, such as lipopolysaccharides (a toxic constituent of Gram-negative bacteria).
- External PAMPs can enter the body through a primary infectious site, whereas endogenous PAMPs enter through the intestinal barrier.
- Intestinal ischaemia can arise due to diseases such as sepsis or due to surgery and vasopressive therapy

DAMPs

- DAMPs are molecules produced by the body itself and are either actively released in response to distress or passively leak from dead or damaged cells

PAMPs and AKI:

- TLRs are trans membrane glycoproteins that can recognize both PAMPs and DAMPs and induce both the innate and adaptive immune system
- There are 10 subtypes of TLRs in humans, of which TLR4 has been studied most extensively with respect to AKI pathophysiology
- ALP does not inhibit TLRs directly, but it has been hypothesized that ALP can prevent activation by the dephosphorylation of TLR ligands

PAMPs and AKI

- The potency of LPS as a TLR4 agonist is determined by a structure inside the LPS molecule called the lipid A subunit
- This lipid A subunit contains two phosphate groups that are vital to its activation but not its binding to TLR4
- Therefore, once the lipid A subunit is dephosphorylated, LPS works as a TLR antagonist

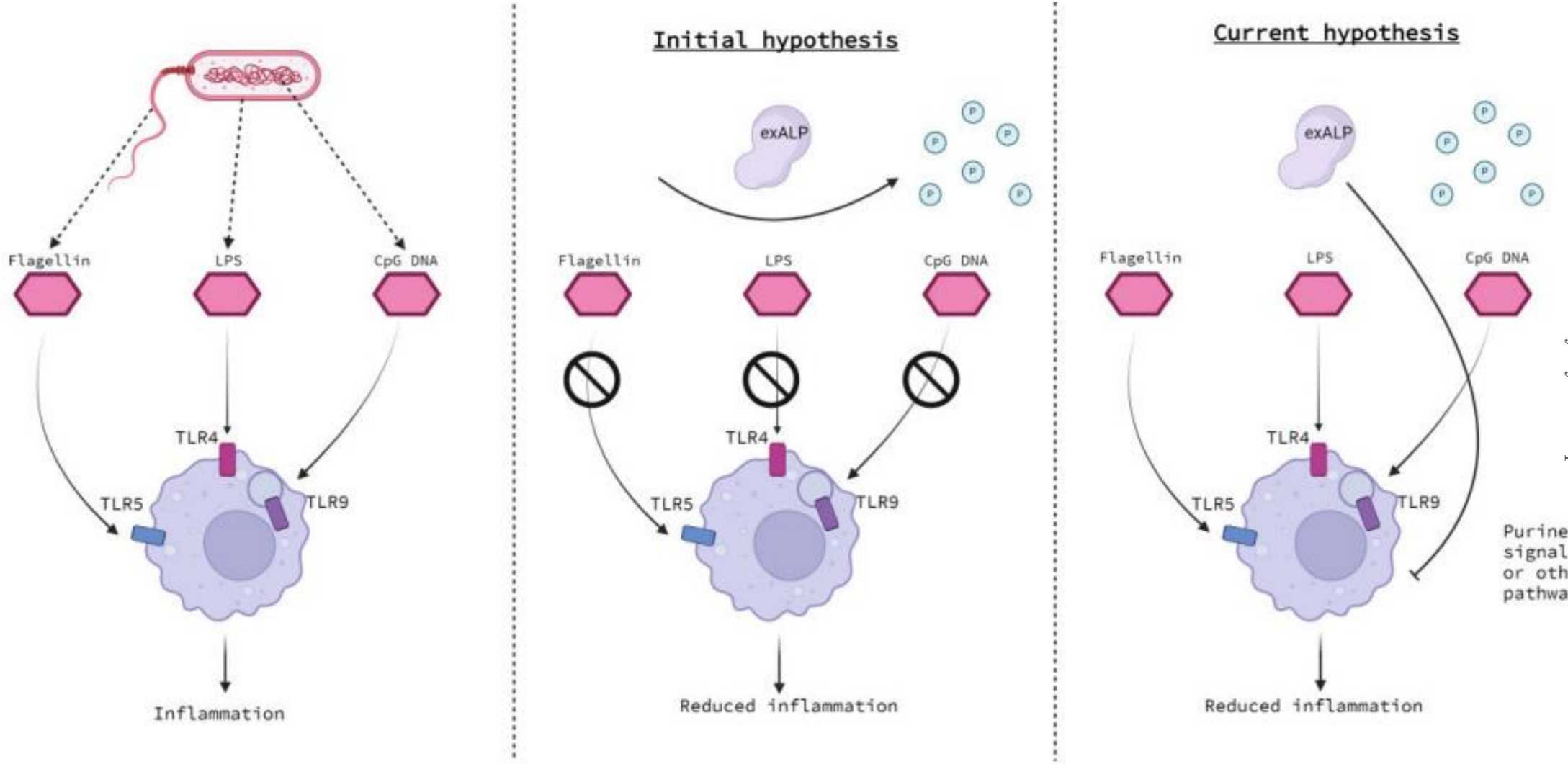
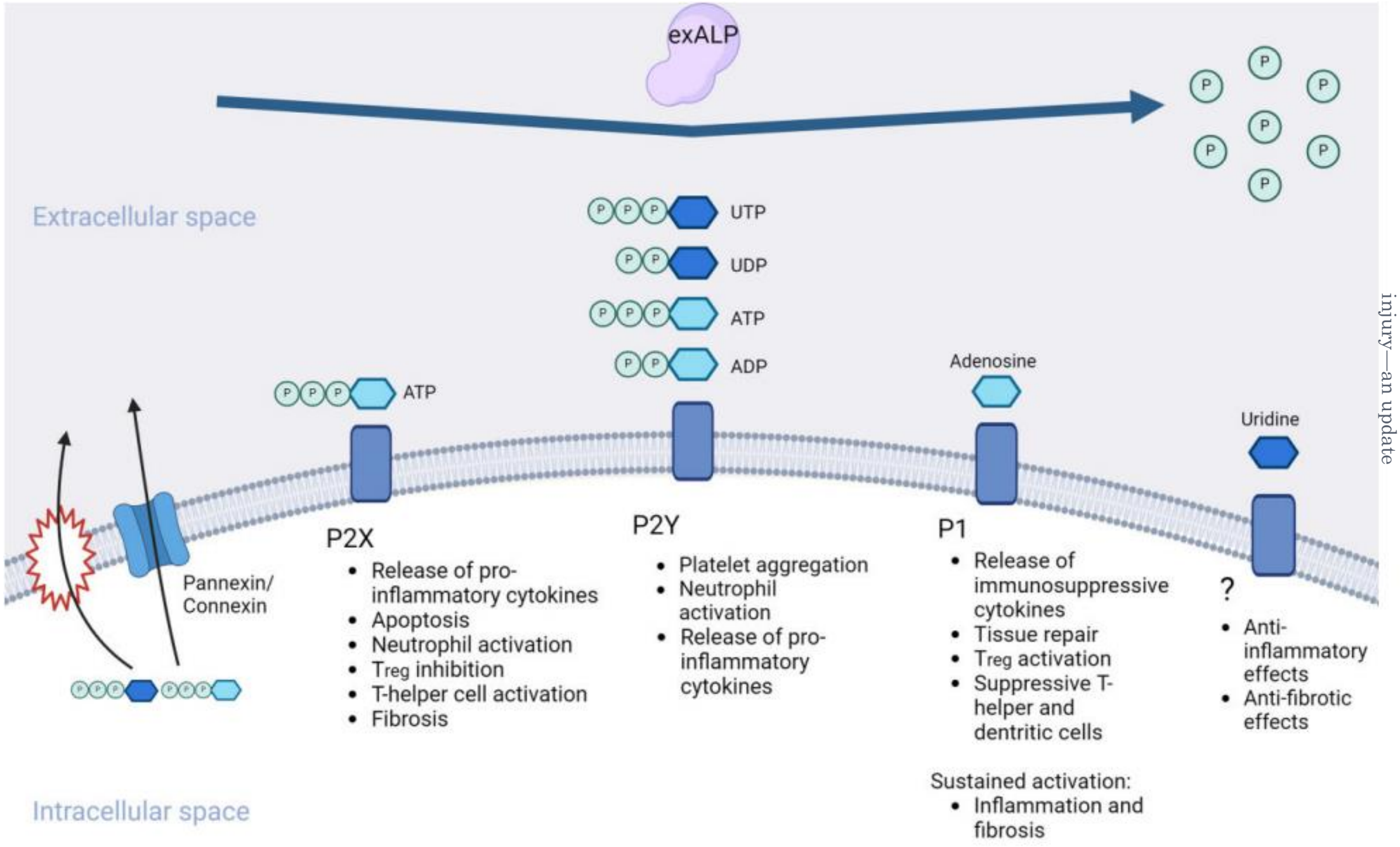


Figure 1: Initial and new hypotheses for interaction of ALP with PAMPs. In this figure, a generic inflammatory cell is depicted. PAMPs released from Gram-negative bacteria cause a potent inflammatory response through TLRs. Despite current evidence pointing away from direct detoxification of the toxic lipid A core in LPS by ALP, treatment with ALP leads to strongly reduced PAMP-induced inflammation *in vivo* and *in vitro*. These effects might be mediated through purinergic/pyrimidinergic signalling or through a yet undetermined pathway. For flagellin and CpG DNA, indirect evidence suggests reduced toxicity, which needs to be confirmed with additional testing. Arrows indicate activation; arrow with flat head indicates inhibition. Created with BioRender.com.

DAMPs and AKI

- Infectious stimuli epithelial and endothelial cells, immune cells and platelets release signalling molecules in the form of extracellular nucleotides and nucleosides (DAMPs).
- A nucleotide is a phosphorylated nucleoside containing up to three phosphate groups, and nucleosides derive from the building blocks of DNA.
- They are released from cells either passively (due to cell damage), through active transport molecules like pannexin and connexin or are formed from other extracellular nucleotides


exALP
Extracellular space
UTP
UDP
ATP
ADP
ATP
Adenosine
Uridine
Pannexin/
Connexin
P2X
• Release of pro-inflammatory cytokines
• Apoptosis
• Neutrophil activation
• Treg inhibition
• T-helper cell activation
• Fibrosis
P2Y
• Platelet aggregation
• Neutrophil activation
• Release of pro-inflammatory cytokines
P1
• Release of immunosuppressive cytokines
• Tissue repair
• Treg activation
• Suppressive T-helper and dentritic cells
Sustained activation:
• Inflammation and fibrosis
?
• Anti-inflammatory effects
• Anti-fibrotic effects
Intracellular space

DAMPs

- ALP can generate nucleosides from nucleotides by dephosphorylation, and these nucleosides exert completely different effects on receptor cells than their nucleotide counterparts.
- Their nucleotide forms are (AMP), (ADP)(ATP) (UDP) and (UTP).

Adenosine

- Adenosine has an inhibitory effect on platelets, reduces inflammation, induces repair of endothelial cells and protects against extravasation of fluids during ischaemia
- It also activates the tubuloglomerular feedback system
- During IRI in kidney transplantation, activation of regulatory T cells by adenosine potentially results in reduced acute and chronic rejection
- In vitro experiments show that exogenous ALP can also remove all phosphate groups from ATP and ADP to produce adenosine

Purines

- At physiologic levels, extracellular ATP stimulates P2X and P2Y receptors, which are vital to normal T cell function.
- At high levels, it activates the P2X7 receptor, which causes tissue damage by pro-inflammatory macrophage polarization and activation of proinflammatory T-helper cells

PLATELET ACTIVATION

- ALP renoprotection is not dependent on a single mechanism of action or on a single adenosine receptor.
- ALP administration also reduces platelet activation by reducing extracellular levels of ADP and increasing adenosine levels.
- Platelets are an important part of the host-response and hyperactive platelets cause inflammation and endothelial damage

Pyrimidines

- The pyrimidinergic nucleotides UTP and UDP exert proinflammatory effects, mainly through P2Y receptors.
- Although no specific receptor has yet been identified for uridine

ALP AND TIGHT JUNCTIONS IN AKI

- ALP is essential to the structural integrity of border tissues (such as endothelium and the tubular epithelium) because of its close relationship with tight junctions (TJs).
- TJ proteins (TJPs) are vital to both endothelial and epithelial barrier function and the preservation of TJP function can protect kidney function against IRI in vivo.

ALP AND TIGHT JUNCTIONS IN AKI

- ALP can prevent leakage of endotoxins into the circulation and prevent them from activating TLRs
- In the kidney, ALP is expressed in both the proximal and distal tubules
- Supplemented exogenous ALP protects the activity of ALP that is already present in the body

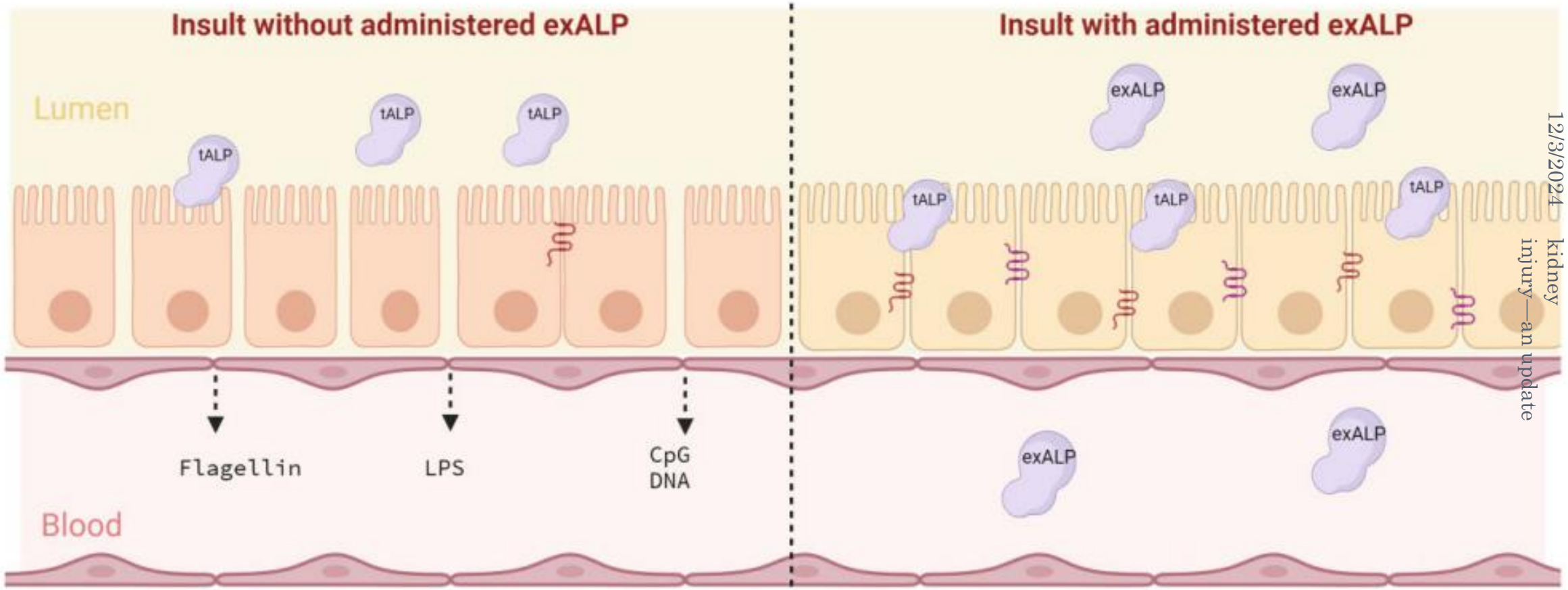


Figure 3: Loss of tissue ALP (tALP) is associated with a loss of intestinal barrier function and increased leakage of endotoxins. Oral and parenteral supplementation with exogenous ALP (exALP) preserves tALP activity and TJPs during (ischaemic) insults and protects intestinal epithelial barrier function. tALP offers protection against bacteria-induced inflammation whereas exALP does not. There are similarities between ALP expression in the intestinal and tubular epithelium, however, its specific functions in the latter have yet to be elucidated. Created with BioRender.com.

Some studies

- Chen et al, demonstrated that cell-bound ALP limits inflammation induced by live bacteria, but exogenous ALP does not.
- This stresses the importance of endogenous ALP protection by ALP supplementation.

Some studies

- In 2019, Davidson et al demonstrated in a piglet model of cardiopulmonary bypass with circulatory arrest that infusion of exogenous ALP protected kidney tissue ALP activity and was associated with a lower incidence of AKI.
- Thus supplementation with exogenous ALP preserves tissue ALP and protects brush border barrier function by preservation of TJP expression and localization

Table 1: List of (possible) substrates for ALP as mentioned in this article.

Substrate/molecule of interest	Product	*In vitro* results of ALP co-incubation	*In vivo* results	Altered by ALP treatment	References
LPS (PEtN)	LPS	Inhibited pro-inflammatory cytokine induction	Reduced damage in LPS-induced kidney injury	Unlikely	Komazin *et al.* [22], Peters *et al.* [23]
Flagellin	Unknown	Pi generation Inhibited pro-inflammatory cytokine induction	Direct evidence unavailable	Possible	Chen *et al.* [26]
CpG DNA	Unknown	Pi generation Inhibited pro-inflammatory cytokine production	Direct evidence unavailable	Possible	Chen *et al.* [26]
ATP/ADP	Adenosine	Inhibited pro-inflammatory cytokine production Inhibited sepsis-induced platelet aggravation	Reduced damage in IRI-induced kidney injury through adenosine production	Certain	Tunjungputri *et al.* [31], Rosin *et al.* [34]
UTP/UDP	Uridine	Inhibited pro-inflammatory cytokine production	Reduced UDP-induced inflammation	Certain	Moss *et al.* [37]
TJPs	Altered phosphorylation State of TJPs	ALP can dephosphorylate TJPs	Increased TJ expression Improved TJ localization	Possible (indirect)	Harmaneh *et al.* [42], Liu *et al.* [47], Plaeke *et al.* [56], Sakakibara *et al.* [48]
Live bacteria	Unknown	Cell-bound ALP limits inflammation Exogenous ALP does not limit inflammation	Direct evidence unavailable	Unknown	Chen *et al.* [26]

Table 2: Placebo-controlled randomized clinical trials with exogenous ALP.

Clinical trial	Setting	Groups	Kidney-related outcomes	Other relevant outcomes
Heemskerk *et al.* [51]	Sepsis	bIALP ($n = 11$), placebo ($n = 5$) (AKI patients)	Improved CCL within 7 days	Reduced renal oxidative stress
Kats *et al.* [57]	Cardiothoracic surgery	bIALP ($n = 32$), placebo ($n = 31$)	None	Lower incidence of severe systemic inflammation in ALP group
Pickkers *et al.* [13]	Sepsis AKI	bIALP ($n = 16$), placebo ($n = 19$)	Stronger recovery of CCL from day 0 to 28	NA
Pickkers *et al.* [52]	Sepsis AKI	RecALP ($n = 111$), placebo ($n = 116$)	Significantly stronger improvement of CCL from day 0 to 28	Negative primary outcome (improvement of CCL within 7 days) 28- and 90-day mortality were significantly lower in ALP group
Keizer *et al.* [58]	Cardiothoracic surgery	bIALP ($n = 27$), placebo ($n = 26$)	None	Inconclusive
Steenvoorden *et al.* [55]	Kidney transplantation – feasibility pilot	bIALP ($n = 5$), placebo ($n = 6$)	Lower expression of urinary kidney injury biomarkers on day 7	NA
REVIVAL [53]	Sepsis AKI	RecALP, placebo ($N = 649$)	Decreased major adverse kidney events by day 90	Terminated early due to futility on primary outcome; 28-day mortality

RecALP: recombinant ALP; bIALP: bovine intestinal ALP; CCL: creatinine clearance

Clinical evidence

- Of the five studies that reported on renal outcomes, four were conducted in patients with Gram-negative sepsis in an intensive care unit setting and one in kidney transplantation.
- The first sepsis study, which was conducted in 2009 with 36 patients (15 patients had AKI), showed reduced renal oxidative stress markers and improved creatinine clearance in the ALP-treated patients with AKI compared with patients with AKI treated with placebo
- These results prompted a follow-up study with 36 patients (all of whom had SA-AKI), which showed a significantly stronger recovery of endogenous creatinine clearance (ECC) in the ALP-treated group compared with placebo

Clinical evidence

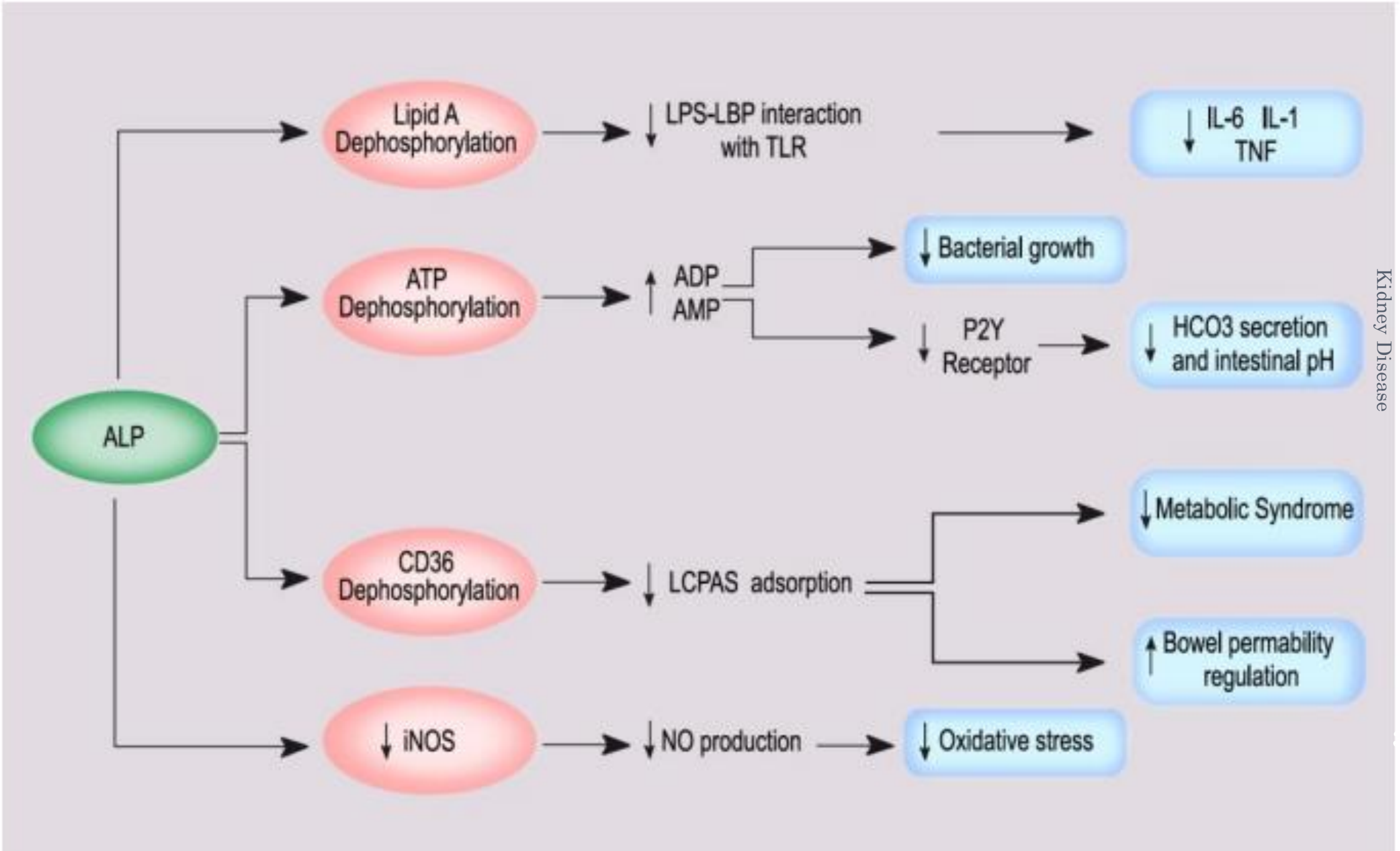
- In the second follow-up study, conducted in 227 patients with SA-AKI, ECC recovery in the first 7 days of AKI was not stronger in the ALP group.
- However, from day 0 to 28, this recovery was stronger and 28- and 90-day mortality were significantly lower in the ALP group [52].
- A larger follow-up trial has since been conducted to confirm the reduced mortality rate at day 28.
- However, a significant reduction in the need for dialysis in the ALP-treated group was described by the researchers

The Role of ALP/BALP in Inflammation, Metabolic Syndrome, and Proteinuria

- Concerning the regulation of the immune and inflammatory response, TNALP is found in neutrophils, macrophages, and some lymphocytes.
- It has been demonstrated that IL-1ß and bacterial endotoxins increase the circulating ALP activity, producing a protective effect against bacterial cytotoxins .
- CRP could suppress ALP and bone mineralization

The Role of ALP/BALP in Inflammation, Metabolic Syndrome, and Proteinuria

- Kaliannan et al. showed that IALP knockout mice had a greater risk of developing metabolic syndrome; moreover, the increase of IALP (endogenous or supplementing it orally) can prevent and reverse metabolic syndrome.
- In a case-control study, compared IALP levels in the stools of 202 patients with type 2 diabetes and 445 healthy controls. Patients with type 2 diabetes had significantly lower IALP levels in their stool (approximately 48%) and obese patients with high IALP values did not develop diabetes.
- These findings suggest that the action of this enzyme could be protective against type 2 diabetes, and that a 50% loss of IALP activity could be predictive for the development of the disease


ALP
Lipid A Dephosphorylation
LPS-LBP interaction with TLR
IL-6 IL-1 TNF
ATP Dephosphorylation
ADP
AMP
Bacterial growth
P2Y Receptor
HCO3 secretion and intestinal pH
CD36 Dephosphorylation
LCPAS adsorption
Metabolic Syndrome
Bowel permability regulation
iNOS
NO production
Oxidative stress

The Role of ALP/BALP in Inflammation, Metabolic Syndrome, and Proteinuria

- Bulum et al. investigated patients with type 1 diabetes and found that serum ALP was associated with glomerular hyperfiltration, proteinuria and progression of CKD in the early stages of diabetic nephropathy
- In a retrospective study on 299 patients with histologically diagnosed diabetic nephropathy, Zhao et al , demonstrating that ALP is negatively associated with eGFR and positively associated with proteinuria
- In fact, patients who had ALP values greater than 97 U/L showed a 138% greater risk of developing ESRD or a 50% reduction in eGFR.
- Patients with nephrotic syndrome had a poor outcome, i.e., 72% experienced a decrease in renal function

The Role of ALP/BALP in Inflammation, Metabolic Syndrome, and Proteinuria

- Circulating ALP was, shown to be independently associated with worse renal outcome.
- The levels of interstitial fibrosis and tubular degeneration were worse in patients with elevated ALP.
- In the kidneys, ALP is expressed on the brush border membranes of proximal tubular cells .
- ALP is also expressed, in physiological conditions, on the endothelial, mesangial, and epithelial cells of Bowman's capsule, and this could also increase the urinary excretion of ALP in hyperfiltration

ALP AND MORTALITY

- Elevated ALP levels increased the risk of all-cause mortality,
- High levels of ALP are associated with various conditions that can increase mortality, such as inflammation, vascular calcification, and CVD

ALP AND MORTALITY

- The association between high levels of ALP, hospitalization, and all causes of mortality was studied in HD patients and in patients with earlier stages of CKD
- Elevated BALP levels were associated with increased mortality even in the long term (4 years)

Dialysis modality on the association of ALP with mortality

- PD patients had an increased mortality risk only when ALP was greater than 150 U/L.
- Combination of high ALP and low PTH values is independently associated with increased cardiovascular and all-cause mortality
- Similar findings were found by Drechsler et al., who demonstrated that hemodialysis patients with high BALP and low PTH had a 2.8-fold increased risk of death within 6 months .
- These findings may suggest that ALP is a better marker than PTH for predicting mortality and CVD.

ALP AFTER KIDNEY TRANSPLANTATION

- In kidney transplant recipients, pre-transplant ALP levels were predictive of post-transplant mortality; patients with high levels of ALP had a 64% risk of mortality and loss of the graft

The Association between ALP, Bone Turnover, and Fracture Risk

- Although levels of PTH and BALP tend to be lower in low-turnover bone disease and higher in high-turnover bone disease, BALP is considered to better reflect bone turnover
- Increased serum PTH levels with low BALP may reflect different degrees of the multifactorial skeletal resistance to PTH, just as the presence of aluminum overload, osteomalacia, Paget's disease, or lytic bone lesions should be excluded

Intestinal ALP in CKD

- Reduced luminal IALP can lead to an imbalance in intestinal functions and gut dysbiosis, with a consequent increase in the risk of developing intestinal inflammation and gut permeability in CKD

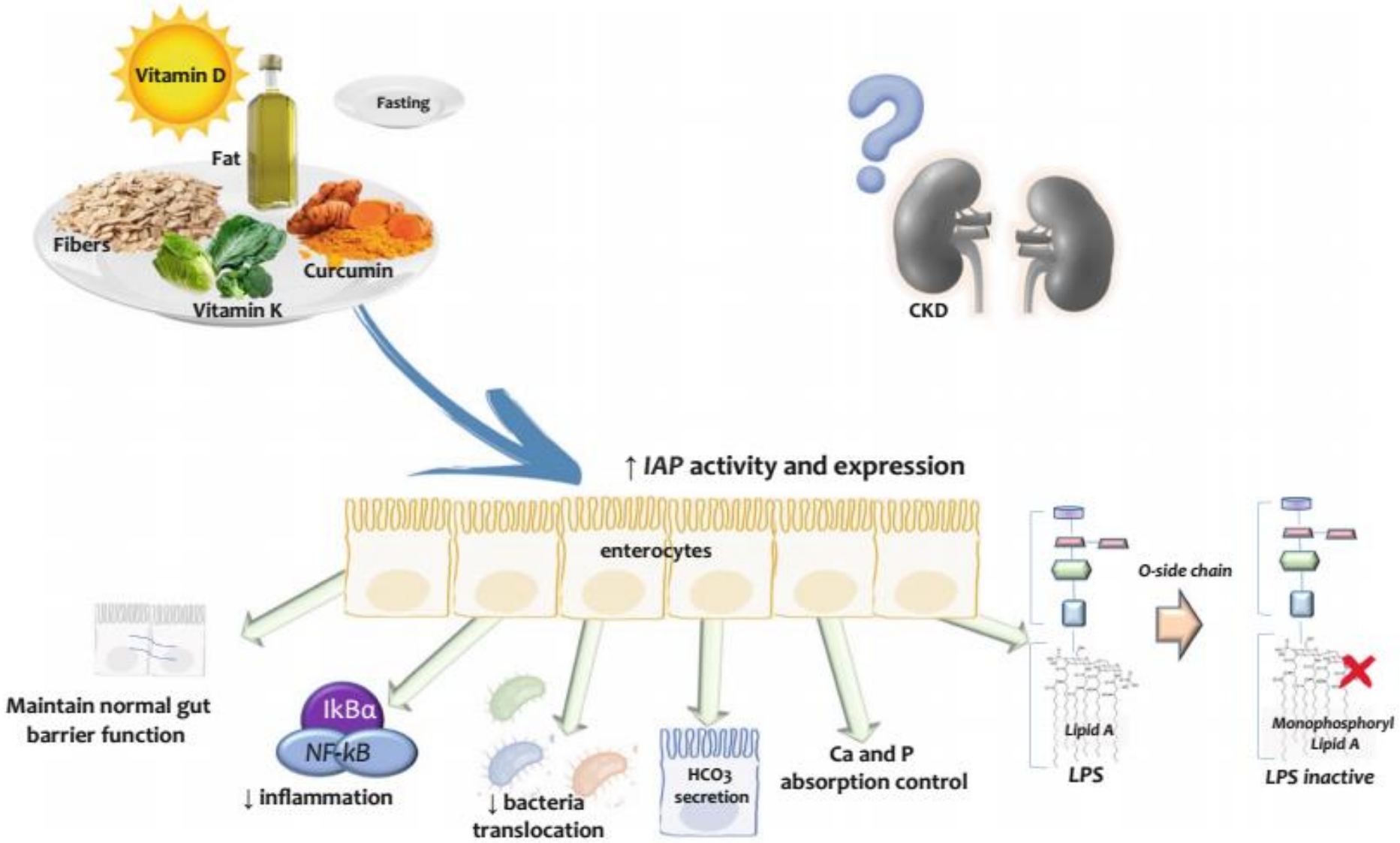


Fig. 1 IAP modulation by food components. Macro- as well as micronutrients and bioactive compounds or fasting influence a range of IAP functions that collectively serve to maintain the homeostasis and structural and functional integrity of the intestine and its transport systems for nutrients, while maintaining the gut barrier function that protects against bacteria translocation. Increased IAP gene expression and activity promoting detoxification of LPS may lead to improvement of both intestinal and systemic inflammation, increase the secretion of bicarbonate, reduce the bacteria translocation and maintain the normal gut barrier function. In patients with CKD, studies are needed to evaluate the possible effects of dietary compounds on IAP activity and expression

nutrients

MDPI

12/2/2024

Review

Alkaline Phosphatase: An Old Friend as Treatment Target for Cardiovascular and Mineral Bone Disorders in Chronic Kidney Disease

Mathias Haarhaus [1,2], Giuseppe Cianciolo [3], Simona Barbuto [3], Gaetano La Manna [3], Lorenzo Gasperoni [4], Giovanni Tripepi [5], Mario Plebani [6], Maria Fusaro [7,8,*,†] and Per Magnusson [9,*,†]

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Alkaline phosphatase treatment of acute kidney injury—an update

Thei S. Steenvoorden [1], Janneke A.J. Rood [2], Frederike J. Bemelman [1], Roberto Armstrong Jr. [3], Henri G.D. Leuvenink [3], Joost W. van der Heijden [4] and Liffert Vogt [1]

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Intestinal alkaline phosphatase modulation by food components: predictive, preventive, and personalized strategies for novel treatment options in chronic kidney disease

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Thank you!