Green Pharmacy Conference

Utrecht 27-October-2017

An endogenous substance to be used as a pharmaceutical

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Alloksys Life Sciences BV and AMRIF BV

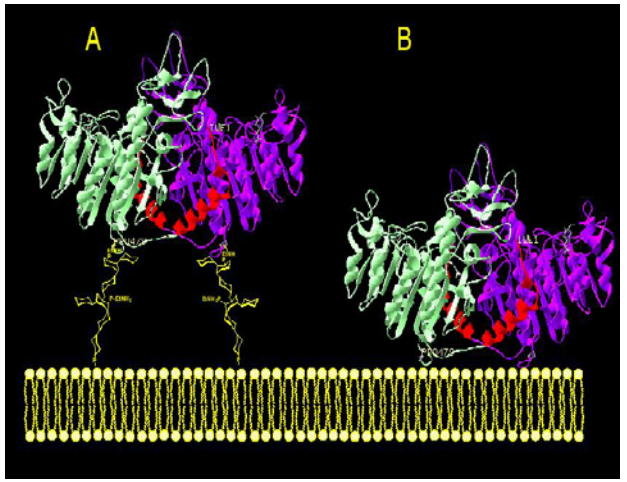
Take Home Message

A healthy gut prevents the use of antibiotics in animal husbandry

Alkaline Phosphatase, an old enzyme newly discovered

the dominant role of intestinal alkaline phosphatase in the protection of gut health

What do you know about Alkaline Phosphatase



01

Superfamily of metalloenzymes that catalyse the hydrolytic removal of phosphate from a variety of molecules.

02

Widely distributed in nature, both in pro- and eukaryotes with exception of some higher plants.

03

Glycosyl Phosphatidyl Inositol anchored membrane bound proteins.

04

Dimers of AP coordinated by 2 Zn^{2+} and 1Mg^{2+} are essential for enzymatic activity. Highest activity at pH>9.7 (where in the body !!)

Mammalian AP’s consist of several isoenzymes

Tissue Non Specific AP’s expressed in liver, bone and kidney (LBK)

Tissue specific AP’s, Intestinal (IAP), Placental (PLAP) and Germ Cell (GCAP)

Cell and Tissue Distribution of AP's

AP is present in barrier systems.

Function: transport across barrier

Highest expression is found in the intestinal barrier system (IAP),in the placental barrier system (PLAP), the blood brain barrier (TNSAP), the lung lining system (TNSAP)

In the liver it is located in bile canaliculi

In the kidneys it is expressed in the brush border of the medullary lining epithelium

Intestinal AP

Expression at gut system level

duodenum, declining in jejunum, ileum to the lowest level in colon.

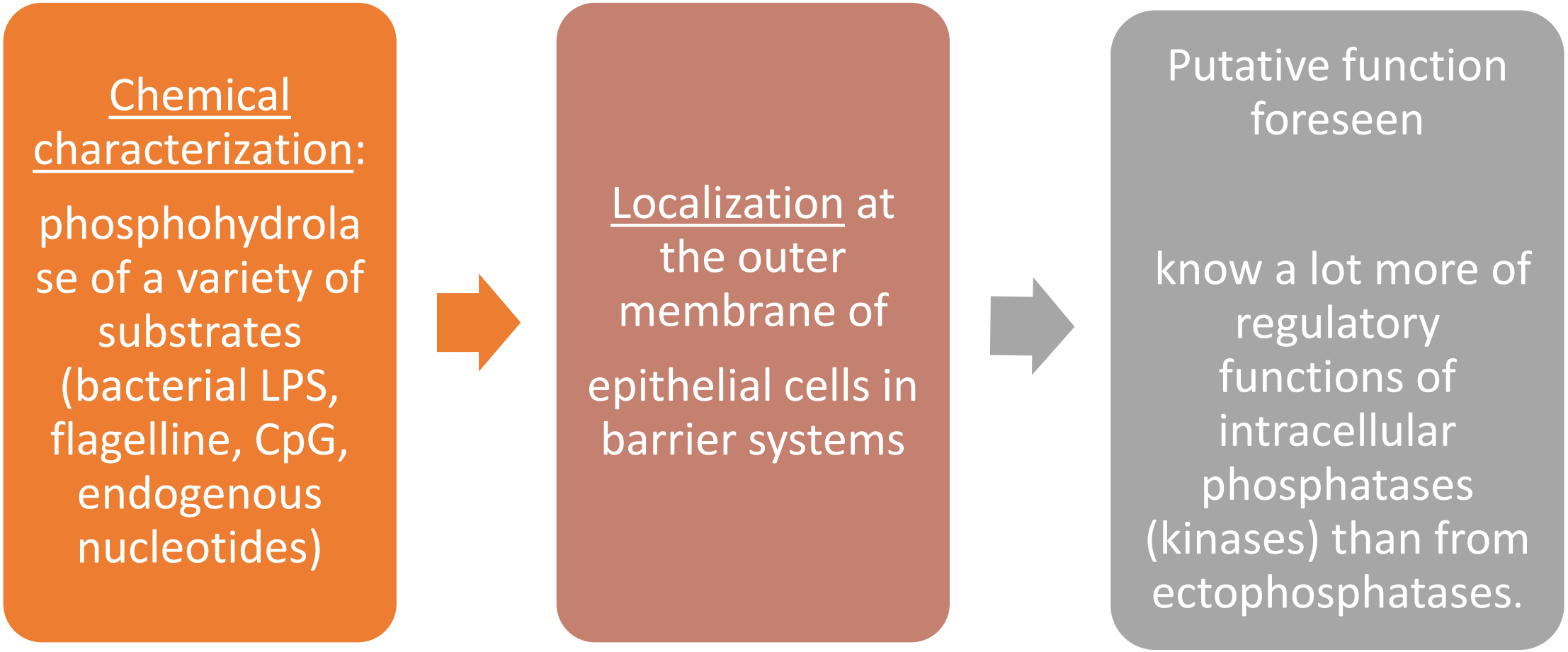
Expression at cellular level

lipid rich microdomains (lipid rafts) of the apical membrane, especially at the top of the villus

Phosphatase activity in lumen

is highest in terminal ileum and still present in the stool.

biological function



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

4

Regulation of gut microbial communities and their translocation across the gut barrier.

5

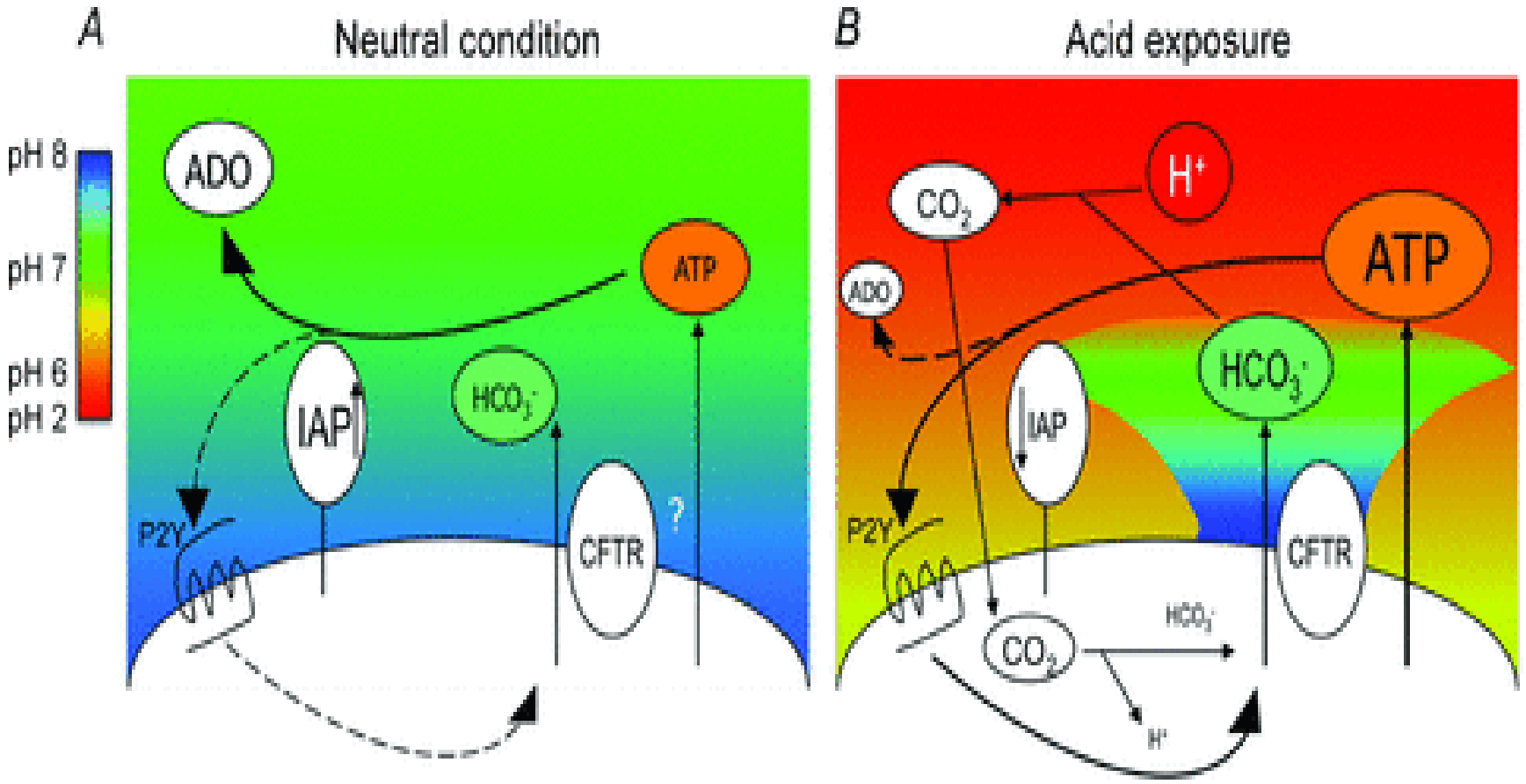
Integrity of Barrier Function

Intestinal alkaline phosphatase regulates protective surface microclimate pH in rat duodenum

- Misa Mizumori, Maggie Ham, Paul H. Guth,Eli Engel, Jonathan D. Kaunitz and Yasutada Akiba
- J Physiol 587.14 (2009) pp 3651–3663

- We conclude that the ecto-purinergic signalling system comprised of IAP, non-lytic ATP release, ATP-dependent HCO_3^- secretion and P2Y receptors regulates pH_o of the duodenal enterocyte, which in turn is important for mucosal protection from acid injury.

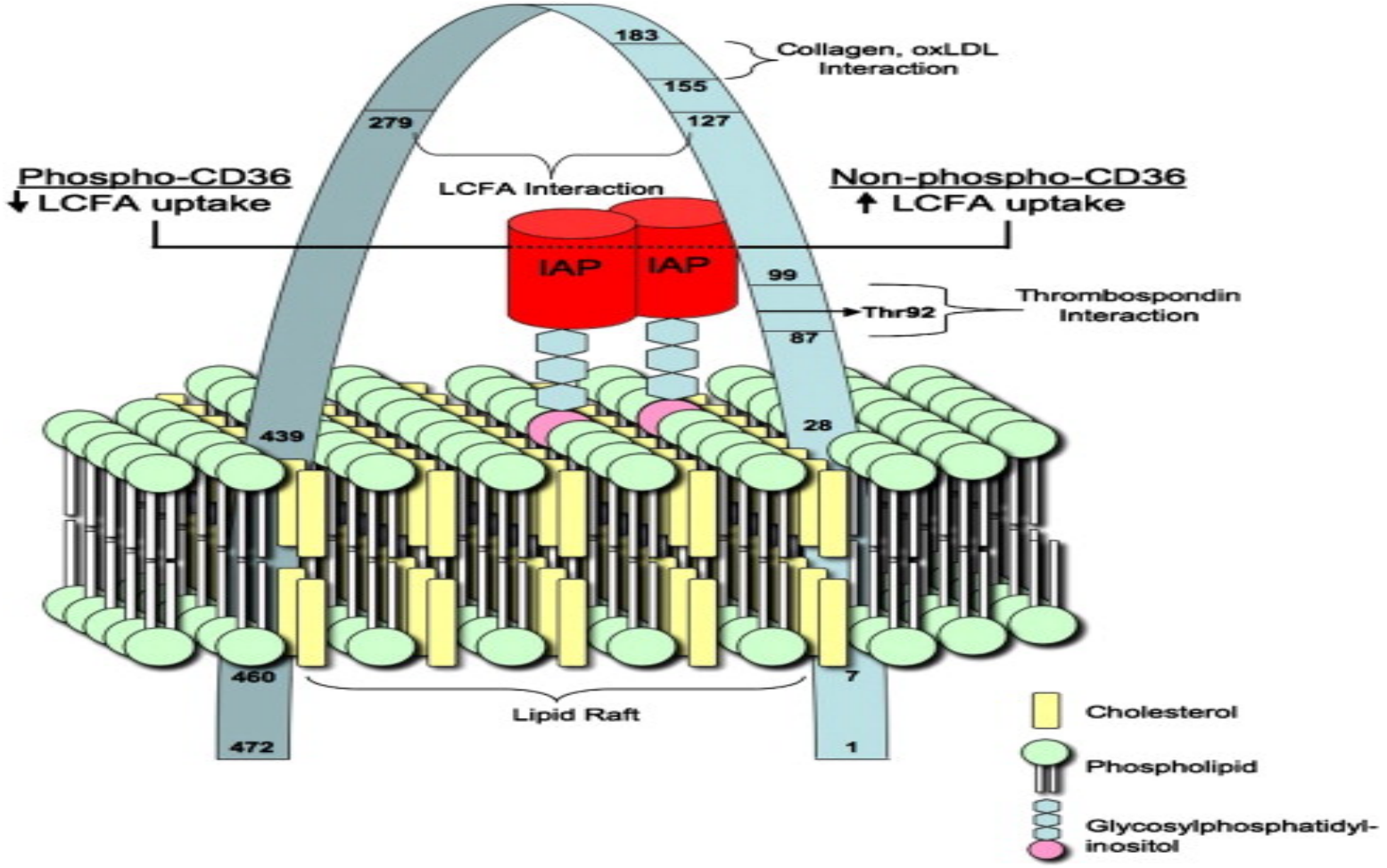
Intestinal alkaline phosphatase regulates protective surface microclimate pH in rat duodenum



The Journal of Physiology
Volume 587, Issue 14, pages 3651-3663, 14 JUL 2009 DOI: 10.1113/jphysiol.2009.172270
http://onlinelibrary.wiley.com/doi/10.1113/jphysiol.2009.172270/full#f8

Modulation of intestinal LCFA absorption

Involvement of CD36 and intestinal alkaline phosphatases in fatty acid transport in enterocytes, and the response to a high-fat diet



lMatthew D.LynesEric P.Widmaier

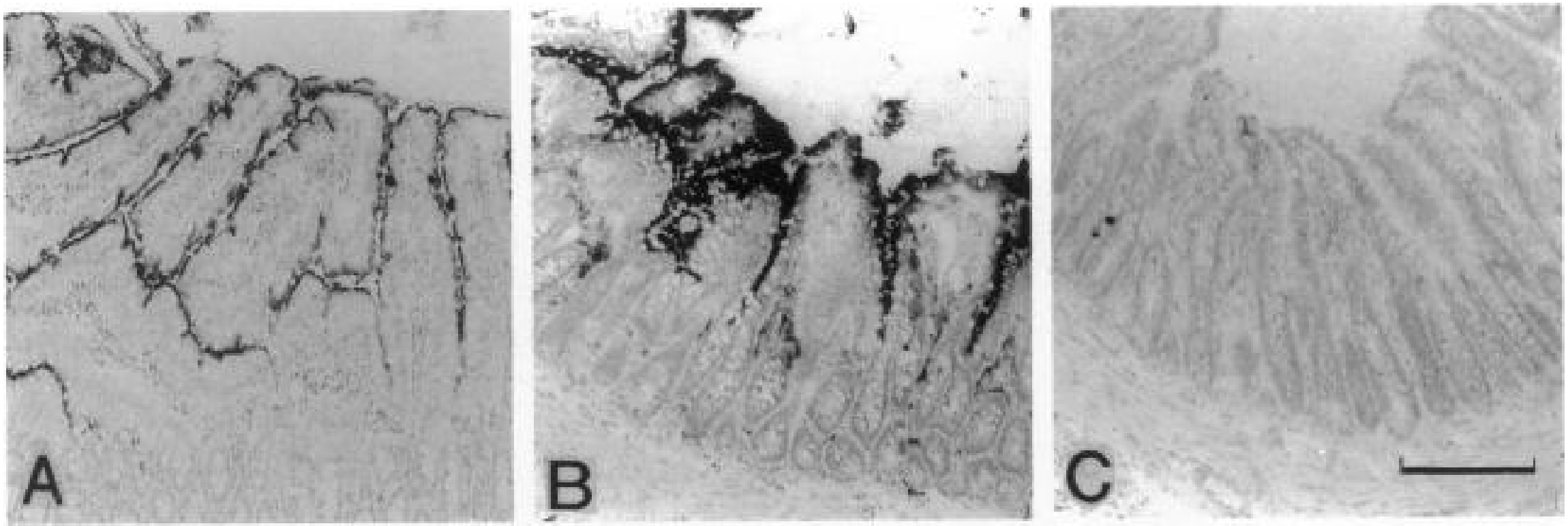
Life Sciences
Volume 88, Issues 9–10, 28 February 2011, Pages 384-391

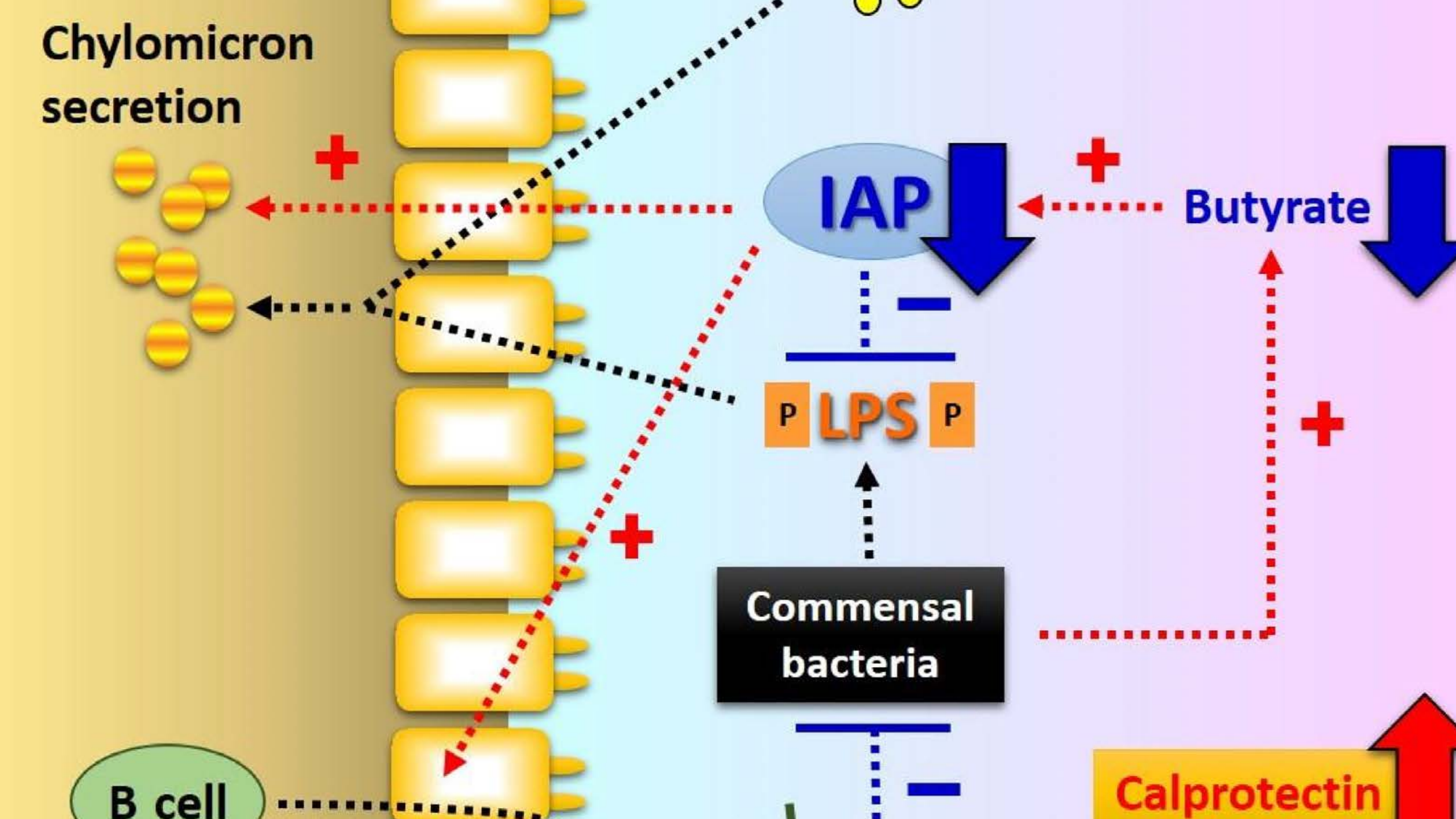
Detoxification of LPS, resulting in amelioration of intestinal and systemic inflammation

Dephosphorylation of Endotoxin by Alkaline Phosphatase in Vivo

Klaas Poelstra,* Winston W. Bakker, Pieter A. Klok, Jan A.A.M. Kamps, Machiel J. Hardonk, and Dirk K.F. Meijer*

American Journal of Pathology, Vol. 151, No. 4, October 1997


A
B
C

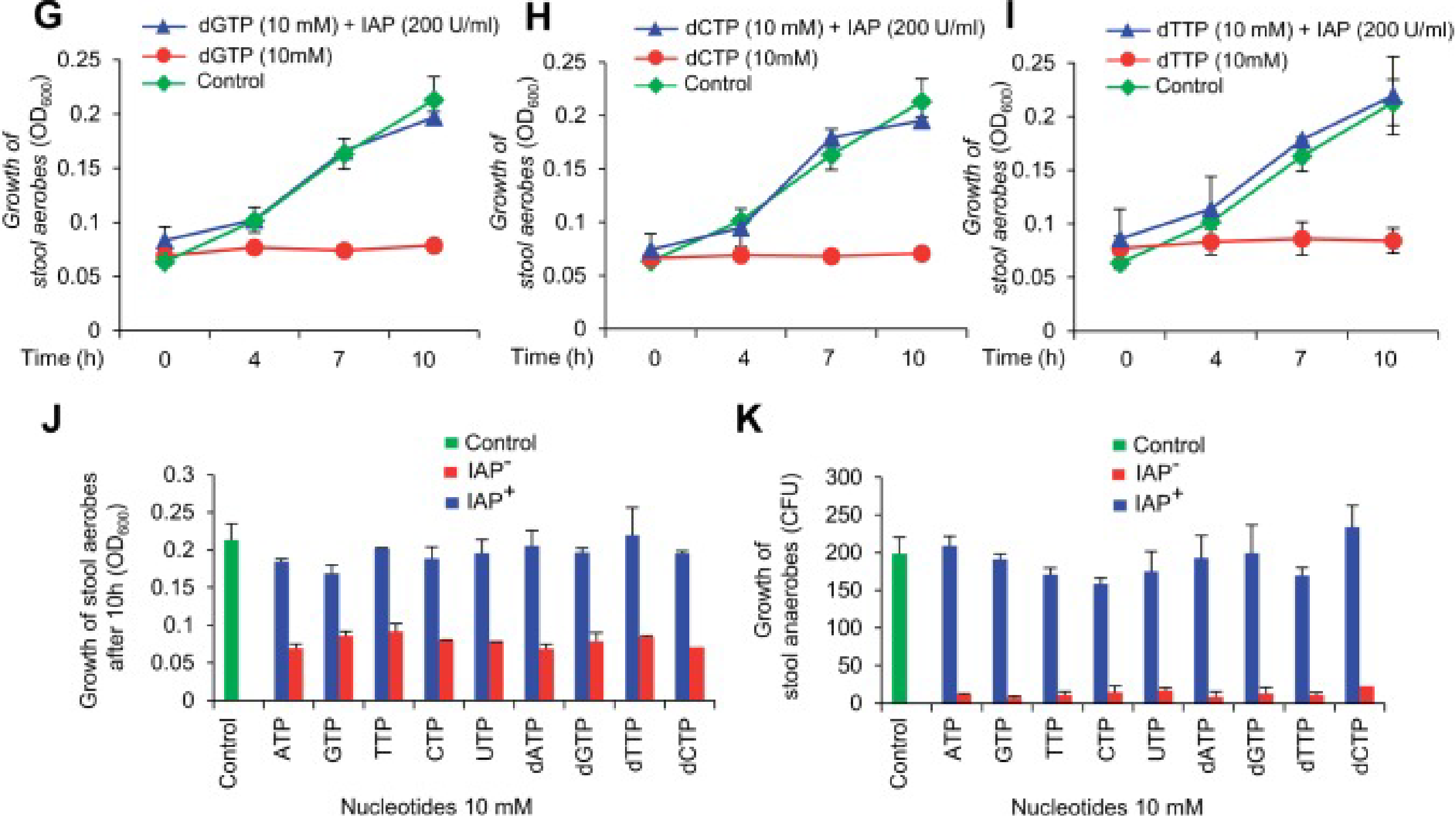

Chylomicron secretion
IAP
Butyrate
P LPS P
Commensal bacteria
B cell
Calprotectin

Regulation of gut microbial communities and their translocation across the gut barrier.

Intestinal alkaline phosphatase promotes gut bacterial growth by reducing the concentration of luminal nucleotide triphosphates

Madhu S. Malo, Omeed Moaven, Nur Muhammad, Brishti Biswas, Sayeda N. Alam, Konstantinos P. Economopoulos, Sarah Shireen Gul, Sulaiman R. Hamarneh, Nondita S. Malo, Abeba Teshager, Mussa M. Rafat Mohamed, Qingsong Tao, Sonoko Narisawa, José Luis Millán, Elizabeth L. Hohmann, H. Shaw Warren, Simon C. Robson, Richard A. Hodin

American Journal of Physiology - Gastrointestinal and Liver Physiology
15 May 2014 Vol. 306 no. 10, G826-G838


G
dGTP (10 mM) + IAP (200 U/ml)
dGTP (10mM)
Control
Growth of stool aerobes (OD600)
0.25
0.2
0.15
0.1
0.05
0
Time (h)
0
4
7
10
H
dCTP (10 mM) + IAP (200 U/ml)
dCTP (10mM)
Control
Growth of stool aerobes (OD600)
Time (h)
I
dTTP (10 mM) + IAP (200 U/ml)
dTTP (10mM)
Control
Growth of stool aerobes (OD600)
Time (h)
J
Control
IAP-
IAP+
Growth of stool aerobes after 10h (OD600)
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
ATP
GTP
TTP
CTP
UTP
dATP
dGTP
dTTP
dCTP
Nucleotides 10 mM
K
Control
IAP-
IAP+
Growth of stool anaerobes (CFU)
300
250
200
150
100
50
0
Control
ATP
GTP
TTP
CTP
UTP
dATP
dGTP
dTTP
dCTP
Nucleotides 10 mM

Integrity of Barrier Function

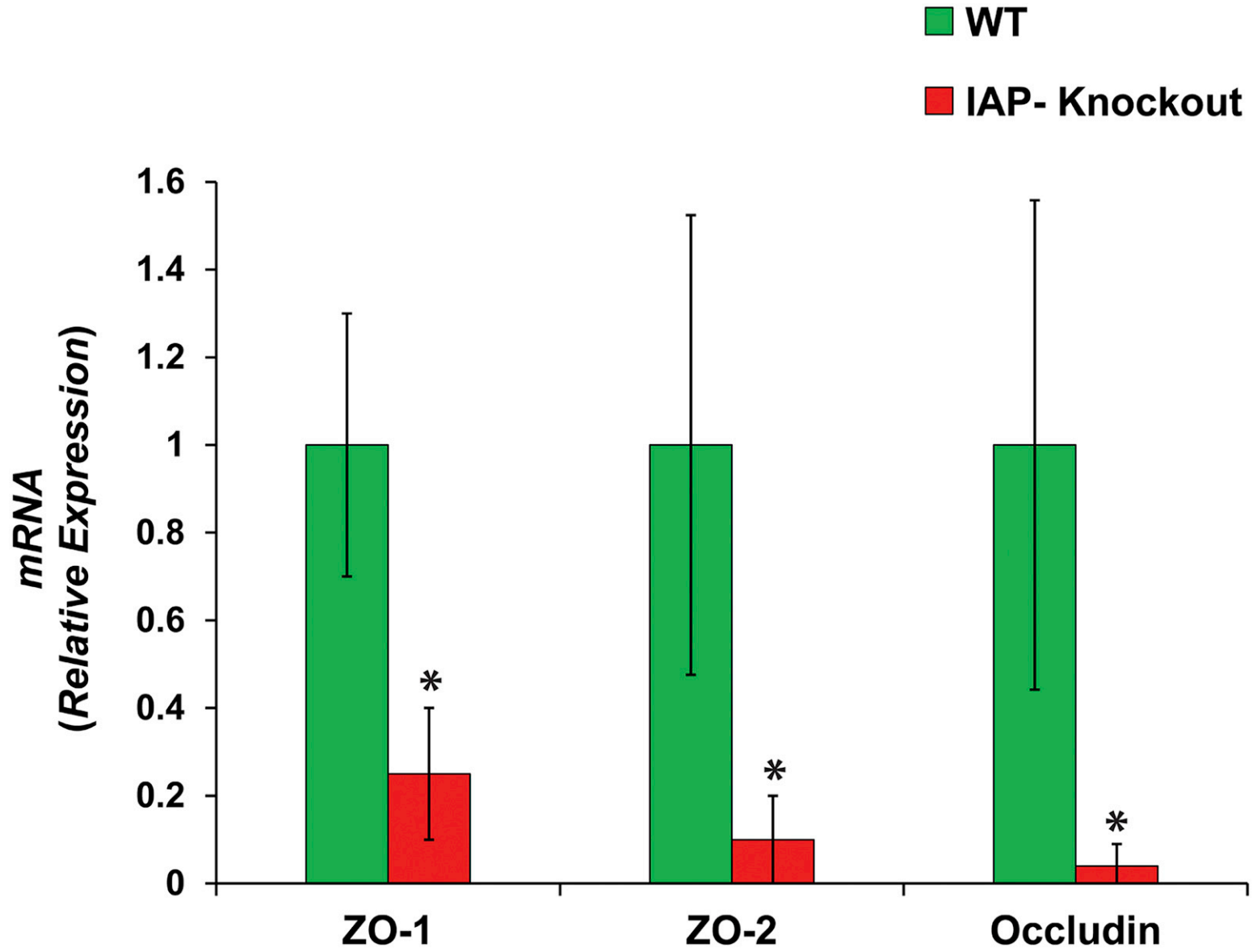
Intestinal Alkaline Phosphatase Regulates Tight Junction Protein Levels

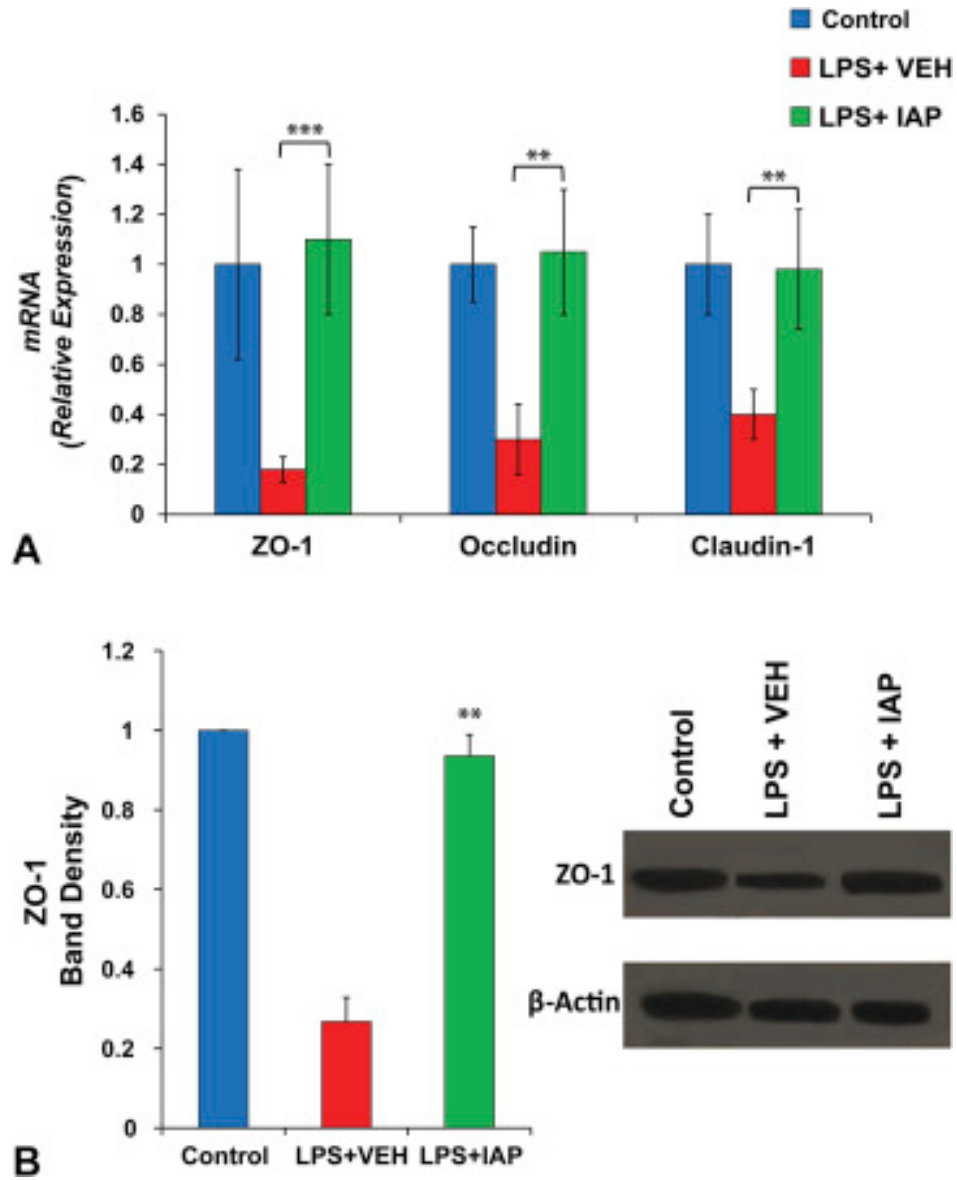
Liu W, Hu D, Huo H, Zhang W, Adiliaghdam F, Morrison S, Ramirez JM, Gul SS, Hamarneh SR, Hodin RA

J Am Coll Surgery. 2016 Jun;222(6):1009-17.

Conclusions

Intestinal alkaline phosphatase is a major regulator of gut mucosal permeability and appears to work at least partly through improving TJP levels and localization. These data provide a strong foundation to develop IAP as a novel therapy to maintain gut barrier function.


WT
IAP- Knockout
mRNA
(Relative Expression)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
*
*
ZO-1
ZO-2
Occludin


Control
LPS+ VEH
LPS+ IAP
mRNA
(Relative Expression)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
***
**
**
ZO-1
Occludin
Claudin-1
A
1.2
1
0.8
0.6
0.4
0.2
0
ZO-1
Band Density
**
Control
LPS+VEH
LPS+IAP
Control
LPS + VEH
LPS + IAP
ZO-1
β-Actin
B

A Novel Approach to Maintain Gut Mucosal Integrity Using an Oral Enzyme Supplement

Ann Surg . 2014 October ; 260(4): 706–715.

Sulaiman R Hamarneh, Mussa M. Rafat Mohamed, Konstantinos P.Economopoulos, Sara A. Morrison, Tanit Phupitakphol, Tyler J.Tantillo, Sarah S. Gul, Mohammad Hadi Gharedaghi, Qingsong Tao,Kanakaraju Kaliannan, Sonoko Narisawa, José L. Millán, Gwendolyn M.van der Wilden, Peter J. Fagenholz, Madhu S. Malo and Richard A. Hodin.

Conclusions—IAP is a major regulator of gut mucosal permeability and is able to ameliorate starvation-induced gut barrier dysfunction. Enteral IAP supplementation may represent a novel approach to maintain bowel integrity in critically-ill patients.

Take Home Message

A healthy gut prevents the use of antibiotics in animal husbandry

Hamarneh SR Mohamed MM Economopoulos KP Morrison SA Phupitakphol T Tantillo TJ Gul SS Gharedaghi MH Tao Q Kaliannan K Narisawa S Millán JL van der Wilden GM Fagenholz PJ Malo MS Hodin RA

Ann Surg . 2014 October ; 260(4): 706–715.

A novel approach to maintain gut mucosal integrity using an oral enzyme supplement.

Conclusions—IAP is a major regulator of gut mucosal permeability and is able to ameliorate starvation-induced gut barrier dysfunction. Enteral IAP supplementation may represent a novel approach to maintain bowel integrity in critically-ill patients.

Description of human alkaline phosphatases (APs)[a]

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