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EDITORIAL

Probiotic-Derived Polyphosphate Prevents Pancreatitis

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Acute pancreatitis (AP) is an acute inflammatory condition of the pancreas that is most commonly caused by alcohol abuse [1]. It is a severe and potentially fatal disease due to its highly morbid local and extra-pancreatic complications such as walled-off pancreatic necrosis, the systemic inflammatory response syndrome, and multi-organ dysfunction. Intrapancreatic activation of trypsin followed by autodigestion underlies the pathogenesis of pancreatitis [1]. Recent studies have suggested that in addition to intrapancreatic activation of trypsin [1], translocation of intestinal microflora to the pancreas due to impaired intestinal barrier function could also acutely injure the pancreas via the activation of pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) [2, 3]. This idea is supported by the observation that intestinal barrier dysfunction and inflammatory injury are associated with the initiation and propagation of severe complications associated with AP. Thus, disruption of intestinal barrier function, followed by the translocation of intestinal microflora into the circulation and the pancreas itself, may increase the severity of AP. Studies have further suggested that increased colonization of beneficial microbiota with the ability to enhance intestinal barrier function or decrease the populations of pathogenic bacteria associated with the disruption of the intestinal barrier function may prevent or ameliorate AP and its serious complications [2, 3].

In this issue of *Digestive Diseases and Sciences* (Fig. 1), Takauji et al. have addressed this issue using beneficial microbiota-derived polyphosphate (poly P). They aimed to clarify whether probiotic *Lactobacillus brevis SBL88*-derived long-chain poly P attenuates AP in a cerulein (a cholecystokinin receptor agonist)-induced mouse model of AP through alterations of the intestinal microflora [4]. They had previously shown that oral intake of poly P efficiently induced clinical remission in patients with ulcerative colitis (UC) through enhancement of intestinal barrier function [5, 6]. Although the beneficial effects of poly P on the gastrointestinal tract have been reported, the effects on distant organs other than the intestine still remain unknown. In this study, the authors examined whether poly P alters intestinal microbiome composition and increases intestinal barrier function, and whether facilitation of the interaction between host and beneficial bacteria induced by poly P attenuates pancreatic inflammation and injury by preventing the translocation of pro-inflammatory bacterial products from the intestine to the portal vein. To address these issues, the authors initially examined whether oral administration of poly P to mice prior to the induction of AP by systemic injection of cerulein altered the composition of the gut microbial flora. Analysis of 16S rRNA gene sequencing data revealed increased colonization of some beneficial bacteria and decreased colonization of the inflammation-associated *Desulfovibrio* species. As for the preventive effects of poly P on the development of AP, oral administration of poly P prior to induction of AP attenuated the development of pancreatic injury. In fact, concentrations of serum amylase and lipase were significantly reduced in mice treated with both cerulein and poly P compared with those treated with cerulein alone. Such preventive effects of poly P on the development of AP could be partially explained by the suppression of pancreatic production of the chemokine, C–C motif chemokine ligand 2 (CCL2), and reduced influx of myeloperoxidase (MPO)-positive granulocytes into the pancreas. Having confirmed the suppressive function of poly P on acute pancreatic injury, the authors turned their attention to alterations in intestinal barrier function. For this purpose, they examined the expression of the tight junction (TJ) proteins zonula occludens (ZO)-1 and occludin in the colon, both components of intercellular TJs that regulate paracellular permeability to ions and small solutes. They found that oral administration of poly P remarkably augments the expression of both ZO-1 and occludin in the colonic epithelial cell membrane layer. Taken together, the data suggested that increased colonization of

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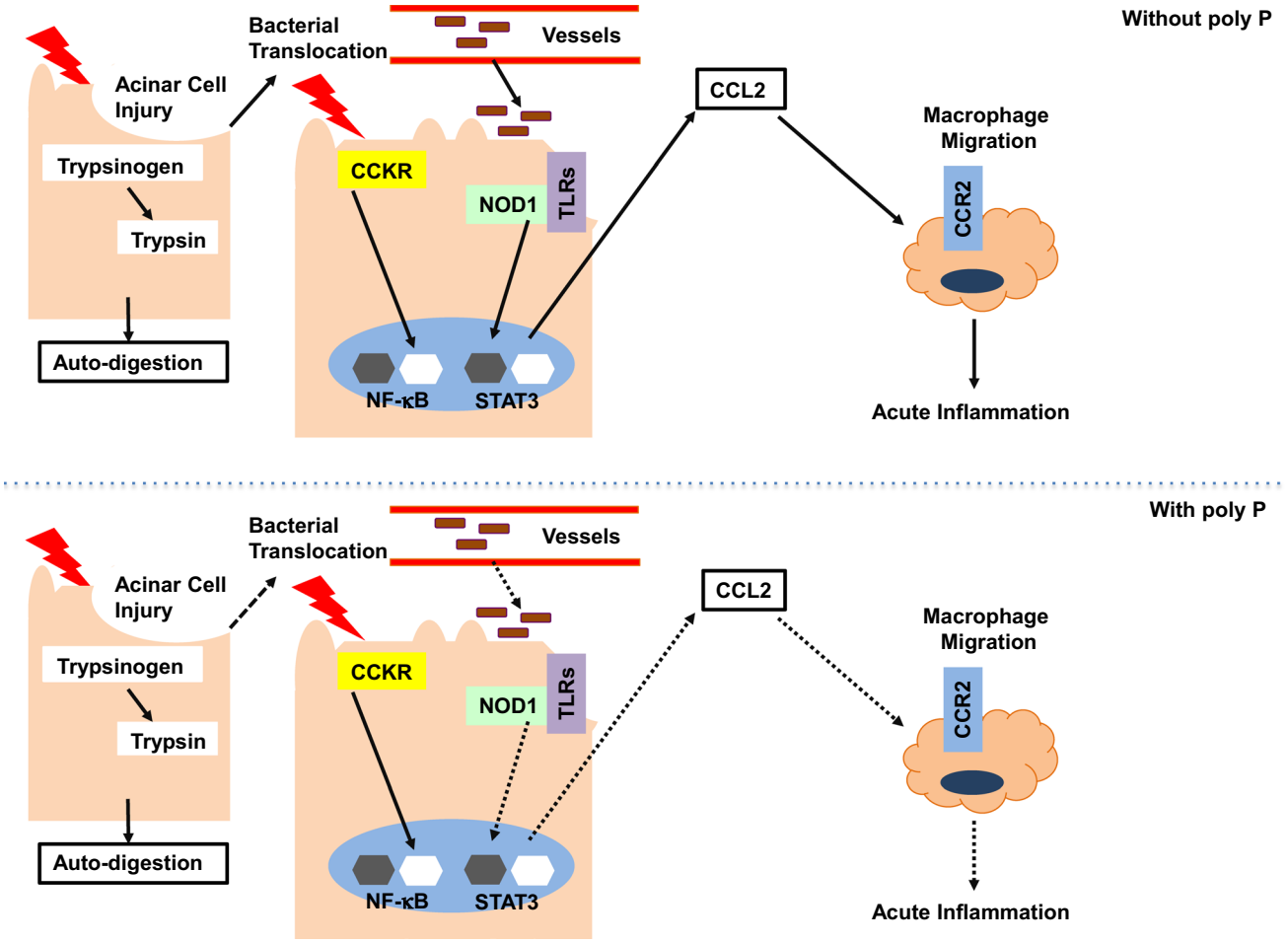

Fig. 1 Suppression of acute pancreatitis by probiotic-derived polyphosphate. Intrapancreatic activation of trypsin through the activation of cholecystokinin receptor (CCKR) causes autodigestion. In parallel, impaired intestinal barrier function leads to bacterial translocation and translocation of pathogen-associated molecular patterns (PAMPs) into the portal vein. Sensing of intestinal bacteria by toll-like receptors (TLRs) and nucleotide-binding oligomerization domain 1 (NOD1) acts in concert with CCKR pathways to produce C–C motif chemokine ligand 2 (CCL2) from pancreatic acinar cells. Pro-inflammatory macrophages migrate into the pancreas to initiate acute pancreatic injury (top panel). Poly P augments intestinal barrier function via alteration of intestinal microbiota composition and reduces CCL2 production presumably by inhibiting translocation of bacteria and PAMPs into the portal vein and the systemic circulation

beneficial bacteria as well as disappearance of pathogenic bacteria induced by poly P attenuates the development of AP via changes in the expression of TJ components.

The findings of this study are of interest to the scientific community since this is the first report to suggest that oral intake of probiotic-derived bioactive molecules can be a potential option for treating pancreatic inflammation. Since the safety of poly P has already been confirmed by its oral administration to patients with UC [5, 6], its clinical application for the prevention of AP and AP-associated complications may be considered feasible. Nevertheless, several issues need to be addressed in order further to elucidate the molecular mechanisms responsible for the suppressive effects of poly P on pancreatic injury. First, whether prior administration inhibits the translocation of intestinal microflora to the systemic circulation or pancreas is unproven. Thus, investigation of bacterial translocation by fluorescence in situ hybridization in living animals, using a universal bacterial probe, would be desirable. Since augmentation of TJ protein expression is only a surrogate for inhibition of intestinal bacterial translocation or alteration of paracellular permeability, quantification of pancreatic bacterial density and direct measurement of paracellular permeability would be required. Although inflammation is considered as a strong driver for increased gut paracellular permeability, it is not known whether reduced expression of TJ proteins associated with inflammation directly induces translocation of intestinal bacteria and their pathogen-associated molecular patterns (PAMPs) via the intestinal paracellular route [7]. In fact, recent studies by Akiba et al. showed that lipopolysaccharide (LPS) derived from Gram-negative bacteria enters the systemic circulation via the epithelial transcellular route, but

not via the paracellular pathway [8]. Therefore, administration of poly P may inhibit the development of AP through a mechanism independent of suppressing bacterial translocation despite the enhanced expression of TJ proteins. In this regard, it would be necessary to examine the action of poly P in the context of sterile inflammation triggered by the release of damage-associated molecular patterns (DAMPs) that occurs after tissue injury. Intrapancreatic activation of trypsin followed by autodigestion induces a robust release of DAMPs including high mobility group box 1 (HGMB1) through acinar cell death [2]. Recent studies highlight the importance of the gut–liver axis in the development of systemic inflammation [9]. In this scenario, HMGB1 release following acinar cell injury activates TLR4 in the gastrointestinal tract after which transport of LPS into the portal vein was accelerated as the result of TLR4-mediated gut mucosal injury [8, 9]. Activation TLR4 expressed on Kupffer cells by LPS translocated from the gut to the portal vein produces pro-inflammatory cytokines with consequent systemic inflammation associated with AP. In addition to bacterial translocation, the molecular mechanisms how poly P inhibits acute pancreatic injury are needed to be examined in terms of its effects on sterile inflammation following activation of the gut–liver axis.

Another issue that needs to be verified is the type of PRRs activated by oral administration of poly P. PAMPs, derived from the intestinal microflora, activate the host innate immune system via PRRs, such as TLRs and NLRs [2, 3]. *In vitro* reporter gene assays would be useful to determine the PRRs activated by poly P. Third, pancreatic pro-inflammatory cytokine and chemokine responses, which are suppressed by the activation of poly P, need to be assessed further. We had previously shown that sensing of intestinal bacteria by intracellular NOD1 expressed in pancreatic acinar cells mediates experimental pancreatitis through the production of CCL2, IFN-β, and IL-33 [2, 3]. Given that oral administration of poly P significantly reduced pancreatic CCL2 expression, poly P is likely to inhibit the migration of macrophages expressing C–C chemokine receptor type 2 and producing pro-inflammatory cytokines, as per our previous reports [2, 3].

In conclusion, this study opens new research vistas that can facilitate the development of prebiotics for AP. AP may be treated with poly P, derived from beneficial microbiota, which can alter the intestinal microbiota composition and possibly intestinal paracellular permeability. Moreover, it would be very interesting to investigate whether poly P inhibits the development of autoimmune pancreatitis, since intestinal dysbiosis is associated with the development of this disorder as well [10].

Compliance with Ethical Standards

Conflict of interest The authors have no relevant conflicts of interest to declare.

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