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ADDENDUM

Inflammatory bactericidal lectin RegIIIβ: Friend or foe for the host?

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ABSTRACT

In the inflamed gut, the bactericidal lectin RegIIIβ is massively produced by intestinal mucosa. RegIIIβ binds peptidoglycan and lipid A respectively, and thus can kill certain Gram-positive and Gram-negative bacteria, including the gut commensal microbiota and enteropathogenic bacteria. Considering the expression pattern and bactericidal activity, RegIIIβ is believed to be a host defense factor for protecting against the infection with enteropathogenic bacteria. However, it was poorly understood how RegIIIβ recognizes the target bacteria and kill them, and how RegIIIβ plays role(s) in infectious diarrhea. Therefore, our recent study has focused on RegIIIβ-target recognition, killing of Gram-negative bacteria, and host protective functions of RegIIIβ for infectious diarrhea inflicted by *Salmonella* Typhimurium. Here, we discuss novel insights into the protective role of RegIIIβ in infectious diarrhea, and propose avenues towards novel therapeutic interventions for *Salmonella* diarrhea.

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Introduction

Gut mucosal defense is critical for prevention of the infection by enteropathogens since the surface of the intestinal tract is continuously exposed to the external environment, and is a frequent entry site for attack. Intestinal epithelial cells equip several mucosal barrier systems for protecting against infection. Epithelial antimicrobial proteins such as defensin exert a barrier function by killing the invading enteropathogens. Furthermore, antimicrobial proteins also display the bactericidal activity against numerous other bacteria, thereby shaping the composition of the gut resident microbiota. These microbial communities are essential for maintaining host homeostasis, including prevention of enteropathogen growth in the luminal gut (referred to as colonization resistance).[1] As the balanced microbiota is absolutely required for the colonization resistance, antimicrobial protein-dependent shaping of the microbiota may be important in host defense against the gut infection with enteropathogens.

The RegIII (regenerating gene family protein III) lectins belong to the antimicrobial proteins, and are expressed in epithelial cells of stomach, small intestine and colon. Subsequently, they are secreted into the gut lumen. Expression of RegIII lectins is dramatically increased in response to bacterial gut colonization and pathogenic infection leading to inflammation.[2] The expression is dependent on bacterial gut colonization which elicits RegIII expression in a toll-like receptor (TLR)-MyD88 dependent fashion.[3,4] In addition, inflammation-elicited IL-22 can elevate the RegIII production dramatically. Thus, the expression of RegIII lectins is inducible.[2,4,5] The inducible expression and the bactericidal activity has led to the hypothesis that RegIII lectins act as innate immune effectors against enteropathogenic bacteria. Indeed, several reports lend support to this hypothesis; RegIII lectins protect mice against infection by some enteropathogenic bacteria.[6–11] However, in spite of these studies, the role of RegIII lectins in bacterial gut infection has not been fully understood. In particular, we

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know little about the remission phase at the end of an acute diarrheal infection and how the pathogen is finally eliminated from the gut lumen. More recently, using the streptomycin mouse model for *Salmonella* diarrhea, we have reported that produced RegIIIβ in the inflamed gut prolongs the duration of gut colonization of *Salmonella* and enteropathy.[12] In this particular case RegIIIβ does not appear to act as a defensive molecule against the gut infection, but rather prolongs transmission. Thereby, it may substantially benefit the pathogen.[13] Controlling the RegIIIβ activity and the RegIIIβ-mediated biological effects might offer new avenues for therapy.

Bactericidal mechanism of RegIII lectins

The murine intestine expresses RegIIIβ and RegIIIγ, whereas HIP/PAP (human hepatocarcinoma intestine pancreas/pancreatitis-associated protein), a human counterpart for RegIII, is expressed in the human small intestine. These lectins are produced by the intestinal epithelial cells and Paneth cells. Pro-RegIII lectins are initially expressed with an inhibitory N-terminal segment, which is subsequently proteolytically removed by trypsin to yield the active form. The two murine RegIII homologues, i.e., RegIIIβ and RegIIIγ are very similar regarding the expression, the regulation and the structure. Nevertheless, they appear to have distinct bactericidal activity. This differential activity may be attributable to the few amino acid differences which exist in the sequences.[14,15] In particular, the amino acid differences in the two distinct loops define the substrate recognition and the bactericidal activity as described below in detail. Earlier studies have suggested that RegIIIγ can kill Gram-positive bacteria but not Gram-negative bacteria whereas RegIIIβ displays bactericidal activity against both Gram-positive and Gram-negative bacteria.[2,3] In addition, the growth phases of the test bacteria seem to have a major effect of the RegIII killing phenotype.[15,16] This featured bactericidal activity is explained by the different recognition how the RegIII lectins bind the target bacteria. RegIIIγ binds to Gram-positive bacteria by recognizing peptidoglycan, compromising the integrity of the peptidoglycan layer in which the mechanism is not fully deciphered, and thereby leading to osmotic lysis with leakage of cytoplasmic content.[3] It is conceivable that RegIIIβ kills Gram-positive bacteria in a similar fashion. In contrast, the bactericidal mechanism of RegIIIβ against Gram-negative bacteria appears to be distinct from that of RegIIIγ against Gram-positive bacteria. The Gram-negative cell envelope (i.e. the outer membrane (OM)) overlays the bacterium, thereby preventing access of RegIIIβ into the peptidoglycan layer. To solve this problem, RegIIIβ appears recognize another target, i.e., the lipopolysaccharide (LPS) which makes up the bulk of the outer leaflet of OM (Fig. 1). RegIIIβ recognizes the carbohydrate moiety of lipid A, a component of LPS.[15]

There is some information about the structural basis explaining the substrate specificity of RegIIIβ. Two distinct loops found in the three-dimensional structure of HIP, are also present in RegIIIβ. This suggests that RegIII lectins have similar loops, designated as “loop 1” and “loop 2”. Furthermore, the respective loops contain the characteristic amino acid motifs: DPT and EPN in loop 1; ERN in loop 2. The EPN motif in loop 1 of HIP/PAP governs specificity for the carbohydrate recognition and the bactericidal activity against Gram-positive bacteria,[14] whereas the EPN motif of RegIIIβ is dispensable for the recognition of substrate target and for the bacterial killing.[15] Instead, the ERN motif and residue Asp-142 in loop 2 contribute to these activities of RegIIIβ. The selective killing by RegIII lectins may be attributable to this difference in role of the respective loops on the substrate recognition and bacterial killing.

It previously has been found that a *Salmonella enterica* serovar Typhimurium mutant lacking O-antigen of

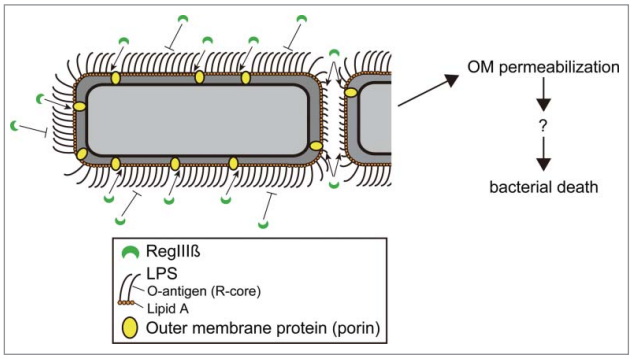

Figure 1. Bactericidal mechanism of RegIIIβ against Gram-negative bacteria. O-antigen of LPS seems to inhibit approaching of RegIIIβ into the target bacteria, i.e., steric hindrance. Therefore, RegIIIβ can approach at the septum of cell division and around porin-inserted site, in which the O-antigen is immature and LPS lacks. The interaction of RegIIIβ elicits outer membrane (OM) permeabilization, and subsequently RegIIIβ traverses OM and interacts with the bactericidal target in the periplasm or the cytoplasmic membrane. Finally, the target bacterium undergoes cell death.

LPS is more susceptible to RegIIIβ than the isogenic wild-type strain.[16] This suggests that the O-antigen of LPS may prevent the access of RegIIIβ to the target lipid A. Furthermore, this can explain the growth phase dependence of the bactericidal activity of RegIIIβ, i.e., *S.* Typhimurium grown in the stationary phase becomes more resistant to RegIIIβ than cells taken from the logarithmic growth phase.[15] Indeed, bacteria in the logarithmic growth phase harbor higher amounts of lipid A on its surface.[16] This also may reflect that bacteria in the logarithmic growth phase have the RegIIIβ-accessible lipid A at the septum of cell division site (Fig. 1). Furthermore, another accessible area can be considered. LPS is though to account for approximate 73% of the outer surface of *S.* Typhimurium.[17] By this fact, it is conceivable that outer membrane proteins such as porin are present in the remaining area (27%). Thus, through this area, RegIIIβ can approach the lipid A without the steric hindrance by the O-antigen (Fig. 1).

The interaction of RegIIIβ with lipid A promotes OM permeabilization, thereby compromising OM integrity (Fig. 1). This appears to enable periplasmic access and explain the ability of RegIIIβ to kill Gram-negative bacteria.[16] At present, molecular mechanism of the OM permeabilization by RegIIIβ is not fully understood. Furthermore, the OM permeabilization is insufficient for explaining how RegIIIβ triggers Gram-negative bacterial death. Upon the OM permeabilization, RegIIIβ traverses the OM, presumably to reach the bactericidal target somewhere in the periplasm or the cytoplasmic membrane (also referred as to inner membrane (IM)). Most of antimicrobial proteins such as defensins kill bacteria by disrupting the peptidoglycan layer and subsequently lysing the IM through forming dimeric pores. Similarly, HIP/PAP (also maybe RegIIIγ) permeabilizes the Gram-positive bacterial membrane by forming a membrane-penetrating oligomeric pore.[18] These findings suggest that RegIIIβ might form an oligomeric pore on the IM, resulting in the bacterial osmolytic lysis (Fig. 1). Clarifying the molecular mechanism of the OM permeabilization and subsequent effects (may be pore formation on IM) in the RegIIIβ-mediated killing will be an interesting topic for future work.

Role of RegIII lectins in intestinal homeostasis

Human α-defensins 5 and 6 (HD-5 and HD-6) are constitutively expressed in the resting intestinal mucosa.[19] They account for most of the intestinal antimicrobials including defensin, RegIII, lysozyme and phospholipase A2.[20] Similarly, cryptidins, counterpart to human α-defensins, are abundantly expressed in the murine mucosa. Thus, α-defensin is believed to significantly impact the microbiota composition and prevent from infection by exogenous pathogens in order to maintain the homeostasis. Indeed, the microbiota composition in defensin-nonfunctional mice (matrix metalloproteinase 7 knockout mice, *MMP7*$^{-/-}$) and HD-5-transgenic mice are apparently different from those in wild-type littermate control animals.[21] Furthermore, the HD-5 transgene was shown to confer resistance to enteric salmonellosis.[21] In contrast, RegIII lectins do not seem to contribute to construction of the balanced microbiota and prevention from infection in intestinal homeostasis. This might be attributable to their low gut luminal concentrations under homeostatic conditions.[12,22] However, RegIII lectins have another important role in the intestinal mucosa. During intestinal homeostasis, secreted RegIIIγ (also RegIIIβ) is not dispersed to the gut lumen, but appears to localize in the inner mucus layer. The RegIIIγ in the mucus layer appears to kill the Gram-positive commensals, and may thereby prohibit the excess interaction of these microbes with the intestinal epithelial cells.[22] This plays an important role in the homeostasis because the excess stimulation by the invaded commensal bacteria elicits unpredictable activation of host immune responses, facilitating injury of the affected host cell. RegIIIγ deficiency goes along with altered mucus distribution, increased bacterial loads contacting with intestinal epithelial cells, and elevated expression inflammatory markers by the mucosa.[10] The RegIIIγ-mediated spatial segregation of microbiota and the intestinal mucosa may be restricted to Gram-positive commensals, but may not pertain to Gram-negatives due to its bactericidal spectrum. It is therefore conceivable that RegIIIβ helps to fill this gap (Fig. 2). In line with this hypothesis, ethanol-fed *RegIII*$\beta^{-/-}$ mice have been found to have significantly higher numbers of commensal bacteria associated with mucus layer compared with ethanol-fed wild-type mice.[23]

Protective role of RegIII lectins in infection with enteropathogenic bacteria

RegIII lectins have been shown to protect mice against some enteropathogenic bacteria. RegIIIγ acts as a

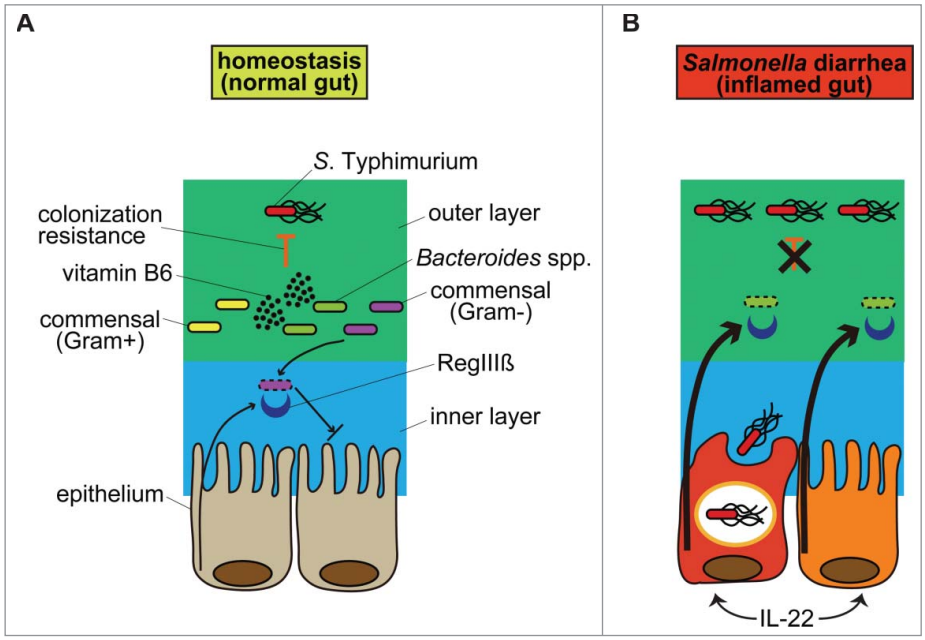

Figure 2. *Bacteroides* spp. killing by RegIIIβ in the inflamed gut leads to decreased luminal levels of vitamin B6, resulting in enhanced *Salmonella* diarrhea. (A) In intestinal homeostasis, *Bacteroides* spp. produces vitamin B6, which seems to contribute to in part the colonization resistance against enteric pathogens. Thus, *Salmonella* Typhimurium cannot infect the normal gut. Furthermore, basal levels of RegIIIβ in the inner layer kill Gram-negative commensal bacteria, preventing from their translocation. (B) In inflamed gut, RegIIIβ is inducibly expressed under the IL-22-dependent manner, and massively secreted into gut lumen, i.e., the outer layer. *Bacteroides* spp. is killed by the secreted RegIIIβ, and thereby luminal levels of vitamin B6 are reduced. As a result, this change contributes to the sustained *S.* Typhimuruium colonization by reduced activity of the colonization resistance, resulting in prolonged *Salmonella* diarrhea.

defensive molecule against infection with Gram-positive bacteria such as *Listeria monocytogenes* and vancomycin resistant *Enterococcus* (VRE).[6,7] The protective effects are attributable to direct killing of the enteropathogens in the intestinal lumen. In addition, alternative mechanisms of protection are conceivable. This is hinted by the fact that RegIIIγ can protect against gut infection by Gram-negative bacteria such as *Citrobacter rodentium* and *Salmonella enterica* serovar Enteritidis.[9,10] The bactericidal activity of RegIIIγ against these pathogens cannot explain for the protection because Gram-negative bacteria are not susceptible to RegIIIγ, at least under the in vitro assay conditions that were applied. How does RegIIIγ prevent the infection with Gram-negative bacteria such as *C. rodentium* or *S.* Enteritidis? Several scenarios can be considered. (A) RegIIIγ may suppress the deep invasion into colonic crypts by undiscovered mechanisms, thus limiting tissue infection. (B) RegIIIγ may fortify barrier functions of the mucus layer. RegIIIγ is able to bind glycan. MUC2, the major component of intestinal mucus layer, is highly glycosylated. Thus, one can hypothesize that the interaction of RegIIIγ with glycan and MUC2 respectively in the mucus layer strengthens the integrity of the mucus barrier. Furthermore, the self-assembly of RegIIIγ may stabilize the frame structure composed of mucin. These may elicit alteration in the structure or distribution of mucus, thereby inhibiting invasion of the mucus layer. (C) RegIIIγ may entrap bacteria in the mucus layer. This may restrict the movement of the invading bacteria in the mucus layer, and may subsequently increase likelihood that other antimicrobials, for example defensins or RegIIIβ, to kill the trapped bacteria. (D) RegIIIγ may affect the composition of microbial communities associated with the mucosal surface. The absence of RegIIIγ has been shown to increase the amount of Firmicutes in the ileal mucosal surface, indicating that RegIIIγ suppresses overgrowth of the Gram-positive commensal bacteria in intestinal mucosal surface.[22] This may indirectly prevent pathogen access to the mucus layer.

RegIIIβ also plays a protective role against Gram-negative enteropathogens. TLR2-dependent RegIIIβ expression appears to contribute to intestinal clearance of *Yersinia pseudotuberculosis*.[8] However, it remains unknown whether the direct killing of *Y. pseudotuberculosis* by RegIIIβ can explain for this protective effect, as recombinant RegIIIβ proteins

have been shown to lack the bactericidal activity towards *Y. pseudotuberculosis*.[8] Moreover, the absence of RegIIIβ confers susceptibility to salmonellosis upon oral infection with *S.* Enteritidis.[11] In conclusion from these studies, it appears that RegIIIβ protects by inhibiting translocation of the pathogens from the gut lumen into intestinal tissue, rather than by killing the pathogens in the gut lumen.

Unexpected role: RegIIIβ prolongs the duration of *Salmonella* diarrhea

Intestinal infection and gut inflammation dramatically change the expression pattern of epithelial antimicrobials. Especially, RegIII lectins are massively expressed in a MyD88- and IL-22-dependent manner.[4,5] Therefore, besides being the protective role towards infection by enteropathogenic bacteria, RegIII lectins seem to have another role in later stages of infection. In this stage, a large amount of RegIII lectins exist in the inflamed gut, and thus the biological effect of RegIII lectins may peak. Based on this hypothesis, we have recently deciphered the role of RegIIIβ in remission of the infectious diarrhea. We employed the streptomycin mouse model for *Salmonella* diarrhea to understand the role RegIIIβ in remission. This model is a powerful tool for studying the interaction between host defense and *Salmonella*.[24] In this model, oral inoculation with *S.* Typhimurium elicits profound colonic inflammation at earlier stages of infection, and the pathogen colonizes gut mucosa with sustained inflammation at the later stages.[25–27] At the end of the infection, *S.* Typhimurium is finally eliminated from the gut, and the enteropathy is restored. Comparative analysis of the *S.* Typhimurium colonization of WT and *RegIII*$\beta^{-/-}$ mice indicated that RegIIIβ prolongs gut colonization by *S.* Typhimurium and the duration of *Salmonella* diarrhea.[12]

Recent work revealed that *S.* Typhimurium cells coated with O-antigen specific IgA cannot separate after completing cell division, resulting in the formation of large monoclonal clumps.[28] This is termed enchained growth, prevents tissue invasion and accelerates the *S.* Typhimurium clearance from the gut lumen.[26,28] Thus, there are at least two different types of defense that are targeting dividing bacteria, i.e. RegIIIβ elicited by innate- and sIgA elicited by the adaptive immune response. It is tempting to speculate that both mechanisms may cooperate in *S.* Typhimurium elimination from the gut. For example, RegIIIβ-mediated killing might kill enchained bacteria and thereby help to accelerate pathogen elimination. On the other hand, we cannot exclude that RegIIIβ may interfere with IgA-mediated enchainment, in particular in the inflamed gut. In fact, as RegIIIβ-mediated OM permeabilization facilitates LPS release from the OM (Miki & Hardt, unpublished data), this might alleviate enchainment by IgA and thereby promote the escape of *S.* Typhimurium from clumps. Deciphering the possible cooperation between RegIIIβ and the O-antigen specific IgA will certainly be an interesting topic for future work.

Recovery from the gut infection with enteropathogenic bacteria is a complex and likely highly regulated (though poorly understood) process which includes the re-establishment of colonization resistance. Indeed, the balanced and dense microbiota is essential for *S.* Typhimurium clearance at the later stages of the gut infection.[25,26] The induced expression of RegIIIβ in the inflamed gut of the streptomycin mouse model apparently impacts the microbiota composition, resulting in reduced proportions of Bacteroidetes in the gut lumen.[12] This is attributable to the bactericidal activity of RegIIIβ towards *Bacteroides* spp. and *S.* Typhimurium. RegIIIβ directly kills *Bacteroides* spp., while *S.* Typhimurium is more resistant. Furthermore, orogastric application of a *Bacteroides* mixture (including *B. acidifaciens*) isolated from murine feces enhances *S.* Typhimurium clearance and remission of the enteropathy.[12]

Gut environmental parameters, i.e., microbiota-derived metabolites are well known to affect the bacterial fitness in the gut. The RegIIIβ-dependent changes of microbiota affect the intestinal metabolism. From the altered metabolic pathways, vitamin B6 metabolism is the most affected, and the expression of RegIIIβ profoundly reduces luminal levels of vitamin B6.[12] The production of vitamin B6 in the gut depends upon the commensal bacteria. Accordingly, antibiotic treatment depleting the microbiota reduces the gut luminal vitamin B6 levels. Similarly to *Bacteroides* spp., supplementation with vitamin B6 facilitates *S.* Typhimurium clearance from the gut and resolution of the enteropathy.[12] In conclusion, in the later stages of the enteric infection, RegIIIβ does not act as a

protective effector. Instead, it rather unexpectedly fuels *Salmonella* gut infection (Fig. 2).

New therapeutic intervention for infectious diarrhea

Our recent findings suggest that RegIIIβ, *Bacteroides* spp., i.e., *B. acidifaciens*, and vitamin B6 are novel targets for therapeutic intervention in the context of *Salmonella* diarrhea.[12] Since the induced RegIIIβ expression can alter the gut environment, thus favoring enteropathogens, limiting the activity of RegIIIβ at later stages of an acute infection might become a new strategy for therapy of *Salmonella* diarrhea. In addition, supplementation of *B. acidifaciens* and/or vitamin B6 might be another promising intervention.

How do *B. acidifaciens* and vitamin B6 contribute to the *S.* Typhimurium clearance and remission from the enteropathy? *Bacteroides* spp. are the most abundant commensal bacteria in healthy human colonic microbiota. As the commensal bacteria can produce vitamins, the microbiota is a main source of vitamins in the gut. Certain commensal bacteria such as Bacteroidetes, but not Firmicutes seem to produce vitamin B6 efficiently in the gut,[29] and *B. acidifaciens* has been shown to contribute to this vitamin B6 production.[12] Therefore, the protective effect of *B. acidifaciens* supplementation on *Salmonella* diarrhea might be explained by the increased levels of the gut luminal vitamin B6. However, it should be noted that alternative scenarios also be considered. Certain *Bacteroides* strains have been shown to protect against the gut inflammation. In *IL2*$^{-/-}$ mouse model, *B. vulgatus* protects against *E. coli*-triggered colitis by unknown mechanism.[30] In other examples, polysaccharide A from *B. fragilis* promotes regulatory T-cell development through TLR2 stimulation while inhibiting Th17-associated proinflammatory responses, leading to protection from colitis.[31] In addition, it is notable that *Bacteroides fragilis* can affect gut colonization by other bacteria via its type VI secretion system (T6SS), a multiprotein complex that kills and inhibits neighboring bacteria.[32] This may confer colonization resistance. It is unknown whether *B. acidifaciens* harbors a T6SS. If this were the case, functional expression of T6SS by such *B. acidifaciens* strains might promote the clearance of *S.* Typhimurium from the gut lumen. In the case of *Clostridium difficile* diarrhea, transplanting a normal healthy microbiota is quite effective for treatment of this disease. This protective effect may be attributable to reactivation of colonization resistance. In addition, normalization of secondary bile acid levels is also critical for treatment of *C. difficile* diarrhea.[33] This also may involve the restoration of colonization resistance.[34] As supplementation with *B. acidifaciens* increases production of bile acid such as cholate and taurine,[35] the increase in the amount of bile acids might be involved in the restoration of colonization resistance, resulting in the enhanced remission of *Salmonella* diarrhea. Furthermore, host immune responses also may affect *S.* Typhimurium clearance. *B. acidifaciens* can modulate host immune responses, i.e., enhancement of IL-6, IL-10, and IgA production and expression of MHC class II molecule.[36] The enhanced IgA production may facilitate the O-antigen specific IgA-mediated *S.* Typhimurium clearance.[28]

Vitamin B6 is a water-soluble micronutrient, and serves as a co-factor for many enzymatic reactions. Moreover, maintenance of vitamin B6-biosynthesis is of great importance for various homeostatic processes in health and disease, including host immune responses.[37–39] In contrast, dysbiosis and infectious diarrhea can dramatically deplete vitamin B6 levels in the gut.[12] In line with this, approximately 30% of patients with inflammatory bowel disease (IBD) show evidence of vitamin B6 deficiency.[40] Therefore, the reduction in vitamin B6-biosynthesis by the intestinal microbiota might be a key common sign of inflammatory diseases including gut infection with enteropathogens and IBD. At present, the precise mechanism by which vitamin B6 can facilitate remission of *S.* Typhimurium gut infection remains unclear. Therefore, one can envision several scenarios. (A) Vitamin B6 may affect the gut luminal growth of *Bacteroides* or *S.* Typhimurium. If vitamin B6 facilitates *Bacteroides* growth, some (unidentified) *Bacteroides* features may fortify colonization resistance, and thereby accelerate pathogen clearance and remission. Moreover, vitamin B6 may interfere with gut luminal *S.* Typhimurium growth. In this case, one can speculate that the *S.* Typhimurium is outcompeted by vitamin B6-dependent members of the microbiota. In other vitamins, vitamin B12 actually enhances the gut luminal growth of *Bacteroides thetaiotaomicron*,[41] whereas *S.* Typhimurium growth and its virulence genes are affected by ethanolamine, a precursor of vitamin B12.[42,43] (B) Alternatively, vitamin B6 may dampen the inflammation, and thereby allow regrowth of *Bacteroides* spp..

The damaged intestinal cells might be repaired by vitamin B6 dependent mechanisms, and thereby facilitate remission. This also may help to reestablish colonization resistance. Consequently, *S.* Typhimurium might be outcompeted by the regrowing microbiota. (C) Finally, vitamin B6 may suppress the expression of virulence genes by *S.* Typhimurium. In either way, controlling the luminal levels of vitamin B6 might be very promising intervention for therapy of *Salmonella* diarrhea.

Intestinal inflammation can boost horizontal gene transfer (HGT), i.e., PII plasmid transfer and phage transfer in the case of *S.* Typhimurium gut infection.[44,45] Thereby, disease appears to promote the evolution of new pathogen strains and the spread of antimicrobial resistances. In contrast, vaccination eliciting the O-antigen specific IgA against *S.* Typhimurium can suppress the HGT.[28,45] Since vitamin B6 supplementation can also reduce the gut luminal pathogen loads and inflammation,[12] vitamin B6 therapy might also help to curb HGT of virulence factors and antibiotic resistance plasmids.

Certain vitamins have been found to affect the gut mucosal defense. Retinoic acid, a metabolite of vitamin A, affects key responses of gut mucosal immunity such as cell trafficking, differentiation, and function.[46] Especially, it is notable that exogenous retinoic acid treatment has protective effects against oral *Salmonella* infection.[47] The retinoic acid-mediated gut homing of T cells and B cells seems to play a critical role in this protection. Vitamin B9 also has been shown to involve the mucosal defense because vitamin B9 deficiency causes decreased resistance to infections by inhibiting the activity of NK cells.[48] Vitamin D induces antimicrobial gene expression such as cathelicidin and β-defensin 2,[49] thus contributing to innate defense against pathogen infection. Furthermore, vitamin D3 fine-tunes activities of the mucosal defense, therefore excess or defect of vitamin D3 causes alteration in intestinal inflammatory responses. This thus confers on host to increased susceptibility to *C. rodentium* infection.[50]

Our recent findings suggest that supplementation with specific microbiota strains capable of producing certain vitamins or direct vitamin supplementation might open new avenues for therapy of bacterial diarrhea. The gut harbors many metabolites derived from the microbiota, which play crucial roles in the intestinal homeostasis. Dysbiosis caused by the infection with enteric pathogen and inflammation drastically alters the metabolomic profile. Deciphering the infection- and inflammation-caused alteration in gut environment will bring about development of new therapeutic strategies to combat infectious agents.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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