Species-specific and pathotype-specific binding of bacteria to zymogen granule membrane glycoprotein 2 (GP2)

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Species-specific and pathotype-specific binding of bacteria to zymogen granule membrane glycoprotein 2 (GP2)

Abstract
With interest we read the paper by Juste et al 1 proposing the amount of zymogen-granule membrane glycoprotein 2 (GP2) on the surface of intestinal bacteria as a Crohn's disease (CD) marker. Indeed, a decreased GP2 level was found on microbes in patients with CD as compared to those of healthy controls. GP2 is a homologue to the urinary Tamm–Horsefall protein demonstrating an antimicrobial function by binding type 1-fimbriated uropathogenic Escherichia coli (UPEC). Likewise, GP2 seems to interact with intestinal bacteria as a specific receptor of bacterial type-1 fimbriae (FimH) on intestinal microfold cells that are partaking in immune responses against such microbes.2 GP2 is overexpressed in the inflamed intestine of patients with CD and has an immunomodulating role in innate and acquired immune responses.3,4 Interestingly, GP2 was identified as autoantigen of pancreatic antibodies in CD.4 Altogether, these findings indicate two major GP2 sources (pancreatic/intestinal) and support a role for GP2 in the interaction between the immune system and intestinal microbiota.3 Thus, loss of tolerance to GP2 could play a role in CD's pathophysiology supposed to be exacerbated by preceding intestinal infections. In general, the findings by Juste et al 1 may be explained by a lower pancreatic GP2 secretion, an impaired GP2 binding to bacteria, or by a higher prevalence of bacteria with poor or no GP2 binding in patients with CD.

Disciplines
Veterinary Microbiology and Immunobiology

Comments
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granule membrane glycoprotein 2 (GP2) on the surface of intestinal bacteria as a Crohn’s disease (CD) marker. Indeed, a decreased GP2 level was found on microbes in patients with CD as compared to those of healthy controls. GP2 is a homologue to the urinary Tamm–Horsfall protein demonstrating an antimicrobial function by binding type 1-fimbriated uropathogenic Escherichia coli (UPEC). Likewise, GP2 seems to interact with intestinal bacteria as a specific receptor of bacterial type-1 fimbriae (FimH) on intestinal microfold cells that are partaking in immune responses against such microbes.2 GP2 is overexpressed in the inflamed intestine of patients with CD and has an immunomodulating role in innate and acquired immune responses.3,4 Interestingly, GP2 was identified as auto-antigen of pancreatic antibodies in CD.4 Altogether, these findings indicate two major GP2 sources (pancreatic/intestinal) and support a role for GP2 in the interaction between the immune system and intestinal microbiota.3 Thus, loss of

**Figure 1** Binding of *Escherichia coli* pathotypes to GP2. Human recombinant GP2 expressed in SF9 cells were immobilised on 96-well plates with anti-FimH antibodies used as positive, or human serum albumin as negative controls. After incubation of bacterial suspensions for 2 h followed by subsequent washing, bound bacteria were stained with a fluorescent dye and counted with an automated fluorescence interpretation system (Aklides, Medipan, Dahlewitz, Germany). Bound bacteria are represented as bacteria per image (bpi, image=0.61 mm²). UPEC: human uropathogenic *E. coli* (n=24), HFEC: human intestinal commensals (fecoal) *E. coli* (n=24), ETEC: human enterotoxigenic *E. coli* (n=24), EPEC: human enteropathogenic *E. coli* (n=24), SAEC: sepsis-associated human *E. coli* isolated from sepsis patients (n=24), EAEC: human enteraggregative *E. coli* (n=17), AFEC: chicken intestinal commensals (avian fecal) *E. coli* (n=24), CAEC: Crohn’s disease-associated *E. coli* isolated from patients with Crohn’s disease (n=20), Salmonella: Salmonella serovar (n=24), Klebsiella: Klebsiella pneumoniae (n=12), Proteus: Proteus mirabilis (n=12), Buttauxella (n=6), Pantoea: Pantoea agglomerans (n=5), Raoultella: Raoultella ornithinolytica (n=7), Serratia: Serratia fonticola/liquefaciens (n=6), and methicillin-resistant Staphylococcus aureus: MRSA (n=24). Data are displayed as bpi in box-and-whisker plots with far out values, defined as values that are smaller than the lower quartile minus 3 times the IQR, or larger than the upper quartile plus 3 times the IQR, displayed as solid triangles. Posthoc analysis, p<0.05 for the following comparisons: UPEC: ETEC, EAEC, Salmonella, Buttauxella, Pantoea, Serratia, MRSA. EAEC: ETEC, EAEC, Salmonella, Buttauxella, Pantoea, Serratia, MRSA. AFEC: ETEC, EAEC, Salmonella, Buttauxella, Pantoea, Serratia, MRSA. CAEC: ETEC, EAEC, Salmonella, Buttauxella, Pantoea, Serratia, MRSA. EPEC: ETEC, EAEC, Salmonella, Buttauxella, Pantoea, Serratia, MRSA. SAEC: ETEC, EAEC, Salmonella, Buttauxella, Pantoea, Serratia, MRSA. AFEC: ETEC, EAEC, Salmonella, Buttauxella, Pantoea, Serratia, MRSA. CAEC: ETEC, EAEC, Salmonella, Buttauxella, Pantoea, Serratia, MRSA.

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Dear Editor,

With interest we read the paper by Juste et al1 proposing the amount of zymogen-
Tolerance to GP2 could play a role in CD’s pathophysiology supposed to be exacerbated by preceding intestinal infections. In general, the findings by Juste et al. may be explained by a lower pancreatic GP2 secretion, an impaired GP2 binding to bacteria, or by a higher prevalence of bacteria with poor or no GP2 binding in patients with CD.

We have a longstanding interest in GP2’s intestinal function and, therefore, we evaluated GP2 as receptor for intestinal bacterial pathotypes by testing the binding of 284 bacteria in a novel binding assay. These bacteria were of 8 *E. coli* pathotypes, 8 *Salmonella* serovars, 6 other Enterobacteriaceae genera (*Klebsiella*, *Proteus*, *Buttiauxella*, *Pantoea*, *Raoultella*, *Serratia*), and methicillin-resistant *Staphylococcus aureus* (MRSA) (figure 1).

Binding rates of isolates ranged from 0 to 2117 bacteria per image (bpi), and *Salmonella* isolates exhibited the significantly highest binding to GP2; whereas, *Proteus*, *Buttiauxella*, *Pantoea*, *Raoultella*, *Serratia* and MRSA isolates demonstrated low binding (Kruskal–Wallis test, p<0.000001). *E. coli* pathotypes and *Klebsiella* expressed medium binding rates. CD-associated *E. coli* (CAEC) demonstrated no significantly different binding to GP2 than human commensal (fecal) *E. coli* (HFEC); sepsis-associated *E. coli*; UPEC, enteropathogenic *E. coli* (EPEC) or chicken commensal (avian fecal) *E. coli*, but a higher binding than enteroxygenic *E. coli* and human enteropathogenic *E. coli* (posthoc analysis, p<0.05, respectively).

Nine out of 10 *fimH*-negative isolates showed low binding to GP2, and one HFEC isolate a high binding rate (473 bpi). GP2 binding of *Salmonella*, *E. coli* and other bacterial groups was mannose-sensitive and not glucose-sensitive as shown by inhibition experiments proving a GP2-FimH interaction.

To investigate the interaction between FimH-protein and GP2 at a molecular level, we sequenced *fimH* genes of 48 *E. coli* isolates: six isolates of each pathotype including two isolates not binding to GP2, two isolates showing medium and two isolates showing strong binding. Thus, we defined 23 different FimH-amino acid sequences. Though there was no specific FimH-amino acid sequence variation for an *E. coli* pathotype, FimH sequences seem to correlate with the strength of binding of GP2 to bacteria (figure 2). Additionally, bacterial binding to GP2 correlated significantly with that to anti-FimH antibodies, indicating that FimH-expression levels are associated with GP2 binding (p<0.0001, see online supplementary data).

We conclude that GP2 is an intestinal epithelial receptor interacting with distinct FimH-positive bacteria. This interaction can be modulated by pancreatic GP2 secreted together with zymogens into the intestine. GP2 binding is selective for bacterial species and pathotypes and, thus, may determine immune responses to the intestinal microbiota. CAEC do not differ in their binding from most *E. coli* isolates supporting the...
assumptions that other bacteria with low GP2 binding are more abundant in CD, or the GP2 interaction with CAEC is impaired.

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Contributors DRo, DRe and PS conceived of the study and participated in its design and coordination and helped to draft the manuscript. SR, RK, EB, and KG carried out the experiments. RH developed the study and participated in its design and coordination. TJ provided the bacterial isolates. All authors read and approved the final manuscript.

Competing interests DRo is a shareholder of GA Generic Assays GmbH and Medipan GmbH. The remaining authors declare that they have no competing financial interests.

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