Cellular & Molecular Immunology (2016) 13, 263–264

www.nature.com/cmi

LETTER TO EDITOR

"LOCK"ing up allergic responses with a Polish probiotic

Yuying Liu and J Marc Rhoads

Cellular & Molecular Immunology (2016) **13**, 263–264; doi:10.1038/cmi.2015.15; published online 23 March 2015

Three relatively new *lactobacillus* species that were developed from fecal samples of a 26-year-old woman (LOCK0900), a 6-year-old girl (LOCK 0908), and a 5-year-old boy (LOCK 0919) have been studied by Kozakova et al.[1] in an attempt to define the mechanism by which the probiotics modify the allergic response. The researchers colonized germ-free (GF) mice with individual or combined strains and demonstrated a reduced allergic response to intraperitoneal sensitization with birch pollen allergen by evaluating intestinal lavage IgA, IgE, and IgG1/G2 levels. Increased Th1 versus Th2 cytokine responses in splenic and mesenteric lymph node cells were also observed. In addition, the ultrastructural appearance of the ileal tight junctions appeared to be improved with increased immunoreactive ZO-1 in the intestinal homogenate, suggesting an impact on the gut epithelial barrier.

It is interesting that three strains of *lactobacilli* activated completely different pattern recognition pathways; *L. rhamnosus* strain LOCK0900 interacted with membrane TLR2, and *L. casei* LOCK 0919 interacted with cytosolic NOD2, while *L. rhamnosus* LOCK0908 interacted with neither of the above pattern recognition receptors. However, LOCK0908 could stimulate production of IL12p70 and IL-10 by an unknown mechanism. Clearly, the cytokine production by stimulated bone marrow dendritic cells is not species (*lactobacillus*)-specific but rather strain-specific. Our group has also found that different strains of *L. reuteri* differentially activated (or repressed) cytokine production in cultured cells.[2] We studied intestinal epithelial cells, whereas Kozakova et al. studied bone marrow dendritic cells.

The authors chose to study a model based on the highly allergenic birch pollen allergen Bet v 1. The model was first described in 2004 by Repa et al.[3] In that study, the subcutaneous route produced the highest levels of specific IgE and the highest splenocyte IgE and IL-5 responses, whereas the intraperitoneal (i.p.) route of sensitization produced the highest levels of serum IgG1 and IgG2 with milder increases in IgE and IL-5. In the current study, the authors chose the i.p. model. One may question the clinical relevance of repeated (every 10 days) antigen exposure via the peritoneum, but the results were indeed dramatic. It is unclear to this reviewer whether the i.p. route was chosen to provide a stronger stimulus toward the development of gastrointestinal allergy and/or a greater impact on the intestinal epithelial barrier compared with subcutaneous or cutaneous exposure. Antigen exposure via the diet would also be of great interest.

This study showed that the different strains of lactobacilli had an impact on the ultrastructure and tight junction protein levels in the ileum of GF mice. These results are not novel and were first reported by Resta-Lenert and Barrett in 2003, who showed that the permeability of cultured enterocyte monolayers injured with enteroinvasive *E. coli* could be rescued by a combination of *S. thermophilus* and *L. acidophilus*.[4] The effects of probiotics on the intestinal barrier not only involve the tight junctions but may also be attributed to the production of mucus and antimicrobial peptides (reviewed comprehensively by Ohland and McNaughton in 2010).[5] It is tempting to speculate that the effects of the different probiotics on serum IgG1, IgG2a, IgA, and IgE levels were produced by the effects on antigen delivery to the epithelium, e.g., via an enhanced barrier. However, increased barrier function cannot be assumed from the microscopic observations by Kozakova et al. alone, as gut permeability studies to establish the barrier function were not conducted.

A recent World Allergy Organization systematic review[6] concurred with an earlier Cochrane Database review[7] in stating that there is not yet an established role for probiotics in preventing or treating allergies. However, the groups emphasized a need for more basic microbial research, such as that presented by Kozakova et al. Evidence for protection against allergic airways disease has been shown in rodent models. The potential mechanisms suggested in the current study include increased systemic levels of IgA, reduced splenic IL-4 levels, and increased levels of circulating and splenic transforming growth factor (TGF)-beta. Oddly, anti-inflammatory IL-10 levels did not change during these studies.

Department of Pediatrics, Division of Gastroenterology, The University of Texas Health Science Center at Houston Medical School, Houston, Texas 77030, USA
Correspondence: J Marc Rhoads, Department of Pediatrics, Division of Gastroenterology, The University of Texas Health Science Center at Houston Medical School, Houston, Texas 77030, USA.
E-mail: J.Marc.Rhoads@uth.tmc.edu
Received date: 26th January 2015; Revised date: 27th January 2015; Accepted date: 27th January 2015

An important consideration for studies focusing on live organisms is to acknowledge that the organisms themselves may not be responsible for the immunomodulating effects. The distinct mechanisms demonstrated in the TLR studies suggest potential additive benefits. The benefit may be via direct effects of the probiotics on the resident microbiota on the surface, effects on the intestinal epithelial cells or their tight junctions, or effects on resident dendritic sensor cells or other lamina propria lymphocytes. Alternatively, the effect could be mediated by secreted factors in the gut lumen, which could affect these cells or shift the microbiota composition.

Finally, the widely used strategies and pharmaceuticals for the treatment of allergic diseases in humans, such as reactive airways disease or gastrointestinal allergy, have included: (a) avoidance of specific triggers, (b) immunomodulation (e.g., steroids), (c) antihistamines, and (d) leukotriene inhibitors. The current study suggests that probiotic organisms could provide a new and powerful clinical tool, but the optimal probiotic or combination of probiotics would likely have to be identified. Additionally, the probiotic would likely need to be consumed for a prolonged period of time because long-term colonization has not been achieved in humans, which necessitates longitudinal safety studies.

In summary, the authors are to be congratulated for an evolving body of work "LOCK"ing up some key findings relevant to allergic disease.

1 Kozakov H, Schwarzer M, Tuckova L, Srutkova D, Czarnowska E, Rosiak I *et al*. Colonization of germ-free mice with the mixture of three lactobacillus strains enhances the integrity of gut mucosa and ameliorates allergic sensitization. *Cell Mol Immunol* 2015; **12**: In press.
2 Liu Y, Fatheree NY, Mangalat N, Rhoads JM. Human-derived probiotic Lactobacillus reuteri strains differentially reduce intestinal inflammation. *Am J Physiol Gastrointest Liver Physiol* 2010; **5**: G1087–G1096.
3 Repa A, Wild C, Hufnagl K, Winkler B, Bohle B, Pollak A *et al*. Influence of the route of sensitization on local and systemic immune responses in a murine model of type I allergy. *Clin Exp Immunol* 2004; **1**: 12–18.
4 Resta-Lenert S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC). *Gut* 2003; **7**: 988–997.
5 Ohland CL, MacNaughton WK. Probiotic bacteria and intestinal epithelial barrier function. *Am J Physiol Gastrointest Liver Physiol* 2010; **6**: G807–G819.
6 Fiocchi A, Burks W, Bahna SL, Bielory L, Boyle RJ, Cocco R *et al*. Clinical use of probiotics in pediatric allergy (CUPPA): a world allergy organization position paper. *World Allergy Organ J* 2012; **11**: 148–167.
7 Osborn DA, Sinn JK. Probiotics in infants for prevention of allergic disease and food hypersensitivity. *Cochrane Database Syst Rev* 2007; **4**: CD006475.