A GUT RESPONSE *to Vaccines*

At the first Keystone meeting in India, researchers discussed how malnutrition and gut health affect the immune system

By Andreas von Bubnoff

A healthy gut and good nutrition are important for a healthy immune system and a good immune response to vaccination. This is likely the reason that oral vaccines have been found to be less efficient in malnourished children with a disordered gut. Still, surprisingly little is known about how nutrition and gut health affect the mucosal immune system in the digestive tract.

The Keystone Symposium on Malnutrition, Gut-Microbial Interactions and Mucosal Immunity to Vaccines, which took place in New Delhi Nov. 7-11, addressed what is known about these issues and how to develop interventions that could improve the gut immunity and response to oral vaccines among children in developing countries.

Vaccine responses

While children in developing countries have a poorer response to oral vaccines, such as oral polio or rotavirus vaccine, than children in developed countries, their response to vaccines that are administered systemically by injection, such as the measles vaccine, is much less impaired.

Gagandeep Kang, professor and head of the Wellcome Trust Research Laboratory at the Christian Medical College in Vellore, India, and one of the organizers of the conference, compared the efficacy of oral rotavirus vaccines in children from developing and developed countries. There are currently two licensed oral rotavirus vaccines that both contain live-attenuated viruses—GlaxoSmithKline's (GSK's) Rotarix and Merck's RotaTeq.

Kang said these vaccines were developed after a study in Mexican children showed that after two rotavirus infections, children had developed complete protection from severe diarrhea caused by subsequent rotavirus infections (*N. Engl. J. Med.* **335**, 1022, 1996). However, when Kang and colleagues did a similar study in children in Vellore, India, they found that after two infections, only 59% of the children were protected from severe rotavirus diarrhea, and only 79% were protected after three infections (*N. Engl. J. Med.* **365**, 337, 2011). The lower level of protection in the Indian children could be partly because they got infected at a younger age when their immune systems were still immature, Kang said. Another possible reason is that they might have received a high level of maternal antibodies, which could have neutralized the incoming virus, inhibiting the development of an immune response.

Based on her results, Kang and colleagues have done modeling studies that suggest the existing oral rotavirus vaccines would have less than 50% efficacy in India, similar to the efficacy for these vaccines in other developing countries. Studies published last year showed that the efficacy of Rotarix was 77% in South Africa and 50% in Malawi (*N. Engl. J. Med.* **362**, 289, 2010), and the efficacy of RotaTeq was between 40% and 50% in Ghana, Kenya, Mali, Bangladesh, and Vietnam (*Lancet* **376**, 606, 2010; *Lancet* **376**, 615, 2010). In contrast, these vaccines have at least 90% efficacy in developed countries.

However, the lower efficacy of the rotavirus vaccines in countries such as India doesn't mean they shouldn't be used there, Kang said. "Even at current levels of efficacy, you will save 50,000 children every year." At the same time, she added, we also need to improve their efficacy or come up with alternate vaccines that are more efficacious in developing countries.

One option is to develop injectable rotavirus vaccines. In the US, several injectable candidates

are currently in preclinical studies, Kang said. Another option is to develop cheaper oral vaccines because such vaccines are easier to deliver, particularly in very young children, making it easier to deliver several doses to improve efficacy, Kang said. Indian scientists are currently developing a cheaper version of a new oral live-attenuated rotavirus vaccine. Kang is involved in a randomized Phase III clinical trial that is testing this vaccine in children in Delhi, Vellore, and Pune. She said an Indian vaccine would most likely cost less than US$1 per dose, compared with about $20-25 per dose that it costs to buy the existing oral vaccines.

Why oral vaccines perform worse

Many children in developing countries have a disordered gut, which contributes to their poor response to oral vaccines, Kang said. They suffer from a complex syndrome that involves gut inflammation, altered gut microbiome, intestinal infections with several putative pathogens, impaired nutrition, and impaired growth, according to Chris Wilson, director of discovery at the Bill & Melinda Gates Foundation. But understanding the underlying causes for this syndrome and why vaccines don't work in this environment isn't easy, he said.

Another problem is that malnutrition and immune system dysfunction likely exacerbate each other, Kang said. Malnutrition seems to cause the gut to fail to maintain its immune and barrier function from pathogens, causing diarrhea and infections. This causes further gut damage and affects the gut's ability to absorb nutrients, which makes the malnutrition worse, Kang said.

Also, it is difficult to study what happens in the gut because doing so typically requires taking biopsies. Therefore, measuring processes in the gut is only possible indirectly, using markers in the blood or stool, such as serum or fecal immunoglobulin (Ig) A levels, Kang said. To address this issue, the Gates Foundation announced US$9 million in grants at the meeting to support research to find better non-invasive biomarkers to assess gut function and health.

Despite these challenges, researchers are beginning to understand what happens in the gut of malnourished children. William Petri, a professor of medicine at the University of Virginia, and his colleagues found that in a group of three-year-old children from Dhaka, Bangladesh, the children with more stunted growth were less likely to respond to oral polio vaccine (OPV), suggesting a possible role of malnutrition. They also found that these children more often have antibodies against bacterial endotoxins, suggesting that their guts are damaged and bacteria are leaking out.

Exposure to endotoxins may be the reason why Evan Newell, a research associate in Mark Davis's lab at Stanford University, and colleagues found evidence of chronic inflammation in children from this group who failed to respond to OPV. Newell and colleagues studied a subgroup of 40 of the children studied by Petri, six of whom didn't develop antibodies to polio after the vaccination. To find out why, they stimulated their white blood cells with inflammatory and anti-inflammatory cytokines that modulate the immune response. They then measured how well the cells could respond to the stimulation by measuring the activation of cytokine receptors. They found that half of the children didn't respond well to the cytokines, including the six that hadn't responded to OPV. Further measurements showed that the white blood cells that failed to respond to the cytokines in the children that didn't respond to OPV also had increased expression of inflammatory cytokine genes.

Together, this suggests that chronic inflammation in these children constantly exposes their immune cells to cytokines, eventually desensitizing them so that they can't respond to cytokines or vaccines anymore. "They are just kind of burned out," Newell said.

Petri said this suggests that in the children who failed to respond to OPV, bacteria are leaking out of the gut and induce an overstimulation of the immune system, which paradoxically resulted in suppression of the response to the vaccine. "[It] supports this idea that chronic inflammation in children, perhaps through exposure to bacterial endotoxin in the gut, is causing these different immune cell populations to be non-responsive to cytokine stimulation," Petri said.

The leaky gut comes from a condition called tropical enteropathy, he added, where the gut is so inflamed that the gut villi fuse together. It is thought to be caused by the onslaught of intestinal infections many children in the developing world are exposed to.

Directing immune responses to the gut

One way to address the underperformance of orally administered enteric vaccines in developing countries is to deliver them through different routes. Conventional intramuscular or subcutaneous immunizations often only induce weak immune responses in the gut, and therefore protect only weakly against gut infections. However, researchers are starting to modify injected vaccines so that they can induce immune responses in

[Tregs IN THE GUT]

Per Brandtzaeg, a professor of medicine at the Oslo University Hospital, provided another possible consequence of the overstimulation of the mucosal immune system in the gut of malnourished children in developing countries. He said that too many regulatory T cells (Tregs) might be generated locally in the gut mucosa of such children. This may explain why their immune response to oral vaccines is suppressed.

Hints that this might be the case come from observations that parasitic infections can result in an excess number of Tregs. Brandtzaeg mentioned a study of a patient who had a parasite infestation that resulted in a large number of Tregs in the gut mucosa. Treatment of the parasitic infestation removed the parasites, but also markedly reduced the number of the patient's Tregs, and the patient developed an over-stimulated local immune response with the features of inflammatory bowel disease.

This suggests that the Tregs induced by the parasite kept the inflammatory bowel disease under control, Brandtzaeg said. If there is a similar expansion of Tregs to dampen the tropical enteropathy in the gut of malnourished children in developing countries, "it could actually dampen the immune system to an extent that they don't get a good response to oral vaccines," he said. However, it's unclear if this actually happens, because nobody has studied the number of regulatory T cells in such children, he added. —*AvB*

the gut. Two approaches presented at the meeting used vitamin A or its derivative retinoic acid (RA) to direct immune responses to the gut in mice.

Swantje Hammerschmidt, a postdoctoral researcher in the group of Reinhold Förster at the Medical School Hannover, reported that when combined with RA, subcutaneous immunization of mice with cholera toxin induced gut homing molecules on activated B and T cells, causing them to migrate to the gut (*J. Clin. Invest.* **121**, 3051, 2011). The mice that had been immunized this way had cholera toxin specific B cells and IgA antibodies in the gut, and had no noticeable fluid influx into their intestine after challenge with cholera toxin, suggesting they were protected from diarrhea.

David Schwartz, a senior scientist at Hackensack University Medical Center in New Jersey, used the RA precursor vitamin A to induce immune responses in the gut when he vaccinated mice with chicken Ovalbumin (Ova) as an immunogen. But in contrast to Hammerschmidt, he vaccinated the mice intradermally, which has previously been shown to induce some immune responses in the gut. He also injected molecules that inhibit vitamin D production in the skin, because vitamin D inhibits the effects of vitamin A, and used hairless mice because the oil mice secrete into their hair contains vitamin D. He also injected the cytokine interleukin (IL)-5 to direct the B cells to make IgA, which is the most important and efficient antibody type in the gut.

Two weeks after the second of two vaccinations that were 20 days apart, Schwartz and colleagues found five- to 10-fold higher levels of Ova-specific IgA antibodies in the gut of mice vaccinated this way, compared with mice that had been vaccinated with Ova alone. The addition of vitamin A, Schwartz said, caused the antigen-presenting cells in the skin to educate B cells to home to the gut.

Next, Schwartz plans to use the strategy to direct immune responses to an HIV vaccine candidate to the gut, first in mice, and if the results are promising, in nonhuman primates.

Improving the gut flora

Bacterial infections and the gut flora are important factors in the complex syndrome of malnourished children with a poorer response to oral vaccines. Therefore, it's important to understand and improve the gut flora in these children.

One way to improve the gut flora is to add probiotic bacteria, the kind of bacteria that are found in yogurt and are believed to improve gut health. Until recently there wasn't much solid evidence that probiotic bacteria can improve gut health, but that is changing, said Kim Barrett, a professor of medicine at the University of California in San Diego, who studies the effects of probiotic bacteria on the gut. "Historically there has been a lot of belief and not so much scientific evidence," Barrett said. "That situation is rapidly changing. There is plenty of data out there now looking at beneficial effects of these probiotic strains on the gut."

Shinji Fukuda, a research fellow at the RIKEN Institute in Yokohama, Japan, reported how a certain type of probiotic bacteria called *Bifidobacterium* that can be found in yogurt can protect mice from dying from infection with a certain serotype of *E. coli* (*Nature* **469**, 543, 2011).

Fukuda and colleagues found that the preventive *Bifidobacterium* type had a gene that enabled it to import fructose, which the bacterium can turn into acetate. Acetate activates an anti-inflammatory response in colon cells.

Fukuda said food manufacturers could now use this finding to test if the *Bifidobacterium* types they add to yogurt also have the protective fructose transporter gene.

Barrett also studies how pathogenic and probiotic bacteria affect the gut in mice. Using a mouse model where *Salmonella* infection causes diarrhea, she found that the *Salmonella* caused diarrhea because the gut of the mice failed to absorb sodium and chloride ions from the gut lumen, which prevented water from being absorbed from the gut. These insights could lead to the development of drugs to treat diarrhea by restoring the absorptive transport of ions, Barrett said.

Using a different model of mice that have ulcerative colitis (inflammation of the colon) and diarrhea, Barrett and colleagues also found that feeding the mice daily for two weeks with *Lactobacillus acidophilus* and *Streptococcus thermophilus*, two probiotic bacterial strains found in yogurt, could ameliorate diarrhea and weight loss in the mice. *In vitro* experiments showed that the probiotics reversed the negative effects of pathogenic bacteria or inflammatory cytokines on ion transport functions of the gut and on its function as a barrier from pathogens.

Next, Barrett wants to better understand if these probiotics can also improve the symptoms of the mice infected with *Salmonella*. If they can, then probiotics could be a possible treatment for diarrhea caused by *Salmonella* in humans. ■