

Discussion

Leader: John Bienenstock, MD, McMaster University, Canada

Dr Bienenstock: No one has mentioned the nervous system, and it would be unwise, in my opinion, to ignore it. There is no question that nerves can control immune response. A recent paper reports that the vagus nerve can control B-cell production indirectly through parasympathetic or sympathetic systems, and it controls B-cell production of immunoglobulins in the spleen. Other research has shown that if you cut the vagus nerve in animals, or if you stimulate the distal end of the vagus nerve, you can have profound effects on some of the things that, for example, Dr Versalovic talked about regarding gut health. Through an alpha-7 nicotinic receptor, in fact, you can control and regulate TNF production with a lipopolysaccharide injection into animals.

The nervous system can, in fact, interact with bicomensal organisms. We found that if you feed a probiotic organism such as *L reuteri* to a conventional rat, a calcium-activated potassium channel is activated in the enteric nervous system. This activation is associated with all of the effects that we see—downregulation of immune response, upregulation of IgA, and down regulation of TNF-alpha production.

Let us discuss timing now. Research in Japan showed that, in the germ-free animal, the hypothalamic-pituitary-adrenal (HPA) axis response to stress was hyperexaggerated. If the investigator then normalized or conventionalized these germ-free animals with either specific pathogen-free (SPF) feces or with a single bacterium—he used *Bifidobacterium infantis*, but he did it before the animals were 6 weeks of age—he reduced the HPA axis response (adrenocorticotrophic hormone [ACTH], corticotrophin-releasing factor [CRF], and

corticosterone) to the normal response to stress. This lasted into adulthood. I think this is an important observation. It opens up the whole biology of these systems and shows that there is a window. Dr Nagler, in some of your studies you referred to a window of vulnerability, which suggests that when we feed probiotic organisms or commensal organisms, when we feed, how much we feed, and the timing are all highly significant. Dr Kuitunen, you have had more experience with this than most of us. Do you have any comments about the timing of the feeding of the particular probiotic you used in your experiments in Finland?

Dr Kuitunen: It seems important to give the probiotic to the pregnant woman so that the infant meets these bacteria very early. Susan Prescott's group published a study 1½ years ago but found no allergy preventive effect. For ethical reasons, however, they could give the bacteria only to the infants, not to the mothers [Taylor AL et al: *J Allergy Clin Immunol* 2007;119:184-191]. We had a clear effect when we started this probiotic feeding a couple of weeks before expected delivery. This outcome points to an early window of opportunity.

Dr Bienenstock: There clearly is a window in rodents, but I think the question of whether there is such a window in humans is still open. There is also discussion about whether, if there is a window, it shuts by the age of say 2 years. If we can change the set point of the neuroendocrine system, keep the window open and maintain it into adulthood, and extrapolate that to the immune system or the nervous system, we have an extraordinary opportunity. But if we miss the opportunity, we will not have that effect. Also, this is an epigenetic phenomenon. Studies have looked at this in terms of the HPA axis from an epigenetic point of view, even localizing it down to the promoter for the benefit of the corticoid receptors. This is not trivial.

Dr Lee: The literature suggests that for the purpose of affecting allergic responses the window seems to be the first 2 years—in fact, the first year. We have seen 13 or 14 obscure studies that

should indicate that effect, but only one or two show a positive effect on people with allergic asthma, or specifically, pollen allergies [Xiao JZ et al: *Clin Exp Allergy* 2006;36:1425-1435]. We do not see others that have achieved any effect on people suffering from allergy.

Dr Bienenstock: But that is treatment, not prophylaxis.

Dr Lee: That is right, it is not prevention. That is a different method altogether. As you say, the timing is important.

Dr Noakes: As already mentioned, timing is important when we talk about treatment versus prevention. A lot of my work has been with Susan Prescott in Australia and Philip Calder in the United Kingdom. We have a number of pregnancy cohort studies going on based on the idea that the window for prevention is within the first 18 months of life. So we are providing different types of omega-3 fatty acids and probiotics during this time. Dr Prescott has published work with Jan Dunstan in which they looked at the effect of interventions with omega-3 fatty acids in a small cohort—40 women in each group [Prescott SL et al: *J Allergy Clin Immunol* 2007;120:200-206; Dunstan JA et al: *J Allergy Clin Immunol* 2003;112:1178-1184]. They saw a general dampening down of Th1-type and Th2-type immune responses overall, rather than skewing in one direction or another. There was a trend toward decreased severity of such conditions as atopic dermatitis in the group that took fish oil, although other larger cohort studies are now going on in which the primary outcomes are clinical outcomes. To reiterate, timing is extremely important in prevention as opposed to treatment. Not to say that there is not a role for probiotics and fish oil in treatment, but from a preventative point of view, we need intervention studies.

Dr Lee: We do not see the effects on the prevention of allergic reactions in adults. Is it a case of colonization of these systems, or is there a change in the maturity of the immune system that results in the differences in the responses in the infant and the adult?

Dr Noakes: Dr Prescott's group also gave probiotics to infants 6 to 18 months of age. They saw trends for a reduction in the severity of atopic dermatitis in those infants who received the probiotics, as well as improved quality of life [Weston S et al: *Arch Dis Child* 2005;90:892-897]. I believe, however, that these results were sustained only for the duration of the intervention. So we also have to think about the duration of the intervention and how long the effects last.

Dr Kuitunen: Yes, duration might be important. Just getting one probiotic bacteria for 6 months and then hoping that for the rest of your life you are safe from allergies might be too naïve. We are now analyzing the 5-year results of our cohort study, and it seems that the allergy preventive effects are not as strong as they were at 2 years. We did a 6-month intervention with four probiotics and found that the colonization is also transient. We can see colonization at 3 months and at 6 months, but at 2 years—1½ years after the colonization intervention—we see no differences between those who received the probiotics and those who did not.

Dr Lee: I would like to mention the work of Kalliomäki, Isolauri, and their colleagues [Kalliomäki M et al: *Lancet* 2001;357:1076-1079; Kalliomäki M et al: *Lancet* 2003;361:1869-1871; Kalliomäki M et al: *J Allergy Clin Immunol* 2007;119:1019-1021]. They used *Lactobacillus* GG (LGG), as well, and they saw effects for up to 7 years.

Dr Bienenstock: Those were effects on atopic dermatitis, but not on asthma.

Dr Lee: And not on sensitization.

Dr Kuitunen: This is the only study—and it is a small study—showing that the effect is sustained. In this study, in fact, the effect was strong at 2 and 4 years and a little weaker at 7 years. It remains to be seen whether this is the case in larger cohorts.

Dr Rueda: A recently published study used the same experimental design as the Isolauri group, and they obtained the opposite results [Kopp MV et al: *Pediatrics* 2008;121:e850-e856]. Another recent study obtained positive results using *Lactobacillus rhamnosus*, giving the probiotic to the mother starting at 35 weeks of gestation and to the infant until 2 years of age. That is a controversial study, however, because if you use the same experimental design as a previous researcher and obtain the opposite results, they might be influencing other factors with that intervention.

Dr Nagler: In all these studies in which probiotics were given to pregnant women, has anyone demonstrated that the bacteria that were fed can be recovered from either the breast milk or from the gastrointestinal tract of the infant?

Dr Bienenstock: There is no question that they can be recovered from the gastrointestinal tract of the infant. The question is, how long you feed the probiotics? I think the longest after termination of feeding that anyone has found the probiotics—I think it was with LGG—was 18 months. I do not think anyone has suggested that these are permanent colonizers, right?

Dr Kuitunen: Right. Studies have been conducted with LGG on adult volunteers, and, in fact, the disappearance is rapid once you stop feeding the probiotics. At 3 weeks it is almost gone [Lassig A et al: *SSA Gut Impact: 3rd Platform Meeting on Foods for Intestinal Health*. Haikko, Finland: 2007, pp 29-31].

Dr Naglar: That same group, I believe, did a study in which they fed pregnant women a specific strain of bacteria and tried to isolate that strain in the breast after birth. I do not know whether they obtained positive results or whether they have published yet.

Dr Versalovic: Dr Bienenstock, I want to go back to something you mentioned at the beginning of this discussion—the connection with the enteric nervous system. I do believe in this window of opportunity to produce health benefits in the first year of an infant’s life. I am intrigued by one study that indicates that the window may be as long as 5 or 6 years [Palmer C et al: [PLoS Biol](#) 2007;5:e1777], but clearly, according to the Stanford metagenomic data set, there is tremendous flux and an opportunity during the first year of life. Flavia Indrio’s group from the University of Bari in Italy has tried to apply probiotics to effects on intestinal motility in infants [Indrio F et al: *J Pediatr* 2008;152:801-806]. They have shown that, with a single strain, they can have a significant effect on gastric emptying and irritability, with a reduction in mean daily crying times. Recently, she shared data from a 4-month follow-up, which showed a reduction in regurgitation in the infants. These data suggest that there may be a real opportunity to affect the function of smooth muscle in the enteric nervous system.

Dr Bienenstock: My colleagues and I have evidence in visceral pain from distention of the colon of the same effects with single organisms, showing that they could completely convert the germ-free disorder of motility in adults, following either single organisms or several organisms.

Annick Mercenier and colleagues have published data on the anti-inflammatory effects of probiotics and the anti-inflammatory, downregulatory mutant, which is diamine-deficient and is much more potent than the proinflammatory ones in terms of pain regulation. We believe that this is due to its generation of IL-10, which is antinociceptive. This is one situation in which the

whole immune system potentially interacts with the nervous system, although this will prove difficult to unravel.