Given how infrequent this observation has been in our cohort of patients, we propose that a large-scale study with a nested case-control design would be necessary to determine whether this clinical observation is representative of a true association. Unfortunately, such a study would be expensive and difficult to carry out, especially because AERD itself is so rare and patients with AERD having gastrointestinal complaints during desensitization are thought to represent less than a quarter of the total number of patients.[3] Therefore, for events with a low frequency of occurrence, such as this, awareness of possible adverse drug reactions in a given cohort of patients and the increase in clinical suspicion that generally follows become even more powerful not only to further validate the underlying association that might have been missed but also to avoid unnecessary morbidity.

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Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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Available online November 10, 2012.
http://dx.doi.org/10.1016/j.jaci.2012.09.035

Gut microbiota diversity and atopic disease: Does breast-feeding play a role?

To the Editor:

Using new technology, the recent report by Abrahamsson et al[1] confirms previous findings that low diversity of the infant gut microbiota is associated with an increased risk for allergic disease. In this study of 40 high-risk infants, the authors report that exclusion of subjects receiving probiotics or antibiotics, which could influence microbiota diversity, did not substantially change the results. On the basis of our own research, we propose that breast-feeding might represent another potential confounding factor that warrants investigation.

Infant diet is an established determinant of gut microbiota composition, and the protective effect of breast-feeding against atopic disease has been a frequent subject of study (and debate) for many years.[2] Consistent with previous studies,[3] in our population-based cohort of healthy full-term Canadian infants, we have detected decreased fecal microbiota richness and diversity in infants who are exclusively breast-fed compared with those who are partially or not breast-fed at the age of 4 months (Table I). This association has been attributed to human milk oligosaccharides, which are found exclusively in breast milk and serve as a selective metabolic substrate to limit the number of gut microbes and reduce diversity.[4]

TABLE I. Richness and diversity of infant fecal microbiota according to breast-feeding status at 4 months of age

		Chao1 Estimator: Richness			Shannon Index: Diversity		
Diet at 4 mo	No.	Mean	SD	*P* value	Mean	SD	*P* value
Exclusively breast-fed	10	9.0	4.1	.006	1.19	0.51	.12
Partially breast-fed	5	12.6	5.3		1.42	0.64	
Not breast-fed	9	15.0	4.0		1.58	0.47	

Comparisons were done by using ANOVA with posttest for linear trend.

Together, these results present an interesting paradox: breast-feeding promotes lower intestinal microbiota diversity, and low diversity is associated with an increased risk of atopic disease, yet breast-feeding is generally considered protective against atopy. Clearly, breast-feeding has physiologic effects beyond the gut microbiota, but these contradictory findings nevertheless signal the need for further research and careful consideration of the clinical relevance of summary measures for microbiota diversity. Abrahamsson et al[1] report that all infants in their study were breast-fed for at least 1 month, but they do not specify whether breast-feeding was exclusive or supplemented with formula. This distinction might be important because, based on our findings, the relative amount of breast-feeding seems to influence microbiota richness and diversity (Table I). Because it appears that exclusivity of breast-feeding was documented in their study,[5] it would be informative for the authors to provide more detailed infant feeding data and present their results after stratification by degree of formula supplementation.

We also note that maternal atopy was present for nearly all infants in the high-risk Abrahamsson et al[1] cohort. Maternal atopy modifies the association between infant feeding and atopic disease, with breast-feeding by atopic mothers actually increasing the risk of child atopy.[6] It has been shown that the breast milk of atopic mothers has an altered microbial composition, which affects the infant gut microbiota.[7]

In summary, new technology is facilitating analysis of the human microbiome in unprecedented detail; however, the development of meaningful and clinically relevant measures of microbiota composition and diversity remains challenging. It will be important to investigate the effects of infant feeding on gut microbiota in larger, population-based cohorts to confirm and better understand the association with atopic disease. We are pursuing such research within the Canadian Healthy Infant Longitudinal Development national birth cohort and look forward to complementary reports from our colleagues around the world.

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Supported by the Canadian Institutes of Health Research and by AllerGen NCE, the Killam Trusts, and Alberta Innovates–Health Solutions.

Disclosure of potential conflict of interest: M. B. Azad has received grants from the Canadian Institutes of Health Research, the Killam Trusts, Alberta Innovates Health Solutions, and the Canadian Allergy and Immune Diseases Advanced Training Initiative and has received travel support from the Canadian Institutes of Health Research, AllerGen NCE, and the National Institutes of Health/International Human Microbiota consortium. M. R. Sears has board memberships with AstraZeneca and Merck; has consultant arrangements with AstraZeneca, Novartis, and Merck; has provided expert testimony for AstraZeneca and Novartis; has received payment for lectures and service on speakers' bureaus from AstraZeneca and Merck; and is the AstraZeneca Endowed Chair in Respiratory Epidemiology. J. A. Scott has received grants from the Canadian Institutes of Health Research and AllerGen NCE. A. L Kozyrskyj has received grants from the Canadian Institutes of Health Research Microbiome Initiative. The rest of the authors declare that they have no relevant conflicts of interest.

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Available online November 27, 2012.
http://dx.doi.org/10.1016/j.jaci.2012.10.044

Reply

To the Editor:

We thank Azad et al[1] for their important points regarding our article entitled "Low diversity of the gut microbiota in infants with atopic eczema."[2] Admittedly, we were satisfied with the notion that all infants were breast-fed until 1 month of age in the original article. The absent increase in diversity from 1 week to 1 month of age also indicated that breast-feeding inhibited diversity development. However, we did not relate microbiota diversity to exclusive breast-feeding at 1 month of age. Reassessing the data, we identified 3 atopic and 4 healthy infants who were not exclusively breast-fed at 1 month of age. As requested, we have analyzed whether exclusive breast-feeding was associated with lower gut microbiota diversity and whether this affected the comparison between the atopic and healthy infants.

As hypothesized, exclusive breast-feeding was associated with low diversity of the total microbiota in infant stool (Table I). Interestingly, the results indicate that the difference was a consequence of low Firmicutes diversity in the exclusively breast-fed infants. Thus although the differences in Firmicutes diversity did not reach statistical significance and only 7 partially breast-fed infants were included in this analysis, formula introduction seems to favor the establishment of new Firmicutes strains. As expected, the relative abundance of bifidobacteria was higher in the exclusively than in the partially breast-fed infants (39% vs 15%, $P = .04$). The relative abundance of the other bacterial phyla and genera did not differ significantly (data not shown).

Our results are consistent with previous reports.[3,4] However, most studies have compared breast-feeding with formula feeding and not with partial breast-feeding. What our findings[2] and those of Azad et al[1] add to the field is that the diversity is higher also among partially breast-fed infants.

Second, we reassessed the diversity in the atopic and nonatopic infants, limiting the comparison to infants who were exclusively breast-fed at 1 month of age. The differences in diversity of the total microbiota, Bacteroidetes, and *Bacteroides* species between atopic and healthy infants in the original study[2] remained (Table I), and there were still no significant differences in relative abundance for any bacteria (data not shown).

There is poor evidence for an association between breast-feeding and allergy. Any allergy-preventive effects seem to be at most marginal.[5] Azad et al[1] argue that the exclusively breast-fed infants in our study might run an increased risk of atopic disease because their mothers had allergic disease. However, the atopic eczema incidence was similar between infants with maternal atopic heredity who were and were not exclusively

TABLE I. Shannon diversity index of the total microbiota and dominant phyla and genera in stool samples obtained at 1 month of age in infants who were exclusively or partially breast-fed and exclusively breast-fed infants who did or did not have atopic eczema during the first 2 years of life

	Exclusive breast-feeding at 1 mo			Atopic eczema (only exclusively breast-fed included)*		
	Yes, median (n = 33 [IQR])	No, median (n = 7 [IQR])	*P* value†	Yes, median (n = 17 [IQR])	No, median (n = 16 [IQR])	*P* value†
Total microbiota	1.57 (1.39-1.85)	1.93 (1.52-2.14)	.03	1.45 (1.13-1.57)	1.63 (1.53-2.12)	.002
Bacteroidetes	0.31 (0.00-0.53)	0.12 (0.00-0.56)	.72	0.06 (0.00-0.42)	0.49 (0.08-0.61)	.04
Bacteroides species	0.12 (0.00-0.48)	0.12 (0.00-0.47)	.86	0.04 (0.00-0.35)	0.30 (0.08-0.49)	.04
Actinobacteria	0.38 (0.22-0.56)	0.33 (0.09-0.46)	.42	0.38 (0.33-0.47)	0.41 (0.17-0.71)	.63
Bifidobacterium species	0.35 (0.18-0.46)	0.32 (0.06-0.37)	.38	0.34 (0.22-0.41)	0.37 (0.16-0.63)	.53
Firmicutes	0.59 (0.35-0.87)	1.16 (0.43-1.80)	.15	0.44 (0.34-0.85)	0.67 (0.48-0.92)	.19
Proteobacteria	0.21 (0.09-0.35)	0.10 (0.06-0.42)	.92	0.17 (0.04-0.35)	0.27 (0.17-0.33)	.38

IQR, Interquartile range.
*Only infants who were exclusively breast-fed at 1 month of age were included in the analyses.
†Mann-Whitney *U* test.