

ORIGINAL ARTICLE

Modelling bacterial transmission in human allergen-specific IgE sensitization

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Abstract

Aims: The impact of bacterial transmission from mother to child on human allergy development is poorly understood. The aim of the present work was therefore to use a temporal collected dataset of 117 mothers and their children to model the potential effect of mother-to-child bacterial transmission on allergy (IgE) sensitization.

Methods and Results: We have recently shown a negative IgE correlation to high *Escherichia coli* levels until the age of 1 year, with a shift to positive correlation to high *Bacteroides fragilis* levels at the age of 2. In the present work, we used the previous published data to model the persistence and interaction effects of *E. coli* and *B. fragilis* with respect to IgE sensitization. Temporal modelling was made by first defining a stochastic model for sensitization state based on Markov chains and regression tree analyses. Subsequent simulations were used to determine the impact of mother-to-infant bacterial transmission. The regression tree analyses showed that *E. coli* colonization within 4 days was negatively correlated to sensitization, while lack of *E. coli* colonization at day 4 combined with *B. fragilis* colonization after 4 months was positively correlated. With Markov chain analyses, we found that *E. coli* was highly persistent in infants until the age of 4 months, while the persistence of *B. fragilis* increased with age.

Conclusions: Simulations showed that the mother's bacterial composition correlated significantly to the child's IgE sensitization state at the age of 2 years. High *E. coli* and low *B. fragilis* levels in the mother were negatively correlated, while low *E. coli* and high *B. fragilis* were positively correlated to IgE.

Significance and Impact of the Study: Our results support that allergy could partly be communicable, being transferred from mother to infant through the gut microbiota.

Introduction

There has been a dramatic increase in IgE-sensitization-associated disorders, such as asthma and allergy, during the last few decades (Gould and Sutton 2008). The hygiene hypothesis stated more than 20 years ago that the clean Western lifestyle and lack of bacterial exposure can be a main underlying cases for the increase in allergy (Bjorksten *et al.* 2001). A wide range of studies have been conducted addressing the hygiene hypothesis, but still no

consensus can be drawn with respect to the effect of bacterial profiles on immune development (Round and Mazmanian 2009).

An important issue not yet embraced in the hygiene hypothesis is the communicability and temporal interactions of bacteria. Microbiota colonization and immune development are both dynamic processes, with the initial gut microbiota being transmitted during delivery and later through a multitude of environmental exposures (Palmer *et al.* 2007; Adlerberth and Wold 2009). Until

now, technological limitations have precluded analyses of these processes. The recent developments of rapid quantitative techniques, such as real-time quantitative PCR, microarrays and next generation sequencing have now opened the potential for large-scale temporal screenings of bacterial transmission and interaction patterns in allergy development (Brugere *et al.* 2009).

Current gut microbiota modelling strategies are generally aimed at describing the metabolic processes and host interactions in the gut (Karlsson *et al.* 2011). To our knowledge, very little work has been performed on modelling of persistence and transmission patterns.

The aim of the current work was to model the potential of allergy inheritance from mother to child through vertical transmission of gut bacteria. This was carried out using data from the prospective IMPACT cohort (Oien *et al.* 2006), for which twelve bacteria were quantified by real-time PCR in mothers and offspring during the first 2 years of life, while the IgE sensitization state of the children was determined at the age of 2 (Storrø *et al.* 2011). If *Escherichia coli* was found to have colonized the gut before the age of 1 year, then the children were less likely to show IgE sensitization, while *Bacteroides fragilis* colonization at 2 years increases the sensitization likelihood. The effects of bacterial transmission and interactions, however, were not investigated in the previous study. Therefore, *B. fragilis* and *E. coli* were chosen as candidates for the transmission and interaction analyses in the present work.

The modelling presented here was based on the analysis of individual bacterial transmission patterns from mothers to children at the age of 2–4 days, 10 days, 4 months, 1 year and 2 years. Transmission effects of *E. coli* and *B. fragilis* on IgE levels were investigated by regression tree analyses to address hierarchical interaction patterns. Based on empirically derived parameters, Markov models were used to determine the effect on IgE sensitization from hypothesized prevalence of *E. coli* and *B. fragilis* in the mother's population. The motivation for using the Markov models was that we could test the effects of *E. coli* and *B. fragilis* relative prevalence outside of what we actually have observed.

We present support for both significant bacterial interactions and mother-to-child transmission in IgE sensitization. These results are discussed with respect to potential transmission and interaction effects on IgE sensitization and allergy through the gut microbiota.

Methods

All statistical analyses were performed using the TIBCO SPOTFIRE S+ 8.1 (TIBCO Software Inc., Palo Alto, CA, USA).

Dataset

The study population consisted of 117 infants and their mothers for whom 12 bacteria were quantified by qPCR, and specific IgE quantified for the children at the age of 2 years. Most children (*c.* 90%) were delivered vaginally, and at the age of 6 weeks, *c.* 95% of the infants were breast-fed. Details about the cohort and IgE analyses are given by Storrø *et al.* (2011). Based on the reasons described in the introduction, we chose qPCR data for *E. coli* and *B. fragilis* for analyses in our work. As we used probabilistic binary models in our simulations, the quantitative data were dichotomized prior to the analyses. A binominal model was used because the samplings were too infrequent for quantitative modelling. The data for *E. coli* and *B. fragilis* were dichotomized based on thresholds determined from the overall quantitative distribution patterns in the cohort by the combined information from measurements both above and below the detection limit. For IgE dichotomization, we chose a cut-off for specific IgE ≥ 0.70 . This represented 31.5% of the children. The rationale was to select children with possible sensitization effects.

Temporal structure

The significance of the pairwise dependencies between the presence/absence of bacteria between one time point and the next were determined using both the Fisher's exact test and McNemar test. Fisher's exact test is a test for independence between the row and column variables of a contingency table, while the McNemar test determines whether there are significant changes between two time points.

The IgE dichotomized data were modelled as a function of the dichotomized bacterial data using regression tree models. The basis for using regression trees was that hierarchical interaction in the time series can be revealed. Tree models were fit by successively splitting the data to form homogeneous subsets. The result is a hierarchical tree of decision rules useful for prediction or classification. These decision rules were finally evaluated in a temporal context, so that the decision rules follow the age structure of the data.

We derived a Markov chain model for the colonization pattern of individual infants based on the formula *E. coli* and *B. fragilis* in Eqn (1):

$$T = \frac{\text{OBS} - E}{1 - E} \quad (1)$$

where T is the individual probability of vertical transfer of a bacterium from one age group to the next; OBS is the frequency of observations where the bacterium is

present in consecutive age groups, given that it was present in the first; E is the frequency of vertical environmental transfer where the bacterium is observed in the last, but not in the first age group.

Simulation model

The simulations were directly based on the Markov model with the frequency of *E. coli* and *B. fragilis* in the mothers as independent variables initiating the Markov chains. The output from the Markov chain was subsequently used as input in a probabilistic model with probabilities derived from the regression tree function of the sensitization. The outputs from the models were -1 if the simulated child was nonsensitized, 0 if sensitization status cannot be determined and 1 for sensitized children. The overall differences between sensitized and nonsensitized children were determined from 10 simulations for each of the 121 (11×11) combinations of prevalence of 0–100%, with steps of 10% for each of the bacterial prevalence in the mothers. The differences were subsequently described as a function of the prevalence of *E. coli* and *B. fragilis* in the mothers. Finally, the direct correlation between *E. coli* and *B. fragilis* in the mother was

determined using ANOVA with the sensitization output as response variable.

Results

Data categorization

There were clear differences in the overall distribution patterns for *B. fragilis* and *E. coli* within the cohort. The *E. coli* population showed low standard deviation of 1.2 for the concentration relative to the total flora, with a positive skew at 0.18. The *B. fragilis* population, on the other hand, showed larger standard deviation of 1.6 and a negative skew at -0.85. From the histogram plot, it seems like *E. coli* was present as a single population, while *B. fragilis* was present as both a low abundant and high abundant population (Fig. 1).

The quantitative data were transformed into categorical data using a relative abundance threshold of -3.5 for *B. fragilis* and -5.4 for *E. coli* (marked in Fig. 1). The rationales for using these thresholds were that for *E. coli*, the threshold represents the detection limit, while for *B. fragilis*, it represents the shift from high- to low-density populations. Furthermore, for *B. fragilis*, it is not possible

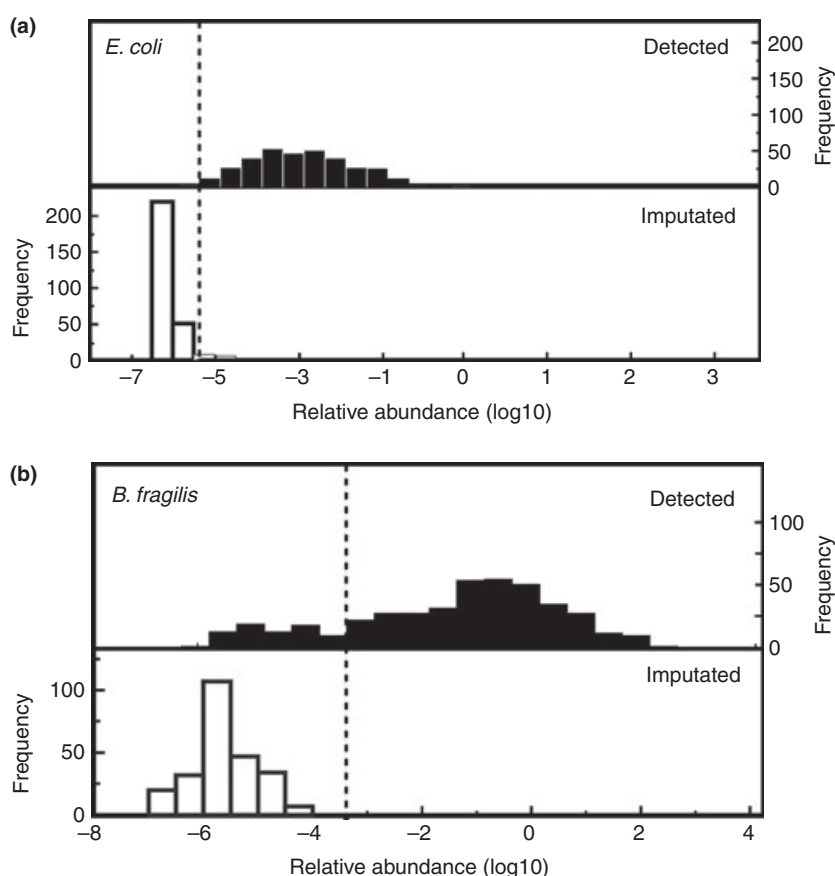


Figure 1 Histogram for the overall distribution of *Escherichia coli* (a) and *Bacteroides fragilis* (b) in the cohort. The dashed line shows the respective thresholds for dichotomization. The abundance is reported relative to the total microbiota as previously determined (Storrø et al. 2011) and represent the complete dataset. The measurements above the detection limit are shown as detected, while the measurements below the detection limit are shown as the imputed data.

to differentiate the low-level population from the population below the detection limit (Fig. 1).

The overall dichotomized dataset is shown in Fig. 2a. The prevalence data for *E. coli* show an increase from 4 days with a peak between 4 months and 1 year, while for *B. fragilis*, there was a decrease from day 4 to 10 and an increase from 10 days to 2 years (Fig. 2b).

Analyses of transmission dependencies

Using the Fisher's exact test, we found that the dependencies were highly significant for *E. coli* up to 1 year, while for 2 years, it was not significant. The dependencies for *B. fragilis*, on the other hand, increased until 2 years. There was also a significant interaction between *E. coli* at

1 year and *B. fragilis* at 2 years (the significant values are marked with black asterisks in Fig. 3).

With respect to individual changes, as determined by the McNemar test, we found significance between all age groups except at 4–10 days for *B. fragilis*, while *E. coli* showed significance between 10 days and 4 months and between 1 and 2 years (significant values are marked with grey asterisks in Fig. 3).

The individual transmission probabilities showed that *E. coli* had high probability of vertical transfer until 4 months and that the horizontal transmission from the environment was high at 4 months and at 1 year. For *B. fragilis*, the probability of vertical transfer increased with age, while the environmental transmission was quite low. In addition, *E. coli* at the age of 1 year increased

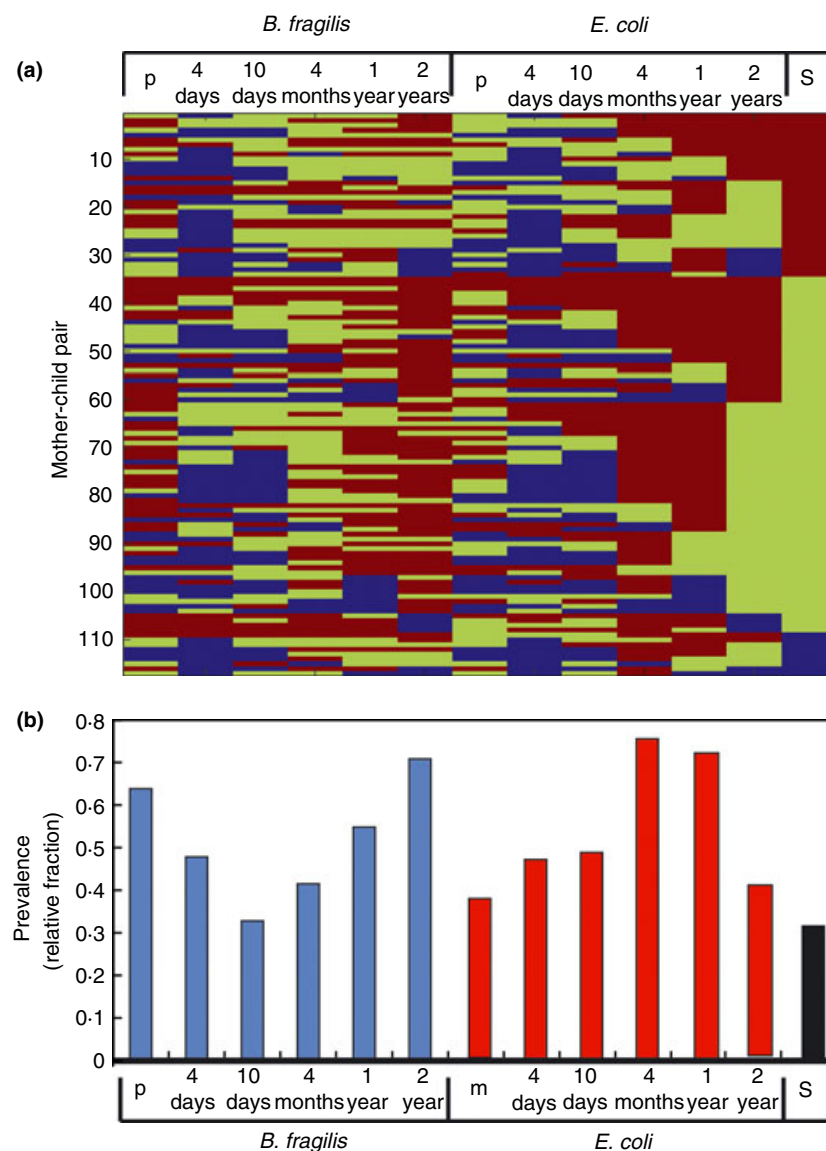


Figure 2 Prevalence data. (a) Prevalence data (presence/absence) were calculated for *Escherichia coli* and *Bacteroides fragilis*, based on the thresholds shown in Fig. 1. Prevalence data for each individual measurement are systemized for the last column – colour at the top indicates presence, in the middle absence, and in the bottom lack of measurement. (b) Summary of the relative frequency data for each age group. (a and b) The following abbreviations were used: p – pregnancy, d – day, m – month and S – sensitized.

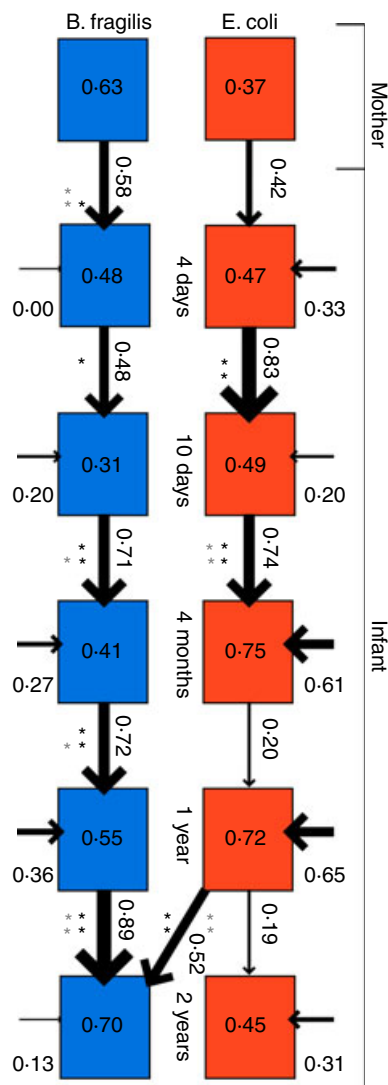


Figure 3 Infant colonization patterns by *Escherichia coli* and *Bacteroides fragilis*. The figure represents the probability of transfer of the bacteria from one age group to the next as a Markov chain. The numbers in the boxes represent the actual prevalence data. The numbers between the boxes show the probability of vertical transfer, while the numbers for the horizontal arrows show the probability of horizontal transfer from the environment. The asterisks indicate significance: * $P < 0.05$, ** $P < 0.01$. The colour code for the significance levels is that black represents the Fisher's exact test, while grey represents the McNemar test.

the probability of *B. fragilis* being present at 2 years (Fig. 3).

Temporal model for sensitization

The temporal trends in sensitization were modelled using regression tree analyses. In the regression tree analyses, we started with a relatively complex model comprising seven

nodes. Inspection of these trees showed that they were overfitted, showing spurious relations. We therefore increased the stringency in the model by enlarging the sizes of groups before splitting a node. Based on a trade-off between complexity and misclassification, we found the best fit (11.5% misclassification rate) to a simple model with only three terminal nodes (Fig. 4). Validation of the model showed that all nine children with *E. coli* at day 4 were nonsensitized, while 75% (six out of eight) of the children lacking *E. coli* at day 4 and having *B. fragilis* after 4 months were sensitized.

The significance of the model was evaluated using an exact binominal test. This test determines if the distributions observed can occur by chance or not. Using the exact binominal test, we found a significant association between the presence of *E. coli* at 4 days and lack of sensitization ($P = 0.05$). The exact binominal test also confirmed that lack of *E. coli* at 4 days and colonization by *B. fragilis* at 4 months were correlated to sensitization ($P = 0.03$).

Effect of the mother's microbiota on sensitization

Our final analyses sought to identify the correlation between the microbiota in mothers and subsequent sensitization of the infants. Direct investigation of the original data by ANOVA indicated a correlation between *E. coli* in mothers and sensitization ($P = 0.06$), while we could not detect any correlation for *B. fragilis* ($P = 0.36$). Fisher's LSD method with a 95% confidence interval for the non-sensitized children showed prevalence estimates of $20 \pm 21\%$ for *E. coli* and $10 \pm 22\%$ for *B. fragilis*.

Based on the lack of significance for the empirical data, we were prompted to explore the potential of correlations between the presence/absence of *B. fragilis* and *E. coli* in mothers and the sensitization state of the children at the age of 2 years using simulations for prevalence data outside that observed. Using the Markov chain derived from the colonization model in Fig. 3, we simulated the sensitization effect of mother populations with *E. coli* and *B. fragilis* prevalence ranging from 0 to 100%, given the regression tree in Fig. 4. Using this simulation, we found the highest probability of sensitization in the situation with low *E. coli* and high *B. fragilis* in the mothers. Low *B. fragilis* and high *E. coli*, on the other hand, correlated to low probability of sensitization (Fig. 5). By ANOVA for the simulated data, we found significance for differences in the prevalence of *B. fragilis* and *E. coli* in mothers with respect to the sensitization outcome ($P < 0.01$ for both). The effect was largest for *E. coli*, where the modelled prevalence was $11 \pm 4\%$ (determined by the Fisher's LSD method with a 95% confidence interval) higher in mothers of nonsensitized children than that of sensitized

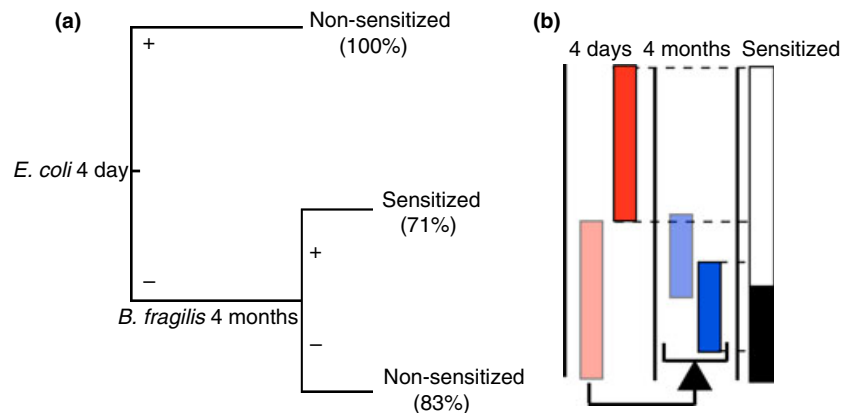


Figure 4 Model for infant sensitization. (a) Regression tree model of sensitization state. The tree shows the bacterial compositions correlating to the sensitization state. The percentages at the terminal nodes show the classification accuracy. (b) A model for sensitization was built based on a regression tree analyses. The filled bars represent presence, while the transparent bars represent absence of *Escherichia coli* and *Bacteroides fragilis* (*E. coli* to the left). With respect to sensitization, the white bars represent nonsensitized, while the black bars represent the sensitized children. The arrow represents the dependencies in the data.

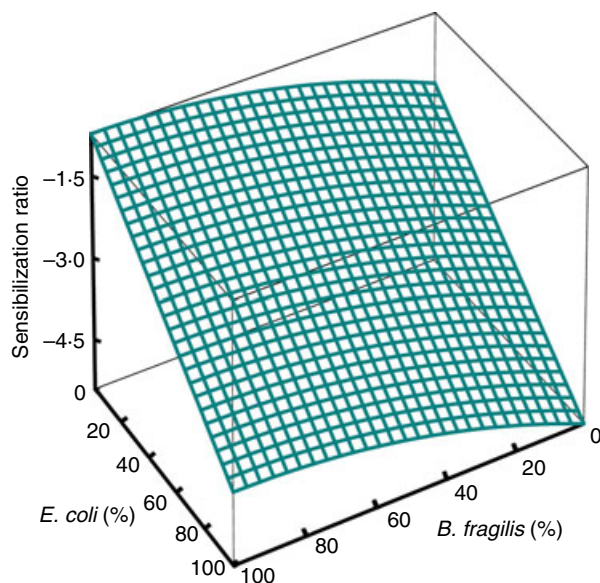


Figure 5 Modelling the effect of *Escherichia coli* and *Bacteroides fragilis* prevalence in the mother on infant sensitization. The predicted effect of *B. fragilis* and *E. coli* prevalence in mothers on the sensitization ratio (outcome of 10 simulations per point) is shown.

children. The modelled effect was opposite for *B. fragilis*, with corresponding differences in mothers' prevalence of $7 \pm 4\%$.

Discussion

We acknowledge that our model represents a simplified view of the transmission and persistence patterns of the infant gut microbiota and that there are uncertainties with our parameter estimates owing to the analysis of

relatively few individuals and bacteria. Gut bacteria may also have strong interaction networks (Trosvik *et al.* 2010), making it difficult to untangle the actual casual interactions. Furthermore, we cannot rule out that the detected colonization pattern is a consequence, rather than a cause of the sensitization state (Spor *et al.* 2011). Theoretically, there could also be underlying genetic differences leading to differences in bacterial colonization susceptibility between the sensitized and nonsensitized children (Junge 2011). Despite these limitations, we believe that deriving models and doing simulations are an important step on the path leading to better understanding of the function of microbes in the human gut.

The main rationales for assuming that the sensitization state is a consequence of the bacterial colonization are the temporal separation of the events leading to sensitization and the actual sensitization state measured at the age of 2 years. Furthermore, we have recently shown that bacterial colonization patterns of infants can be explained by bacteria–bacterial interactions with no major host interference (Trosvik *et al.* 2009). Taken together with the fact that adaptive immunity is not well developed for infants (Brandtzaeg 2003), this supports the interpretation that sensitization is a consequence of bacterial colonization rather than a cause of it.

Previous works have shown that both *E. coli* and *B. fragilis* can modulate the immune system (Lodinova-Zadnikova *et al.* 2010; Troy and Kasper 2010). *E. coli* is a strong trigger of the innate immune system (Heimesaat *et al.* 2007), while *B. fragilis* has an immunosuppressive effect (Bannerman *et al.* 2004). We therefore propose a sensitization model where early immune stimulation by *E. coli* protects for sensitization, irrespective of later colonization patterns. Without an early stimulation, our model

suggests that immune suppression at about 4 months of age would trigger sensitization.

There has been a long-standing debate of whether vertical transmission of bacteria from mother to infant is important in allergy development. The actual transmission of both *B. fragilis* and *E. coli* from mother to infant has recently been suggested by us (Bjerke *et al.* 2011; de Muinck *et al.* 2011). Here, using simulations, we also found support for the importance of vertical transmission of bacteria from mother to child in IgE sensitization. The conclusion concerning the protective effect of *E. coli* in the mothers seemed quite robust with both observational and simulated data, indicating the same protective tendency. The situation for *B. fragilis*, however, is not that conclusive. The observational data showed no effect, while the simulations indicated that high *B. fragilis* in mothers is the cause of the sensitization state. Here, additional studies are needed to determine whether an effect has been overlooked in the observational data.

We observed in our data a change in persistence between *E. coli* and *B. fragilis* at about 4 months. *Escherichia coli* showed a relatively high persistence between 10 days and 4 months, while the persistence was low above 4 months. *Bacteroides fragilis*, on the other hand, showed an increased persistence from 4 months to 1 year and a further increase from 1 to 2 years. Interestingly, the change in persistence corresponded with the development of the adaptive immune system (Brandtzaeg 2003). Thus, the persistence switch between *E. coli* and *B. fragilis* could reflect a switch to selection by the adaptive immune system, with escape of *B. fragilis* by phase variable surface epitopes and immune modulation (Nakayama-Imaohji *et al.* 2009; Round *et al.* 2011).

Of future interest will be to investigate the effect of modulating the bacterial transmission from mother to child in the actual birth process on allergy development. This process represents a particular challenge, because the child should be protected against pathogens, at the same time as being exposed to bacteria that promote a proper immune development. Further definition of the bacteria and processes that are needed for proper immune maturation, however, could help in defining more targeted approaches in exposing children to bacteria, combating both infectious diseases and allergy-related disorders.

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