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Research Article

Effects of antibiotic exposure and immune system challenge on the development of allergic asthma

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Abstract. In previous studies, experimenters have found that the risk of atopic disease is increased due to less exposure to infection and increased antibiotic use during immune system development. The data have supported this finding in accordance with hay fever, eczema and atopy, but are inconclusive regarding asthma. This study aims to further research the effects of antibiotic use and immune system challenge early in life on the development of allergic asthma. This was done retrospectively by collecting data from labor and delivery records of mothers who delivered vaginally, pediatric records up until age two, and participant completed questionnaires concerning family medical history and home environment information. A total of 206 women and 277 children, of which 18.4% have asthma, were enrolled in the study. Children who were prescribed antibiotics anytime after birth until two years of age were statistically more often diagnosed with asthma than those who were not given antibiotics (OR 2.884, CI 1.093-7.637). Results also suggest that babies who were born pre-term (<37 weeks gestation) were statistically more likely to be asthmatic which may reflect insufficient lung development (OR 3.048, CI 1.191-7.801). Birth order and exposure to antibiotics administered to the mother during delivery, which can correspond to immune system challenge, were determined to have no statistical relationship with asthma diagnoses. This research supports that allergic asthma is dissimilar to other atopic diseases in relation to birth order. More data are currently being collected to further study these relationships.

Introduction

According to the Global Initiative for Asthma (Braman, 2006; Masoli et al., 2004), approximately 300 million people globally suffer from asthma and it is the cause of 1 in every 250 deaths worldwide. An increase of approximately 100 million more cases is projected to occur by 2025. It is not only a major health problem, but also an economic burden due to the cost of hospitalizations and treatments needed to control symptoms (Braman, 2006; Kozyrskyj et al., 2007; Masoli et al., 2004;). Asthma is defined as a chronic inflammatory disorder of the airways which increases airway hyper-responsiveness causing wheezing and coughing. This airflow obstruction varies in severity and is often reversible (Braman, 2006; Masoli et al., 2004). An asthmatic phenotype is expressed based on a genetic predisposition and the interaction between genes and environmental stimuli (Romagnani, 2004). The inflammation associated with asthma is due to a skewed ratio of immune cells and cytokines and their effects on lung tissue. Two major hypotheses are commonly used to explain the causes of asthma with a third

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hypothesis recently being developed (Kozyrskyj et al., 2007; Romagnani, 2004). These hypotheses are based around the evidence that asthma has become more prevalent over the last few decades as countries became more urbanized, and that there is a greater incidence of allergic disease in "westernized" countries as compared to developing countries (Braman, 2006; Kozyrskyj et al., 2007; Masoli et al., 2004; Romagnani, 2004).

The hygiene hypothesis suggests that lack of exposure to viral and bacterial infections early in life results in insufficient production of T helper type 1(Th1) cells (Kozyrskyj et al., 2007). Recently, it has been determined that many bacterial and viral components interact with receptors called Toll-like receptors (TLRs) on innate immune cells (Sabroe et al., 2003). These cells then produce certain cytokines, the most influential to the hygiene hypothesis being IL-12, which signals T helper cells to differentiate into Th1 effector cells by turning on promoters (T bet) to produce IFN- γ (Manetti et al., 1993; Szabo et al., 2000). A Th1 response occurs due to IFN- γ produced by natural killer cells and the IFN-α produced by dendritic cells (DCs) (Maggi et al., 1992). The subsequent production of IL-2, IFN- γ, and TNF-β by Th1 cells prevents differentiation of Th cells into type 2 (Rengarajan et al., 2000; Romagnani, 1994). Therefore, a lack of bacterial and viral exposure during immune system development can retard Th1 differentiation which leads to a Th2-dominated environment. Cytokines released by Th2 cells (IL-4, IL-5, IL-13) signal to B cells to isotype switch and produce IgE, predisposing an individual to allergic asthma, along with other atopies (Kozyrskyj et al., 2007; Romagnani, 1994; Romagnani, 2004; Yazdanbakhsh et al., 2002).

A second hypothesis is the microflora hypothesis which credits the disruption of the commensal gut microflora as the cause of abnormalities in the microbiota-mediated mechanism of immunological tolerance. Antibiotics and certain diets can have profound effects on the bacterial community of the gastrointestinal (GI) tract (Noverr and Huffnagle, 2005). Supporters of this hypothesis speculate that commensal microbial flora in the GI tract are needed to aid in the development of immunological tolerance through the action of Treg cells. These cells develop when dendritic cells (DCs) present antigens in a non-inflammatory environment, like that of the GI tract when a normal bacterial community is present. DCs signal for naïve T cells to differentiate into Treg cells which produce anti-inflammatory molecules, including IL-10 and transforming growth factor-β (TFG-β). This hypothesis assumes that oral tolerance and airway tolerance are very closely coupled so that allergic asthma can result if immune tolerance to common antigens is not generated in the GI tract (Noverr and Huffnagle, 2005). Support for this hypothesis comes from mouse models in which the GI flora were altered and increased allergic airway disease was the result (Kozyrskyj et al., 2007; Schumann et al., 2005; Noverr et al., 2005). It has also been observed that germfree mice are unable to generate oral tolerance (Maeda et al., 2001).

Another more recent hypothesis posed to elucidate the immunological basis of allergic asthma combines the regulatory component of the microflora hypothesis and the exposure component of the hygiene hypothesis. Studies have found that increased exposure to helminth infections, which induce a predominately Th2 response, decrease the risk of allergic disease (Romagnani, 2004; Wills-Karp et al., 2001; Yazdanbakhsh et al., 2002). This contradicts the idea behind the hygiene hypothesis that an overabundance of Th2 cells contributes to asthma. Therefore, it is now being hypothesized that antigen exposure early in life that elicits either a Th1 or Th2 response can decrease the prevalence of allergic disease due to regulatory cytokines (mainly IL-10) produced by many immune cells. Lack of exposure to common antigens during immune system development can lead to low amounts of the 'anti-danger' signal provided by regulatory functions of IL-10 which can result in stronger immune responses and symptoms of allergic disease (Wills-Karp et al., 2001; Yazdanbakhsh et al., 2002).

This study was designed with these hypotheses in mind to investigate the effects of

antibiotics administered and antigen exposure to children during the development of the immune system on the development of allergic asthma. The purpose is to provide information to physicians and parents that may help prevent the occurrence of allergic asthma by altering certain practices, if possible.

Materials and Methods

This study was designed as a retrospective cohort study. Potential participants were identified, in accordance with IRB protocol, by individuals not associated with the study in the billing office at Penn State Hershey Medical Center. In order to participate, women had to have delivered one or more children vaginally at PSHMC from 1996-2008. Through the Penn State Ambulatory Research Network (PSARN), the primary care physicians of these eligible participants were contacted and they were asked to invite patients that they felt would be appropriate for the study. These primary care physicians at their discretion dismissed those patients who had children that did not survive until the age of two or for various other reasons were not good candidates for the study.

Each approved, prospective participant was sent an invitation letter from her primary care provider inviting her into the study. This letter contained information about the basic study aims (written in a manner so as to limit selection bias), contact information for questions, and directions with an identification number to complete an online questionnaire. This questionnaire was designed to collect information concerning each participant's pregnancy, race, home environment of the child(ren) (e.g., whether there were pets or smokers in the home), and family health history (including familial history of asthma). The questionnaire was formatted by the Center for Opinion Research (COR) at Franklin and Marshall College. Paper copies were made available upon request for participants who did not have access to the internet so as not to discriminate against any class.

Medical release forms were also included in the information packet sent to eligible participants. These release forms gave consent for investigators trained in confidentiality to access medical records of the children of participants from 0-2 years of age. Consent was assumed for investigators to access each mother's labor and delivery records upon completion and signing of the child's medical release forms. Once a participant completed both the questionnaire and returned completed medical release forms, she was remunerated.

The labor and delivery records of confirmed participants were requested from Penn State Hershey Medical Center and data surrounding the birth and newborn status were then collected. In order to obtain the data from the children's medical records, each child's primary care physician (as reported by the mother) was contacted. Medical record releases were made available to the staff at each health care office. Once access was granted, investigators traveled to each health care facility to abstract data from each child's records from 0-2 years of age. This included weight and height at 2, 6, 9, 12, 18, and 24 months, any documentation of asthma, eczema, or allergies, and any antibiotics administered.

Data analysis was then performed using only participant information that was complete, meaning the questionnaire was completed, labor and delivery record data was abstracted, and childhood record data was abstracted. If any of these elements were missing, that case was not used in this preliminary analysis. To ensure equal gender distribution, a Chi-squared analysis was performed. Odds ratios were calculated between children with asthma and antibiotics administered to the mother during delivery, antibiotics prescribed to the child between 0-2 years of age, pre-term deliveries ($<$37 weeks), whether the child had older siblings or pets, smoking preferences of mother during pregnancy, and parental asthma (mother or father suffered from asthma).

Results

The data collected from questionnaires and the medical records of 277 mother/child pairs were used in this analysis. There were 155 male

and 122 female children in this study. A total of 51 of the children were diagnosed with asthma according to medical records, 33 males and 18 females. No significant difference was observed between males with asthma and females with asthma, so all data were analyzed without gender consideration ($x^2=1.942$). There were 56 sets of siblings in the dataset. The age of the mothers ranged from 18-45.

The odds ratios between children diagnosed with asthma and possible risk factors are presented in Table 1. Statistically significant associations ($p<0.05$, CI 95%) were found between asthma diagnoses in children and antibiotics administered before 2 years of age (OR 2.884, CI 1.093-7.637), pre-term deliveries (OR 3.048, CI 1.191-7.801), maternal smoking during pregnancy (OR 4.804, CI 1.336-17.274), and parental asthma (OR 4.755, CI 2.408-9.39). When parental asthma was further analyzed, children with asthma showed significant associations with mothers with asthma as well as fathers with asthma (OR 3.169 and OR 4.767, respectively).

There was not statistical significance between asthma diagnoses in children and antibiotics delivered to the mother during delivery, the presence of a cat or dog in a child's home, or a child having one or more older siblings (All had ORs <1 and/or CIs including values <1).

Discussion

Statistical significance between childhood diagnoses of asthma and administration of antibiotics 0-2 years of age (Table 1) may support the microflora hypothesis. According to this hypothesis, the microbe community being developed early in life is disrupted by antibiotics. This lowers diversity in the GI tract and inhibits the development of immunological tolerance to common molecules leading to allergic disease, including asthma (Noverr and Huffnagle, 2005). Previous studies comparing the intestinal microflora of children with atopy and those without have discovered that there are differences that may contribute to atopy development (Bjorksten et al., 1999; Kalliomaki et al., 2001). A meta-analysis has found that the risk of childhood asthma increases two-fold if antibiotics are administered in the first year of life (Marra et al., 2006). The odds ratios calculated for antibiotics administered before one year and asthma diagnoses was greater than that for antibiotics administered before two years of age (3.099 and 2.884, respectively). The more significant association before one year supports previous findings (Marra et al., 2006). While these results may support the idea that antibiotics are the cause of asthma development, as opposed to the asthma being a cause of the infections requiring antibiotic administration, it is also possible clinicians prescribe antibiotics for symptoms masking atopic disorders (e.g., wheezing, cough, skin disorders). Therefore, it is not possible to determine the actual cause and effect relationship in a retrospective study.

There was no significant association between antibiotics administered during delivery to mothers and asthma development (Table 1). In this analysis, the start time of antibiotic administration was not taken into consideration. If there was not sufficient time for the antibiotics to be passed from mother to child before delivery then intrapartum administration of antibiotics would have no effect on the child. Future analysis will separate participants by time between the start of intrapartum antibiotic administration and delivery. Previous studies have determined that approximately two hours is required for antibiotics delivered to mothers to affect a child (de Cueto et al., 1998).

Assuming that the antibiotics prescribed to children up until the age of two were given for significant bacterial infections, those children receiving antibiotics would have experienced more exposure to bacteria than those who were not prescribed antibiotics. Therefore, the significant association between antibiotics given and asthma diagnoses (Table 1) counters the idea that any exposure to antigen early in life decreases the risk of developing allergic asthma. The administration of antibiotics and the exposure to bacterial and viral infections are coupled. A possible explanation to reconcile the results of this study with both hypotheses is that antibiotic administration and antigen exposure both affect immune tolerance, but that imposed

Table 1. Associations with asthma for proposed environmental and genetic risk factors. Statistically significant relationships are shown in bold font.

Risk factors	Non-asthmatic (n=226)	Asthmatic (n=51)	OR (95% confidence interval)
Antibiotics administered during delivery	63	11	0.7115 (0.344-1.473)
Antibiotics administered up to 2 years of age	**172**	**46**	**2.884 (1.093-7.637)**
Antibiotics during delivery or up to 2 years of age	191	48	2.932 (0.865-9.939)
Antibiotics administered up to 1 year of age	**122**	**40**	**3.099 (1.514-6.348)**
Pre-term delivery	**13**	**8**	**3.048 (1.191-7.801)**
One or more older siblings	132	35	1.558 (0.815-2.978)
Cat or dog	145	29	0.736 (0.397-1.365)
Mother smoked during pregnancy	**5**	**5**	**4.804 (1.336-17.274)**
Parental asthma	**29**	**21**	**4.755 (2.408-9.39)**
Mother	**12**	**20**	**3.169 (1.434-7.006)**
Father	**10**	**11**	**4.767 (1.901-11.953)**

by antibiotics is stronger. Lack of a significant relationship between the absence of pets and/or older siblings in the home environment (these variables were used to gauge bacterial and viral exposure) and asthmatic children further contradict the regulatory exposure hypothesis.

A non-association between lack of pets and/or older siblings and asthma diagnoses also provided evidence opposing the hygiene hypothesis. The presence of pets and/or older siblings in a child's home was assumed to correlate with more bacterial and viral exposure. Our results are in accordance with other studies that have determined no statistical relationship between birth order and increased risk of asthma before the age of two (McKeever et al., 2001; Strachan, 2000). These studies did link birth order to increased risk of other atopies, indicating that asthma may be developmentally different from eczema or hay fever, for example.

Maternal smoking was more often associated with children diagnosed with asthma than those non-asthmatics. Nicotine is a known immunosuppressive component of cigarette smoke (Sopori, 2002). With this in mind, it is reasonable to suggest that when the nicotine passes from the mother to the child in utero, immune cells are affected which could have major implications in immune tolerance. As discussed in the introduction, allergic asthma can occur when immune tolerance is not sufficiently developed (Noverr and Huffnagle, 2005).

Pre-term delivery was another factor found to be positively associated with childhood asthma diagnoses (Table 1). Being born before 37 weeks gestation shortens lung development time and increases a child's risk of asthma. Previous studies have found this connection between pre-term children and asthma (Jaakkola et al., 2006).

The significant association between asthma in the child and parental asthma is not a surprising result. Although, the genetic component in the development of asthma is not completely clear, several chromosome regions and genes have been identified in the causation and development of asthma. Like many diseases, the environmental and genetic factors are intertwined and not completely understood (Malerba and Pignatti, 2005). Because there are numerous immune cells and cytokines involved in inflammation, the basis of asthma symptoms, it is extremely difficult to pinpoint exactly how asthma is inherited.

Analysis of the data collected in this study, when considered as a whole, does not support any of the proposed three hypotheses fully. It was determined that antibiotic administration to children before the age of two was associated with the development of asthma, but antigen exposure (as was estimated by pets and older siblings) did not seem to be a major contributing factor. It is possible that select components of each hypothesis are influential in asthma development. Because of the complexity of the development of asthma, there may be confounding factors that cannot be directly

observed using a retrospective study. There may also be some inaccuracy in this data due to the fact that some of the information is self-reported. One conclusion that does seem to be supported is that there are both genetic and environmental factors that come into play, making it ever more difficult to understand the mechanism of asthma development.

Additional analysis of the study data will be performed in order to further investigate possible association between other variables (e.g. breastfeeding, mother's age at delivery, APGAR score) and the development of asthma. More in-depth analysis focusing on genetically predisposed individuals will also be performed to determine if some environmental variables are more influential than others in triggering the development of asthma.

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