Background

According to the Centers for Disease Control and Prevention's National Center for Health Statistics, the rate of cesarean section rose to 29.1% in 2004 in the United States (a > 40% rate increase since 1996) [1]. Because cesarean section has been associated with increased risks of asthma [2-5] and atopy [6-8], further understanding of the relationship between mode of delivery and immune system ontogeny is needed.

Several studies have shown immunological differences between children with and without atopy at the time of birth. For example, increased cord blood levels of IL-13 have been positively associated with atopy among children with a family history of atopy [9-11]. Although less consistent, increased cord blood levels of IFN-γ have been associated with atopy among children with a family history of atopy [11,12]. In children at risk for atopy, increased neonatal levels of IL-10 have been associated with reduced risk of egg allergy[10] but increased risk of atopic dermatitis [11,12]. Among children unselected for family history, detectable neonatal IL-10 was associated with a reduced risk of asthma at age 6 years[13].

We hypothesized that mode of delivery influences neonatal immune responses. Specifically, we examined whether cesarean section results in neonatal secretion of cytokines associated with increased risk of atopy and/or asthma in childhood. We were also interested in exploring potential mechanisms for any observed association between mode of delivery and neonatal immune responses.

In murine models [14], oral exposure to lipopolysaccharide (LPS) during passage through the birth canal triggers gut epithelial cell activation, as measured by production of the chemokine MIP-2 and activation of the transcription factor NF-κB. In contrast, activation of gut epithelial cells does not occur in mice delivered by cesarean section. These findings suggest that microbial exposure during passage through the birth canal may trigger immune responses leading to tolerance in mice.

During the natural birthing process of humans, neonates transition from the sterile environment of the womb to a nonsterile environment where they are exposed to microbes originating from their mother and the surrounding environment. Neonates born by vaginal delivery acquire most of their intestinal flora by swallowing their mother's vaginal fluid at birth. In contrast, children born by cesarean section are not exposed to the maternal vaginal flora at birth. We examined whether specific microbes in the maternal intestinal flora (which is closely correlated with the maternal vaginal flora) [15-18] has different influences on neonatal immune responses depending on mode of delivery.

Methods

Study cohort

Pregnant women were recruited between July 2003 and November 2005 from three outpatient facilities affiliated with Brigham and Women's Hospital in Boston at their 24-week prenatal visit. Inclusion criteria were maternal age between 18 years and 44 years; plans to deliver at Brigham and Women's Hospital; and maternal ability to speak English or Spanish. Informed consent was obtained from participating mothers. The study was approved by the Institutional Review Board of the Brigham and Women's Hospital.

Questionnaire and review of medical records

A questionnaire was administered to each participating woman between her 24-week prenatal visit and delivery to obtain information on demographics, general health, and history of allergic diseases and/or symptoms in herself and the father of her child. Information on labor and delivery (including the white blood cell count of participating mothers) was obtained from review of medical records.

Isolation of Cord Blood Mononuclear Cells

Cord blood samples were collected by needle/syringe from the placental side of the umbilical vein after the newborn was delivered but prior to placental delivery. Samples were processed within 24 hours, and cord blood mononuclear cells (CBMCs) were isolated from umbilical cord blood by density gradient centrifugation with Histopaque (Sigma-Aldrich, St. Louis, MO).

Cytokine measurements

Aliquots of 4×10^5 CBMCs were incubated in triplicate in 96-well round-bottom tissue-culture plates (Corning, Acton, MA) at 37°C in 5% CO_2. At the start of the culture, cells were either unstimulated (media) or stimulated with each of the following antigens: dust mite allergen (*Der f* 1) at 30 μg/ml, cat dander allergen (*Fel d* 1) at 10 μg/ml (Indoor Biotechnologies, Charlottesville, VA), phytohemagglutinin (PHA) at 10 μg/ml, and LPS at10 μg/ml. Cell supernatant fluids were harvested 24 hours after stimulation and analyzed in duplicate for cytokine (IL-13, IFN-γ, IL-10) production by ELISA according to the manufacturer's instructions (Pierce Biotechnology, Inc., Rockford, IL). The sensitivities of the assays were < 2 pg/ml for IFN-γ, < 7 pg/ml for IL-13, and < 3 pg/ml for IL-10.

Stool collection and culture

A stool sample was collected from participating women between their 24-week prenatal visit and delivery. More than a gram of stool was collected into a sterile specimen container and frozen for transport to the laboratory. Samples were weighed, serially diluted (10^{-2} to 10^{-7}) with sterile phosphate-buffered-saline (PBS) in an anaerobic