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*J Immunol* 1998; 160:3578-3584; ;
http://www.jimmunol.org/content/160/7/3578

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B Cell Sensitization to Helminthic Infection Develops In Utero in Humans

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Human neonates are generally deficient in their ability to generate humoral immunity. This deficiency is thought to reflect physiologic immaturity of T and B cell function and lack of previous exposure to exogenous Ags. To determine whether neonatal humoral immunity can be modified by maternal helminth infection during pregnancy, we assessed Ig production by cord blood lymphocytes from healthy newborns of mothers living in an area of Kenya where schistosomiasis, bancroftian filariasis, and geo-helminth infections are endemic. Twelve of 40 and 17 of 39 cord blood lymphocyte preparations from healthy newborns in Coast Province, Kenya, spontaneously made polyclonal IgE (range, 0.15–21 ng/ml) and IgG (1.6–10.1 ng/ml) in vitro. In vitro IgE synthesis by cord blood lymphocytes (CBL) was, on the average, 10-fold less than that of PBMC of Kenyan mothers (1.1–98 ng/ml) and was undetectable for CBL from newborns delivered in the United States. Schistosome and filarial Ags stimulated a 3- to 100-fold increase in the production of polyclonal IgE and parasite-specific IgG Abs by lymphocytes from 10 of 40 and 6 of 39 Kenyan newborns, respectively. CBL observed to have helminth Ag-driven B cell responses were more likely to be from newborns of schistosome- or filaria-infected mothers than from uninfected mothers (p < 0.05). These data indicate that the human fetus can be sensitized in utero to produce helminth-specific B cells and that neonatal B cells are intrinsically capable of IgE and IgG production.


Multiple studies indicate that human newborns are immunologically naive and that T and B cells are functionally immature (1–4). Accordingly, immunity against infectious pathogens encountered during the first few weeks to months after birth is thought to be mediated primarily by maternal IgG Abs obtained transplacentally or from colostrum (5, 6). Nevertheless, neonatal T cells are capable of producing cytokines (7, 8) and expressing costimulatory molecules necessary for B cell Ig isotype switching (e.g., CD40 ligand) if appropriately activated (9, 10), suggesting that the fetus itself may be able to generate polyclonal and pathogen-specific Abs in utero. This prenatal sensitization to infectious pathogens runs counter to the idea that in utero exposure to Ags engenders immunologic tolerance (11, 12).

Several lines of evidence suggest that maternal infection or prenatal exposure to soluble Ags in humans leads to immunologic sensitization of the developing fetus. The offspring of women with mumps or toxoplasmosis during pregnancy exhibited Ag-specific Ab and memory T cell responses to viral and toxoplasma Ags (13, 14). Babies whose mothers during pregnancy were immunized with tetanus toxoid, streptococcal, or meningococcal vaccines had anamnestic Ag-specific Ab and T cell responses when childhood vaccines were administered (15–17). Finally, since Ig isotypes other than IgG normally do not cross the placenta, the presence of parasite-specific IgE and IgM Abs in cord blood has been taken as evidence that prenatal sensitization can occur in the context of chronic parasitic infections such as schistosomiasis and filariasis (18–21) and atopic diseases (22–24). These latter studies examined only IgE levels in cord sera and not in vitro Ig production by cord blood lymphocytes. The influence of maternal parasitic infections on immunity during early infancy may be particularly important in developing countries where helminthiases and malaria are prevalent. For example, epidemiologic studies of subjects with a history of atopy (25) or helminth infections (26), and investigations of helminth-infected mice (27, 28) indicate that type 2 responses can bias immunity to new Ags, such as those included in childhood vaccines.

We recently reported that CD4+ T cells from Kenyan newborns and their helminth-infected and mycobacteria-exposed mothers have similar patterns of T cell cytokine responses, including the capacity to make IL-4 and IL-5 (29). Polyclonal and Ag-specific IgE were also detectable in some cord blood samples. The functional maturation of neonatal B cells function was, however, not directly evaluated. To address this issue and determine the relationship between maternal helminth infection and the capacity of newborns to make IgE and IgG, we examined polyclonal and Ag-driven B cell responses by a different cohort of newborns and their mothers living in an area of Kenya where schistosomiasis, filariasis, and geo-helminth infections are endemic.

Materials and Methods

Study population

Thirty-nine paired cord and maternal blood samples were collected at Msambweni District Hospital in Coast Province, Kenya. An additional sample of cord blood was obtained from an uncomplicated delivery, but blood was not available from the mother of this newborn.
Women who attend the prenatal clinic at Msambweni District Hospital live in neighboring communities where there is a high prevalence of Schistosoma haematobium, Wuchereria bancrofti, and/or mixed intestinal helminth infections, predominantly hookworm sp., Trichuris trichiura, and Ascaris (30, 31) (P. Mungai, J. Ouma, and C. L. King, unpublished observations). Umbilical cord blood was collected from full-term newborns of uncomplicated pregnancies. This population is a different group of newborns and mothers than that reported previously (29). For comparison to newborns whose mothers were not infected with parasitic helminths, cord blood was obtained from five offspring of healthy American mothers who delivered their children at University Hospitals of Cleveland. Informed consent for donation of blood was granted by pregnant women before delivery.

Helminthic infection status of pregnant women was determined 3 mo or less before delivery. Several diagnostic methods were used. First, urine and anticoagulated blood were passed through a Nucleopore filter (Nucleopore Corp., Costar, Cambridge, MA) to identify eggs of S. haematobium (32) and microfilariae of W. bancrofti, respectively. Second, serum filarial Ag was measured by ELISA using mAb Og4C3 (Trop-Ag W. bancrofti assay, JCU Tropical Biotechnology Pty. Ltd., Townsville, Australia). Third, active and/or recent schistosome or filarial infection was assessed by the presence of elevated IgG4 Abs to soluble lysates of adult S. haematobium (SWAP) or Brugia malayi worms (BmA) (33, 34). Fourth, infection with intestinal helminths was diagnosed by microscopic examination of a single stool specimen using the formal ether method. Using these criteria, 31 of 39 pregnant Kenyan women were infected with one or more helminths. Eleven individuals had S. haematobium infection, nine had W. bancrofti infection, and 18 women had one or more geohelminth infections (stool samples were available from only 28 of 39 eligible subjects).

In none of the newborns was there any sign of infection based on the presence of microfilaria or filarial circulating Ag using the Og4C3 assay in cord blood. Urine samples from newborns were difficult to obtain. None of the few neonates examined (n = 9) had ova in their urine.

**Ags and mitogens**

SWAP and BmA were prepared as saline extracts of adult-stage parasites (35, 36). Endotoxin in these preparations was <0.5 ng/ml (5- to 50-fold less than that required for LPS stimulation of cytokine production by human lymphocytes) (37). Pokeweed mitogen (PWM) was obtained from Sigma Chemical Co. (St. Louis, MO).

**Cell culture conditions for in vitro cytokine production**

Studies were performed using freshly isolated mononuclear cells separated from heparinized maternal venous blood (PBMC) or cord blood (CBL) by Ficoll-Hypaque density gradient centrifugation. The cell preparations were suspended in Iscove’s DMEM supplemented with 10% FCS, 4 mM L-glutamine, 25 mM HEPES, 80 µg/ml gentamicin, and ITS (insulin, transferrin, and selenium; BioWhittaker, Walkersville, MD). Cultures were set up at a density of 2 × 10^6 cells/ml in a total volume of 1 ml as previously described (38). Medium alone, SWAP (1–20 µg/ml), BmA (1–10 µg/ml), or PWM (5 µg/ml) was added to duplicate aliquots of cells, and incubation was conducted at 37°C in 5% CO2 for 12 to 14 days. Supernatants were immediately frozen at −70°C for subsequent determinations of Ig and parasite-specific Ab levels. Separate cultures were used for SWAP and BmA stimulation. The data expressed in the manuscript are values for Ag-driven Ig of the greatest of the two Ag responses for a particular individual. The same tissue culture supplements and Ag preparations were used for both Kenya and Cleveland.

**ELISAs for polyclonal and parasite-specific IgE and IgG**

Polyclonal IgE in culture supernatants and sera were measured by an avidin-biotin-amplified ELISA as previously described (38). Helminth-specific IgE Abs produced by maternal PBMC or CBL cultures in vitro were not evaluated because pilot studies using PBMC from helminth-infected Kenyans failed to demonstrate parasite-specific Ig of this isotype using the current methodology (C. L. King, and I. Malhotra, unpublished observations). Polyclonal and parasite-specific IgG levels in culture supernatants and serum parasite-specific IgE and IgG4 levels were quantified as previously described (39, 40). Polyclonal IgM was performed as described previously (39).

**Statistics**

Results are expressed as the mean ± SEM using log-transformed data unless otherwise stated. Experimental conditions were compared using Fisher’s exact test, and ordered data were evaluated for significance of differences by Spearman’s rank correlation.

**Results**

**Polyclonal and helminth-specific IgE in sera from maternal and cord blood**

Polyclonal IgE was not detectable in sera from cord blood of North American newborns. In contrast, 17 of 40 samples obtained in Kenya had elevated levels of polyclonal IgE, with values ranging from 12 to 990 ng/ml. Ninety percent of serum samples from mothers of these newborns had elevated polyclonal IgE (Fig. 1).

A proportion of the IgE in sera from Kenyan newborns and their mothers was directed against schistosome and filarial Ags. In the case of SWAP, Ag-specific IgE was present in sera from 52% of the neonates (range, 2–30 ng/ml) and 75% of the mothers (range, 2.5 to >1000 ng/ml). BmA-specific IgE was present in 69% of neonates’ sera (range, 0.8–70 ng/ml) and 83% of maternal serum samples (range, 2–105 ng/ml; Fig. 1).
Because IgE does not cross the placenta, we considered the possibility that fetal blood was contaminated with maternal IgE by admixture of the fetal and maternal circulation at the time of birth. To assess whether this had indeed occurred, the ratios of parasite-specific to polyclonal IgE in paired cord and maternal sera were calculated and compared with each other (21). In adults, serum parasite-specific IgE constituted <5% of polyclonal IgE, whereas the proportion of helminth Ag-specific Ab was much higher in serum from cord blood. This difference reflects the limited Ag exposure of the neonate relative to that of the mother. If the ratios were similar for paired cord blood and maternal samples, this would suggest that significant admixture between the maternal and fetal circulation had occurred at the time of birth. Similar ratios of BmA-specific to polyclonal IgE were observed in 3 of 20 cord blood-maternal serum pairs in which IgE was detected in newborn serum. In 17 of the 20 pairs, however, the ratio of BmA-specific to total IgE in cord blood (range, 0.07–0.82) was at least threefold greater than its maternal counterpart (range, 0.008–0.09). Similar results were observed for SWAP-specific IgE in cord sera (data not shown).

Spontaneous in vitro IgE production by neonatal CBL and maternal PBMC

CBL from babies delivered in Cleveland did not spontaneously produce IgE (Fig. 2). IgE production by PBMC from healthy mothers of these newborns was not evaluated, but earlier studies had shown that spontaneous production of polyclonal IgE by North American adults without clinical allergy was <150 pg/ml (41). CBL from Cleveland produced 8.2 ± 2.7 μg/ml of polyclonal IgM in response to PWM (spontaneous, <0.2 μg/ml), indicating their capacity to produce Ig.

In contrast to American newborns, CBL from 12 of 39 Kenyan newborns spontaneously made polyclonal IgE in vitro. The levels were generally less than those of maternal PBMC (ranges, 0.15–18 and 0.14–721 ng/ml, respectively). It is unlikely that the CBL preparations were contaminated with maternal B cells, since 11 of 12 CBL that generated IgE in vitro had ratios of BmA- and/or SWAP-specific serum IgE to total IgE that were at least threefold greater than those of the corresponding maternal sample (see above). This was further substantiated by the lack of correlation between the level of spontaneous IgE production by CBL and matched maternal PBMC (data not shown). There was not a significant relationship between the level of serum polyclonal IgE and spontaneous in vitro IgE production by CBL or between serum polyclonal IgE and spontaneous in vitro IgE synthesis by maternal PBMC.

Spontaneous and mitogen-stimulated IgG production by neonatal CBL and maternal PBMC

CBL from North American newborns did not spontaneously produce detectable polyclonal IgG in vitro (<1 ng ml). IgG synthesis by these cells also was not induced by PWM (Fig. 3). In contrast, spontaneous polyclonal IgG production was demonstrable for 17 of 32 (53%) Kenyan CBL examined. The proportion of Kenyan CBL preparations that made polyclonal IgG increased to 26 of 35 (74%) when PWM was included in the cell cultures (p < 0.05 compared with medium alone; Fig. 3). The levels of spontaneous and PWM-driven polyclonal IgG were two- to fivefold greater for maternal PBMC than those for CBL. The level of spontaneous or PWM-induced IgG production by CBL did not correlate with that of the corresponding maternal PBMC culture (data not shown).

Helminth Ag-driven IgE and IgG production by CBL and maternal PBMC

Addition of SWAP or BmA to cultures of Kenyan CBL stimulated in vitro polyclonal IgE production ranging from 300 pg/ml to 18.4 ng/ml in 10 of 40 children. In eight cases, CBL incubated with medium alone did not produce IgE at a level detectable in our assay (<100 pg/ml; Fig. 4). CBL from North Americans failed to produce polyclonal IgE when incubated with SWAP or BmA.

Seventeen of 38 PBMC from Kenyan mothers produced IgE in vitro when incubated with SWAP or BmA. The levels of helminth Ag-stimulated IgE production were 2- to >50-fold greater than those with medium alone in 13 cases (Fig. 4). SWAP or BmA-specific IgE production in vitro was not detected for any of the CBL or maternal PBMC preparations.

There was a positive correlation between the amount of BmA- or SWAP-induced IgE production by CBL and the level of parasite-specific IgE in sera from cord blood (r2 = 0.67; p < 0.001; Fig. 5). This suggested that helminth Ag-specific T and B cells in CBL account in part for the elevated parasite-specific IgE observed in sera from newborns.
Unlike IgE, 7 of 39 Kenyan CBL synthesized parasite-specific IgG Abs following in vitro stimulation with SWAP or BmA (Fig. 6, upper left panel). When PWM was included in the cell cultures, the number of individuals having B cells that made parasite-specific IgG doubled (n = 14; Fig. 6, upper right panel). These data contrast with the results of experiments using CBL obtained from Cleveland newborns. None of five synthesized IgG when incubated with medium alone or with SWAP, BmA, or PWM (Fig. 6, middle panels).

PBMC from Kenyan mothers demonstrated augmented helminth Ag-specific IgG production when stimulated with SWAP or BmA (11 of 34 cases) or PWM (25 of 34 cases; Fig. 6, lower panels). The level of helminth Ag-driven IgG production by neonatal CBL did not significantly correlate with the corresponding maternal PBMC culture.

Relationship of maternal infection status to helminth Ag-driven IgE and IgG production by CBL

To examine the possible contribution of maternal helminth infection to neonatal Ag-specific B cell immunity, we determined the correlation between SWAP/BmA-driven production of polyclonal IgE and parasite-specific IgG Abs by CBL and maternal infection with schistosomiasis and/or filariasis. Of a total of 14 CBL that made helminth Ag-driven polyclonal IgE and/or parasite-specific

FIGURE 4. Helminth Ag (SWAP and/or BmA)-induced polyclonal IgE production by CBL and maternal PBMC. Lymphocytes were cultured under the conditions described in Figure 2. IgE in culture supernatants was measured by ELISA. Dots connected by lines represent paired culture Ags from a single individual with and without addition of helminth. Only Kenyan individuals are shown, in whom IgE production increased with addition of helminth Ag.

FIGURE 5. Relationship between helminth Ag-induced polyclonal IgE production in vitro by Kenyan CBL and level of parasite-specific IgE in serum from cord blood. Each point represents a single individual. BmA- or SWAP-induced IgE production is correlated with cord blood serum levels of IgE directed to the same Ags.

FIGURE 6. Helminth Ag stimulation of in vitro synthesis of parasite-specific IgG by cord blood and maternal lymphocytes. Lymphocytes were cultured under the conditions described in Figures 2 and 4. Points connected by a line represent parallel cultures incubated with and without helminth Ag or PWM from a single individual. Only individuals for whom an increase in parasite-specific IgG production was observed are shown.
though it has been observed that maternal lymphocytes can contaminate cord blood (49, 50), karyotypic analysis of cord blood cells suggests that this rarely occurs (51). Moreover, the present study failed to show a correlation between the capacity of maternal PBMC and neonatal CBL to produce IgE and IgG in vitro. If mixing of maternal and fetal lymphocytes did occur, a vigorous graft-vs-host reaction with nonspecific lymphocyte activation and proliferation should develop (52). This was not observed in the cell cultures. Finally, some infants have been followed up to 1 yr after birth, and we have found that babies primed to parasite Ag in utero retain immunologic memory without evidence of infection within the first year of life (C. L. King and I. Malhotra, personal observations).

In a recent study of different newborns from this area of rural Kenya, we reported that helminth- and mycobacterial Ag-specific CD4+ T cells develop in utero to produce a cytokine profile similar to that of adults (29). In that study, cord blood samples from offspring of mothers recently or actively infected with S. haematobium or W. bancrofti were more likely than offspring of uninfected mothers to have Ag-specific memory T cells. However, the correlation between maternal schistosomiasis or bancroftian filariasis and neonatal Th cell maturation was not absolute, suggesting that other chronic helminthiases may alter or prime the neonatal immune system. In the current study, we examined this possibility by assessment of mothers for infection with not only S. haematobium and W. bancrofti, but also geohelminths such as T. trichiura, A. caris, and hookworm. The prevalence of intestinal helminth infection exceeds 80 to 90% in coastal Kenya (30), and 64% of subjects in the current study were infected with one or more intestinal helminths based on a single stool examination (18 of 28 pregnant women from whom fecal specimens were available were infected). Since the propensity of neonatal CBL to make SWAP- or BmA-driven polyclonal IgE and/or parasite-specific IgG in vitro was associated with maternal intestinal helminth as well as S. haematobium or W. bancrofti infection, we speculate that cross-reactive helminth Ags and/or helminth glycoproteins can lead to in utero sensitization of fetal B cells and promote IgE production. Several common immunogenic helminth molecules that may produce these effects include nematode phosphocholine (53), "ladder" proteins reactive with IgE (54), and carbohydrates with structural similarities to the Lewis blood group (55). The lack of polyclonal IgE and/or helminth Ag-specific IgG production by CBL from newborns of all helminth-infected mothers may result from a lack of in utero sensitization or the failure to detect Ag-reactive lymphocytes because of low precursor frequencies.

It is not possible to determine the precise mechanism by which intestinal or tissue-invasive helminthic infections lead to prenatal B cell sensitization in humans. Unlike viral infections, blood-borne helminths such as W. bancrofti microfilariae rarely if ever cross the placenta (56), and worms limited to the intestinal tract obviously have no access to the fetal circulation. The intravascular location of schistosome and filarial parasites ensures that their metabolic products and secretions enter the circulation, which has been shown by detection of circulating Ag in the serum, milk, and urine of infected individuals (57–60). Therefore, it is possible that soluble parasite Ags pass from the maternal to the fetal circulation. Alternatively, transplacental transfer of maternal helminth-specific anti-idiotypic Abs (19) and/or maternally derived cytokines may influence neonatal sensitization to parasite Ags. Cord sera obtained from children born of infected mothers all had schistosome- and/or filaria-specific IgG. However, cord sera from a few neonates whose mothers did not have active schistosome and/or filarial infection also had detectable schistosome- and/or filaria-specific IgG Abs (three cord sera). In these cases the mothers may have been
sensitized to helminth parasites, but lost their active infections. This might account for the ability of CBL from four neonates to produce filarial and/or schistosome Ag-induced Ig production although their mothers were not actively infected (see Table 1). Indeed, two of these four children had SWAP-specific IgG in their cord plasma. Only 1 of 17 infants whose CBL failed to produce helminth Ag-induced Ig had helminth-specific IgG in their cord plasma. This finding is consistent with the hypothesis that anti-idiotypic Abs stimulate fetal lymphocytes; however, the numbers are too small to draw firm conclusions. In any event, it is likely that these or hitherto unknown maternal-fetal interactions promote IgE production by neonatal B cells, since there is apparently no intrinsic deficiency in the capacity of newborns’ T cells to express CD40 ligand (9, 10) or produce cytokines such as IL-4 (7, 29).

The biologic and health implications of the development of helminth Ag-specific T and B cell responses in utero are not yet known. Transplacentally acquired immunity to protozoan parasites such as malaria has been attributed to maternal IgG Abs, which confer partial resistance to asexual blood-stage infection for the first few months after birth. In contrast to IgG Abs, IgE Abs do not cross the placenta and have been implicated in protection against infection by several helminths, including schistosomiasis haematobium in humans (61, 62) and intestinal nematodes in experimental animals (63). Thus, in areas of the world where newborns and babies encounter a wide variety of helminths in the soil, mothers’ milk, and through invertebrate vectors, the presence of IgE Abs at the time of birth and the augmented production of this Ig isotype and parasite-specific IgG by primed B cells may limit the infection burden. In utero development of B cells with the capacity to make IgE may also predispose an infant to the development of allergic responses to environmental or vaccine Ags. The latter may be especially relevant to the efficacy of vaccines in which bias of the immune response toward the type 2 functional phenotype is deleterious (64).

Finally, the results of this and other studies suggest that neonatal tolerance may be limited to self Ags and selected viral infections, such as hepatitis B (65–67). In the case of helminthic infections and malaria, soluble Ags acquired from the mother during gestation may be processed by fetal APCs, which prime the immune system to recognize organisms encountered shortly after birth. This priming not only may be important for generating robust immunologic responses to natural infection, but may also be used to enhance the immunogenicity and efficacy of vaccines administered to newborns and infants. More generally, these and other data (15) suggest that vaccination during pregnancy may benefit the unborn child through Ag-specific priming of the developing fetus. Continuing studies are therefore directed at understanding how parasite Ag-primed neonatal T and B cells modify immunity to naturally occurring infection in infants and whether maternal helminth infection during pregnancy influences the immune response to vaccines delivered during the first year after birth.

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