

Fetal sensitization to cow's milk protein and wheat: cow's milk protein and wheat-specific TNF- α production by umbilical cord blood cells and subsequent decline of TNF- α production by peripheral blood mononuclear cells following dietary intervention

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We present a case of fetal sensitization to cow's milk protein (CMP) and wheat, resulting in non-IgE mediated food allergy (NFA). Fetal sensitization was indicated by onset of NFA symptoms shortly after birth and CMP/wheat-specific tumor necrosis factor- α (TNF- α) production by cord blood mononuclear cells. Following dietary intervention, we observed a decline of TNF- α production by peripheral blood mononuclear cells with stimuli of these dietary proteins (DPs) but recurrence of reactivity was observed following viral gastroenteritis, while interleukin-10 production with these DPs persisted during his first 5 yr of life. This finding may indicate active suppressive mechanisms for maintaining oral tolerance.

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Adverse reaction to food can occur by immune mediated or non-immune mediated mechanisms [food allergy (FA) and food intolerance, respectively] (1). FA refers to sensitization to food components [mainly dietary proteins (DPs)] via IgE mediated or non-IgE mediated mechanisms. Non-IgE mediated FA (NFA) is more frequently observed in infants and young children (1).

In infants/children with NFA, immune reactivity is thought to be mediated by cellular immunity as demonstrated by the production of type 1 T-helper (Th1) cytokines [γ -interferon (IFN- γ) and tumor necrosis factor- α (TNF- α), especially TNF- α] with stimuli of causative DPs

(1–5). Clinical features of NFA are generally limited to gastrointestinal (GI) symptoms, typically occurring 6–24 h after intake of offending DPs (1, 2). Cow's milk protein (CMP) and soy protein, the two major proteins in infant formulas, are the most common DPs causing NFA (1, 2). NFA symptoms typically develop several weeks after birth, indicating postnatal sensitization to DPs.

However, some NFA infants reveal GI symptoms shortly after birth (within a week), too soon to be explained by postnatal sensitization to DPs. Neonates are extremely unlikely to be exposed to foods causing food intolerance but their clinical

features are consistent with NFA. Thus these NFA babies could be sensitized in the prenatal period. Fetal sensitization to DPs has been indicated previously by presence of cell proliferative responses to food antigens (Ags; mainly DPs) by cord blood mononuclear cells (CBMCs) (3, 6). In fetuses and neonates, Th-cell responses are skewed toward Th2, followed by a shift toward Th1 upon exposure to microbes after birth (7). However, it is poorly understood whether fetal sensitization can cause cell-mediated immune responses such as NFA and how such aberrant immune responses become controlled after birth. Herein we report a case of NFA whose fetal sensitization to CMP/wheat was indicated by elevated DP-specific TNF- α production by CBMCs. His cellular reactivity to DPs was closely monitored after his birth and our observation may indicate induction of active suppressive mechanisms for inducing tolerance to DPs.

Case report

The presented case is a Caucasian boy born at full term via spontaneous vaginal delivery without a complication. Birth weight was 6.1 lb. Pregnancy was uneventful. Secondary to severe NFA symptoms observed in his older brother within 2–3 days after birth, his mother started avoiding dairy and wheat products a few weeks prior to his delivery. He tolerated breast feeding and discharged to home within 48 h after his birth. Newborn screening for metabolic disorders was negative. He had been exclusively breast fed since birth with his mother being on a dairy-free, wheat-free diet. However, at 7 days of age, he revealed similar symptoms as observed in his brother 6–24 h after his mother took wheat products. He was irritable and crying with arching indicating severe abdominal pain. Afterwards, he continued to reveal various GI symptoms including severe colic, diarrhea, and vomiting with distended abdomen, resulting in poor weight gain.

At 2 wk of age, breast feeding was switched to formula feeding using a free amino acid formula (Neocate[®]; Nutricia North America, Gaithersburg, MD, USA). This was due to severe GI symptoms and positive cellular reactivity to CMP and wheat by his CBMCs (see the laboratory finding section). Cellular reactivity to DPs by his CBMCs was initially assessed due to family history (FH) of severe NFA in his older brother who also revealed onset of NFA symptoms shortly after birth.

Introduction of Neocate[®] quickly (days) led to weight gain and resolution of GI symptoms.

Following several weeks of formula feeding with Neocate[®], breast feeding was resumed with his mother being on a strict wheat-free, dairy-free diet. Breast feeding was continued until 1 yr of age with introduction of solid food beginning around 6 months of age. He maintained a wheat and dairy-free diet until 2 yr of age. During this time there was no recurrence of GI symptoms. After confirming no significant cellular reactivity to wheat *in vitro*, wheat products were slowly introduced to his diet without complication. Accidental exposures to dairy products occurred on several occasions and always resulted in GI symptoms including diarrhea and abdominal cramping. Thus the intake of dairy products continues to be kept to a minimum. The patient continues to thrive with normal physical and cognitive development, tolerating all the DPs except CMP.

Family history

Family history is significant with his older brother having developed severe NFA shortly after birth and diagnosis was made at 4 months of age. His brother was born at full term with uneventful pregnancy/delivery and had been exclusively breast-fed for the first 4 months. His GI symptoms similar to those observed in the presented case became substantially worse when he was switched to hypoallergic formula (Pregestimil[®]; Mead Johnson, Evansville, IN, USA) from breast feeding. His GI symptoms persisted until implementation of a wheat-free, dairy-free diet at 11 month old. His peripheral blood mononuclear cells (PBMCs) revealed elevated TNF- α production with stimuli of CMP and wheat (gliadin) prior to dietary intervention. FH is otherwise negative for atopic disorders, autoimmunity, or IgE mediated FA.

Laboratory findings

Cord blood mononuclear cells from the case study subject revealed elevated production of TNF- α with stimuli of CMP and its major components [α -lactalbumin (α -LA) and β -lactoglobulin (β -LG)] (Fig. 1). His CBMCs also produced >300 pg/ml of TNF- α with gliadin *in vitro* (Fig. 1a). Interleukin-5 (IL-5), a Th2 cytokine was undetectable. Following his birth, his cellular reactivity to these DPs has been monitored by measuring production of Th1 (IFN- γ and TNF- α), Th2 (IL-5), and regulatory (IL-10, IL-12p40) cytokines by PBMCs in response to CMP/ α -LA/ β -LG/gliadin. Reference values of

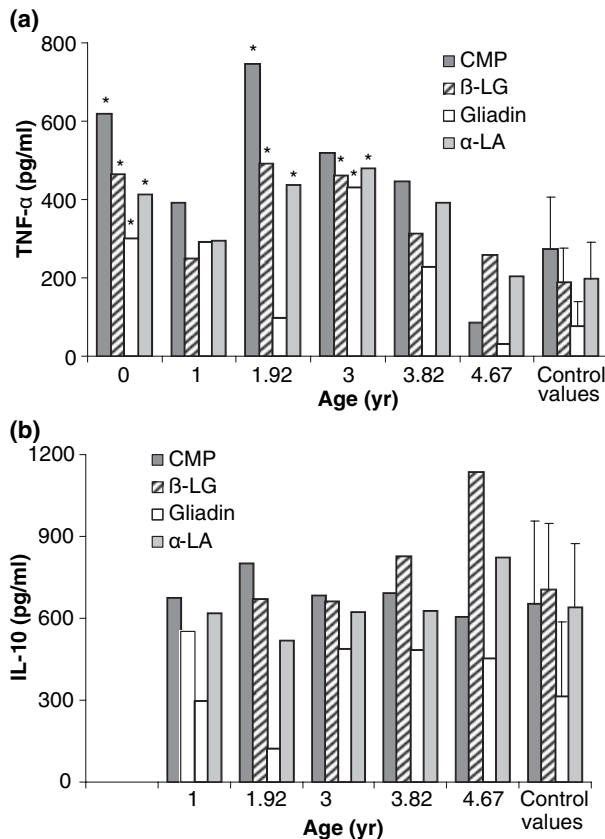


Fig. 1. Levels of tumor necrosis factor- α (TNF- α) (panel A) and interleukin-10 (IL-10) (panel B) produced by cord blood mononuclear cells (CBMCs) and peripheral blood mononuclear cells (PBMCs) between 0–5 yr old in response to cow's milk protein/ β -lactoglobulin/ α -lactalbumin/gliadin (CMP/ β -LG/ α -LA/gliadin). Endotoxin level in CMP/ β -LG/ α -LA was <1 ng/ml as reported before (17). Cytokine production was assessed by incubating cells (10^6 cells/ml) for 4 days with these stimuli as described before (17). Reference values were obtained from 20 typically developing children without non-IgE mediated food allergy (NFA) (Age: 1–6 yr, median 3.9 yr) and shown as a mean value with standard deviation (s.d.). *, higher than a control mean value + 2s.d.

IL-10 and TNF- α (pg/ml) from 20 control children (median 3.9 yr) were shown in Fig. 1.

Tumor necrosis factor- α production by PMBCs with CMP/ α -LA/ β -LG declined gradually with age (Fig. 1a), but increased following accidental exposure to dairy products at 1.92 yr old and when he suffered from viral gastroenteritis at 3 yr old. TNF- α production with gliadin revealed a rapid decline (Fig. 1). However, TNF- α production with gliadin increased temporarily after wheat was reintroduced to his diet as well as when he suffered from acute viral gastroenteritis at 3 yr of age (Fig. 1a). In contrast, IL-10 production by his PBMCs tended to persist with stimuli of these DPs but these values are equivalent to controls (Fig. 1b). Moreover, IL-10 production with gliadin increased after re-intro-

duction of wheat to his diet (Fig. 1b). His TNF- α and IL-10 production against soy protein or casein to which he did not reveal clinical symptoms were equivalent to control values at all the time points tested (data not shown).

We did not observe higher than within normal range of IFN- γ production with the DPs tested. Production of IFN- γ , IL-10, TNF- α , IL-5, and IL-12p40 with T-cell mitogens and other recall Ags was within normal limits (data not shown). His immunoglobulin levels and antibody (Ab) titers against diphtheria/tetanus toxoid were also within normal limits at 3 yr of age.

Discussion

The case presented here appears to reveal clinical features of fetal sensitization as indicated by the onset of characteristic GI symptoms within 24 h when the mother ingested offending food despite exclusive breast feeding and rapid resolution of symptoms with dietary intervention. Fetal sensitization to CMP and wheat was also indicated by TNF- α production by CBMCs with CMP and gliadin. The presented case illustrates an evidence of fetal sensitization to DPs and induction of NFA symptoms with exposure to small amounts of offending food derived through breast milk. In addition, our data also indicate that changes in his immune reactivity to these DPs after birth may be partly associated with active suppressive mechanisms via IL-10.

Immature fetal T cells are less likely to be sensitized to maternally-derived foreign Ags, but transfer of antigenic foreign proteins into cord blood via placenta has been documented (8). Fetal sensitization with food Ags and aeroallergens has also been indicated with Ag-triggered proliferative responses and cytokine production by CBMCs (6). During pregnancy, the mother's immune system is in suppressive state and skewed to Th2, which is thought to prevent rejection of fetus (7, 9). This Th2 skewed status persists in the newborn period (7, 9). Thus fetal sensitization to environmental Ags was implicated with a risk factor for development of Th2-mediated (IgE-mediated) atopic diseases (10).

In contrast, NFA is thought to be mediated by cell-mediated immune responses with TNF- α being the major mediator in case of NFA to CMP (2–5). In children with NFA to CMP, TNF- α production by PBMCs is reported to positively correlate with clinical features of NFA (3, 4, 11). Therefore, TNF- α production with CMP in these children declines following dietary intervention (a dairy-free diet) in parallel to the resolution of GI symptoms. However, it is

unclear whether prenatal sensitization of DPs could cause similar pattern of cytokine production by CBMCs. The presented case indicates that fetal sensitization to DPs may induce similar cellular reactivity indicated by elevated production of TNF- α regardless of the Th2 favored fetal environment. Genetic predisposition may contribute to this type of sensitization as demonstrated by positive FH of NFA in this case. It has yet to be determined why these children appeared to have developed such marked cellular reactivity despite the fact that mother even avoided a dairy and wheat products before the birth of second child.

It is possible for infants to be sensitized to trace amounts of common DPs (cow's milk, soy, and egg protein) present in human breast milk (12, 13). There is also a possibility that DP-specific cytokine production may not reflect fetal development of T-cell memory. That is, Thornton et al. (14) reported that a significant component of the reactivity of human neonatal T cells against environmental Ags likely represent a default response by recent thymic emigrants, providing an initial burst of short-lived cellular immunity in the absence of conventional T-cell memory. It is hard to determine what extent of cytokine production we observed against CMP/gliadin (higher than control mean value + 2s.d.) can be attributed to such default mechanisms; in their study all the CBMCs were obtained from normal full-term babies and presence or absence of NFA symptoms in the newborn period were not documented (14). However, in his CBMCs, we did not observe such elevated production of TNF- α with stimuli of other DPs including soy protein and casein. It is also of note that the child was exclusively breast fed while the mother was avoiding wheat and dairy products but the symptoms started shortly after (<24 h) his mother took wheat products. Therefore, it is most likely that DP-specific T cells sensitized before birth were effectively activated by trace amounts of DPs present in the mother's breast milk shortly after mother's intake of offending food, causing severe GI symptoms. Memory T cells are more easily activated with less dependence on co-stimulatory signaling than naïve T cells (15). This case indicates that even with fetal sensitization, memory T cells may be more sensitive to Ag stimuli. However, his persistent reactivity to CMP/ β -LG/ α -LA may also be associated with several accidental exposures to dairy products after his birth.

In patients with NFA against MP, it has been shown that following dietary intervention (a

dairy-free diet), TNF- α production with CMP declines in parallel to resolution of GI symptoms. Ag-specific, CD4⁺, CD25⁺ regulatory T (Treg) cells appear to play a major role in establishment of immune tolerance to CMP, imposing active suppression (11). The suppressive action of Treg cells is partly mediated by the production of regulatory cytokines (IL-10 and TGF- β). Others report persistent IL-10 production and temporary TGF- β production following Ag challenge in CMP tolerant children (11).

In the presented case, we also found gradual decline of TNF- α production but persistent IL-10 production by his PBMCs with stimuli of MP/ α -LA/ β -LG. IL-10 production with gliadin was low while he was on a wheat-free diet, but increased following induction of wheat at 3 yr of age. Interestingly, no clinical symptoms were observed. These findings suggest a role of IL-10 in the establishment of oral tolerance in this case. Pathogen-induced immune activation can overcome suppressive signaling imposed by Treg cells (16). In the presented case, we observed a temporarily increase of TNF- α production with gliadin/CMP when he suffered from viral gastroenteritis at 3 yr of age. Such clinical observation may indirectly support the role of Treg cells in maintenance of tolerance in this case. However, in the presented case, cellular source of IL-10 was not studied and thus it is difficult to assess whether Treg cells are involved in gut mucosal tolerance induction in the presented case.

In summary, we present a case of possible fetal sensitization to DPs whose cellular reactivity to DPs has been closely monitored by measuring production of Th1 and regulatory cytokines since birth. The presented case indicates persistent presence of DP-specific memory T cells and induction of immune tolerance by active suppression.

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