

Balance between early life tolerance and sensitization in allergy: dependence on the timing and intensity of prenatal and postnatal allergen exposure of the mother

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Summary

Allergens can be maternally transferred to the fetus or neonate, though it is uncertain how this initial allergen exposure may impact the development of allergy responses. To evaluate the roles of timing and level of maternal allergen exposure in the early life sensitization of progeny, female BALB/c mice were given ovalbumin (OVA) orally during pregnancy, lactation or weekly at each stage to investigate the immunoglobulin E (IgE) antibody production and cellular responsiveness of their offspring. Exposure to OVA during pregnancy was also evaluated in OVA-specific T-cell receptor (TCR) transgenic (DO11.10) mice. The effect of prenatal antigen exposure on offspring sensitization was dependent on antigen intake, with low-dose OVA inducing tolerance followed by neonatal immunization that was sustained even when pups were immunized when 3 weeks old. These offspring received high levels of transforming growth factor- β via breastfeeding. High-dose exposure during the first week of pregnancy or perinatal period induced transient inhibition of IgE production following neonatal immunization; although for later immunization IgE production was enhanced in these offspring. Postnatal maternal antigen exposure provided OVA transference via breastfeeding, which consequently induced increased offspring susceptibility to IgE antibody production according to week post-birth. The effect of low-dose maternal exposure during pregnancy was further evaluated using OVA transgenic TCR dams as a model. These progeny presented pronounced entry of CD4⁺ T cells into the S phase of the cell cycle with a skewed T helper type 2 response early in life, revealing the occurrence of allergen priming *in utero*. The balance between tolerance and sensitization depended on the amount and timing of maternal allergen intake during pregnancy.

Keywords: breastfeeding; immunoglobulin E antibodies; *in utero* priming; maternal allergen exposure; mice; neonatal; sensitization; tolerance

Introduction

Early childhood is a phase of life with a high risk of allergic sensitization – it is the time when initial allergen sensitization frequently occurs.^{1,2} The immature status of the immune system in early life,^{3,4} and the predisposition towards T helper type 2 (Th2) skewing of immune function during fetal and neonatal periods^{5,6} may collectively contribute to the initial development of an allergic response subsequent to allergen exposure. Maternal

allergen exposure seems to be a risk factor for early atopic disorders, influenced by genetic and environmental factors.^{7,8} Therefore, it is important to understand the relationship between the amount and timing of initial allergen exposure as a factor in the early development of sensitization.

Maternally derived dietary allergen exposure has been associated with the presence of the allergen in fetal circulation, implying that the fetus may be exposed to dietary allergen during pregnancy transplacentally or

transamniotically.⁹ Transplacental transfer of nutritive and inhalant allergens has been demonstrated in an *ex vivo* model of placenta perfusion,^{10,11} in which most of the allergen did not cross the feto–maternal interface but was retained in the placental tissue.¹² Allergen-specific reactivity of cord blood against both inhalant and food allergens has been described;^{13,14} however, these ubiquitous lymphoproliferative responses do not appear to predict the development of the allergy.^{15–17} Therefore, the relationship between aeroallergen exposure in early life and immunoglobulin E (IgE) sensitization may be non-linear.¹⁸ The key remaining questions are whether there is passage of some allergens to the fetus and how this might impact developing immune responses, including the induction of tolerance versus sustained immunity.

In contrast to the uncertain relationship between *in utero* allergen priming and the outcome of IgE sensitization, early postnatal exposure to high levels of allergen maximizes the risk for subsequent expression of allergic reactivity to that allergen in adult life.^{19,20} Postnatal exposure to a maternally derived dietary allergen, by breastfeeding, has long been known to provoke food-associated allergic symptoms.²¹ Therefore, dietary exclusion or allergen avoidance measures during lactation have been the focus of studies of primary allergy prevention.²²

Our aim in this report is to examine the amount and timing of maternal exposure to ovalbumin (OVA) during fetal development or in the postnatal phase to investigate the balance between tolerance and sensitization in mice. The neonatal period is a time when mammals are susceptible to tolerance induction,^{3,23} so we have also analysed the development of IgE antibody responses in offspring during the weaning period. According to previous observations regarding the influence of maternal immune status on the neonatal immune response,^{24–26} we further evaluated the effect of maternal allergen exposure during pregnancy on the offspring's CD4⁺ T-cell response in an OVA-specific transgenic T-cell receptor (TCR) system that features the presence of OVA-specific CD4⁺ T cells.

Materials and methods

Animals

BALB/c and DO11.10 mice of both sexes (8–10 weeks old) were obtained from the animal facilities of the São Paulo University Medicine School and the Institute of Biomedical Sciences. Wistar Furth rats of both sexes, 3–4 months old and bred in our own laboratory's animal facilities, were used for passive cutaneous anaphylaxis (PCA) reaction studies. All experiments were approved by the Ethics Committee for Animal Research of the Institute of Biomedical Sciences.

Experimental protocols

Prenatal OVA exposure regimen (Protocol I) Female BALB/c mice were mated with males, then 1.5 or 9.0 mg OVA in 0.5 ml saline solution (grade V; Sigma-Aldrich, St Louis, MO) was administered orally using an oral feeding needle, divided into five doses at intervals of 4 days, during pregnancy. Also, groups of female DO11.10 transgenic (Tg) mice were mated with males and submitted to oral administration of 1.5 mg OVA, divided into five doses, during pregnancy.

Postnatal OVA exposure regimen (Protocol II) Female BALB/c mice were mated, then given 1.5, 3.0 or 9.0 mg in OVA orally, divided into three doses, in their first week of breastfeeding corresponding to the second, third and fourth days of lactation. Pups were maintained with their mothers until weaning at the age of 3 weeks.

Timing of maternal OVA exposure in pre- or postnatal periods (Protocol III) To verify the effect of maternal OVA oral administration in each week of gestation or of the breastfeeding period, female BALB/c mice were mated with males, then given 9.0 mg OVA orally, divided into three sequential doses, in the first, second or third gestational week or in the first, second or third week of breastfeeding.

Offspring immunization Three-day-old or 25-day-old mice of both sexes were intraperitoneally (i.p.) immunized with 10 or 200 µg OVA, in 1.25 or 0.6 mg Al(OH)₃, respectively, as described previously.²⁶ Ten days later, offspring received an i.p. injection of 100 µg OVA in saline solution, and were bled after a further 7 days. Others groups were i.p. immunized at 3 days old with 50 µg hen egg lysozyme (HEL; Sigma-Aldrich, St Louis, MO) in 0.6 mg Al(OH)₃, and boosted with a further 50 µg HEL 10 days later.

Collection of amniotic fluid, fetal and maternal sera, and milk

The pregnant mice were subjected to Caesarean section at full term (21 days). During the Caesarean procedure samples of amniotic fluid, fetal sera and maternal sera were obtained and stored at –70° until use. Milk samples were harvested as previously described.²⁴

Passive cutaneous anaphylaxis

Immunoglobulin E antibodies were estimated by PCA in rats according to Mota and Wong.²⁷ Serum dilutions were inoculated intradermally (100 µl) on the shaved backs of the rats. After 18 hr, the rats received 0.5 mg

OVA in 1.0 ml (0.5%) Evans Blue solution through tail vein injection. The PCA titres were expressed as the reciprocal of the highest dilution that gave a spot bigger than 5 mm in diameter.

Assay for OVA measurement by enzyme-linked immunosorbent assay

For OVA measurement in maternal fluids and milk, 96-well microplates (A2, High binding; Costar, Cambridge, MA) were coated with rabbit anti-chicken egg albumin IgG antibody (Calbiochem, La Jolla, CA) and blocked; then samples and standards were applied. After binding, the plates were incubated with goat anti-chicken egg albumin IgG antibody (ICN/Cappel, Aurora, OH), and detected with peroxidase-labelled porcine anti-goat IgG (Biosource, Camarillo, CA) and 3,3',5,5'-tetramethyl-benzidine (Zymed, Carlsbad, CA). The reaction was stopped with 1 M sulphuric acid and read at 450 nm in an enzyme-linked immunosorbent assay (ELISA) microplate reader (Molecular Devices, Sunnyvale, CA). Optical density (OD) units were correlated to the protein concentration using an OVA standard curve. The sensitivity of the assay was 35 pg/ml.

Determination of antibody levels

The OVA-specific IgG1 and IgG2a antibody levels or total IgE, IgA and IgG1 and IgG2a antibody levels were measured by ELISA. The results of antigen-specific antibody levels were expressed as titres with reference to serial dilutions of a titrated serum pool from immunized adult mice with high levels of anti-OVA antibody. Total immunoglobulin concentration was correlated with a standard curve from purified immunoglobulin (Southern Biotech. Ass., Birmingham, AL).

Proliferation assay with tritiated thymidine

Spleen aseptically collected from 20-day-old mice was pressed through a cell strainer (BD Biosciences, Bedford, MA) in RPMI-1640 supplemented with 10% fetal calf serum (Hyclone III, Lotan, CT). The red blood cells were lysed using ACK Lysing Buffer (Biosource, Rockville, MD) for 90 seconds. Cells (2.0×10^5 cells/0.2 ml) were cultured with OVA (200 µg/ml; Sigma) or with anti-CD3 (2 µg/ml; PharMingen, San Diego, CA) at 37° in a humidified 5% CO₂ incubator. Thymidine incorporation was measured on day 4 of culture after 18 hr being pulsed with 1 µCi [³H]thymidine (Amersham Biosciences AB, Uppsala, Sweden).

Cytokine ELISA

The ELISA for transforming growth factor- β_1 (TGF- β_1 ; Promega, Madison, WI) in milk samples was performed

following the manufacturer's recommendations. Interleukin-4 (IL-4), IL-10 and interferon- γ (IFN- γ ; OPTeTM PharMingen) were measured in supernatants of spleen cell cultures with anti-CD3 monoclonal antibody (2 µg/ml; PharMingen) for 24 hr or OVA (200 µg/ml; Sigma) and HEL (200 µg/ml; Sigma) for 72 hr, as previously described²⁸ whereupon cell-free supernatants were stored at -70° until the cytokine assay by means of ELISA. Detection limits of IL-4, IL-10, IFN- γ and TGF- β_1 were 3.9, 31.2, 15.6 and 32 pg/ml, respectively.

Cell cycle analysis by bromodeoxyuridine incorporation

Cell cycle analysis of CD4⁺ spleen cells was performed using bromodeoxyuridine (BrdU) incorporation according to the manufacturer's instructions (BrdU Flow Kit; BD Biosciences). Spleen cells at 2×10^6 /ml were cultured with OVA (400 µg/ml) for 48, 72 or 96 hr. The BrdU (10 µM/ml) was added 12 hr before the end of each culture period. The cells were stained with phycoerythrin-labelled anti-CD4 monoclonal antibody (PharMingen), fixed and permeabilized with buffer (Cytotfix/Cytoperm buffer), then treated with DNase. Afterwards, cells were incubated with anti-BrdU antibody and resuspended in a solution of 7-amino-actinomycin D. Cells were analysed on FACSCalibur (BD, San Jose, CA) and the data were analysed using CELL QUEST PRO.

Statistical analysis

Differences between groups were considered significant when *P*-values were < 0.05, using the Mann-Whitney test.

Results

Maternal allergen transference following pre- or postnatal allergen administration

The study aimed to verify whether maternal OVA intake during pregnancy or lactation permitted OVA transfer by transamniotic, transplacental or breastfeeding routes. Ovalbumin was undetectable in the amniotic fluid, placental serum and milk from mothers that received oral administration of low (1.5 mg) or high (9.0 mg) doses of OVA during pregnancy (Protocol I). In contrast, when evaluating milk samples from mothers that received antigen during the breastfeeding period (Protocol II), OVA was detectable in milk samples in proportion to the mother's intake dose (Fig. 1).

Effect of maternal pre- or postnatal OVA oral administration on the offspring IgE response

To investigate the effect of maternal allergen exposure during pre- or postnatal periods in the development of

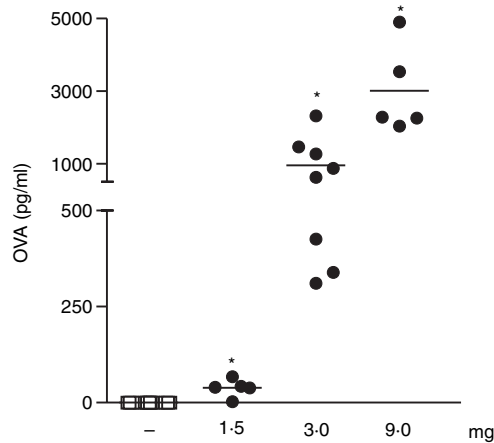


Figure 1. Determination of ovalbumin (OVA) levels in milk after postnatal maternal antigen exposure. Female BALB/c mice were given OVA orally during the first week post-birth (1.5, 3.0 or 9.0 mg), divided into three doses (Protocol II). Breast milk samples were obtained from the stomach of 5-day-old newborn mice. OVA in milk samples was assessed by enzyme-linked immunosorbent assay. Horizontal line represents means. * $P \leq 0.05$ when compared with offspring from non-exposed mothers.

type I hypersensitivity responses, offspring from mothers orally administered with OVA during pregnancy (Protocol I) or lactation (Protocol II) were immunized with OVA at 3 days or at 3 weeks of age. Figure 2(a) shows that neonatal immunization with OVA revealed inhibition of anti-OVA IgE antibody production in offspring from mothers that had received high-dose OVA during either the pre- or postnatal period. The inhibition of the IgE antibody response in progeny of mothers that received high-dose OVA was transient because immunization of these mice at 25 days (Fig. 2b) resulted in an enhanced IgE response compared with offspring from control mothers. In contrast, investigation of offspring from mothers exposed to low-dose antigen (1.5 mg) during pregnancy showed decreased IgE antibody production. These findings indicate that when mothers are subjected to large amounts of allergen, in either the pre- or postnatal period, transient inhibition of the IgE response is promoted in the progeny following neonatal immunization, but at infant age, the pups become susceptible to allergen sensitization. Antigen exposure during the breastfeeding period was related to offspring allergen sensitization, irrespective of the maternal antigen dose. Curiously, only low-dose maternal exposure during pregnancy maintained the tolerogenic effect in the offspring.

Timing of antigen exposure in the pre- or postnatal period and neonatal IgE development

To verify the effect of maternal OVA (9.0 mg) intake during each individual week of pregnancy or of the

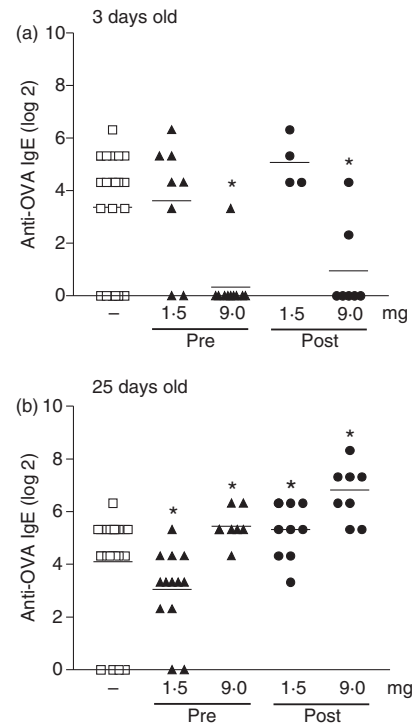


Figure 2. Effect of maternal allergen exposure on the development of the immunoglobulin E (IgE) response of offspring. Three-day-old (a) or 25-day-old BALB/c (b) offspring from mothers exposed to ovalbumin (OVA; 1.5 or 9.0 mg) during the prenatal or postnatal periods, described as Protocol I and II, respectively, in the *Materials and methods* section, were immunized with OVA. Anti-OVA IgE antibody was evaluated by means of a passive cutaneous anaphylaxis reaction. Horizontal line represents means. * $P \leq 0.05$ when compared with offspring from non-exposed mothers.

postnatal period, mothers received OVA in the first, second or third weeks of pregnancy or breastfeeding (Protocol III) and IgE antibody production after neonatal immunization was evaluated. Maternal OVA exposure in the first week of gestation and the first week post-birth significantly inhibited the anti-OVA IgE production of immunized neonates, compared with pups from non-exposed mothers (Fig. 3). Nevertheless, postnatal maternal exposure during the second or third weeks enhanced the IgE antibody response. These results indicate that maternal high-dose allergen exposure during the early phase of fetal development or in the perinatal stage may induce transient IgE inhibition, contrasting with the susceptibility to allergen sensitization that occurred with exposure during the post-birth stage.

Low-dose maternal OVA oral administration during pregnancy induces offspring tolerance

To further verify the effect of low-dose maternal OVA exposure during pregnancy on neonatal immunization,

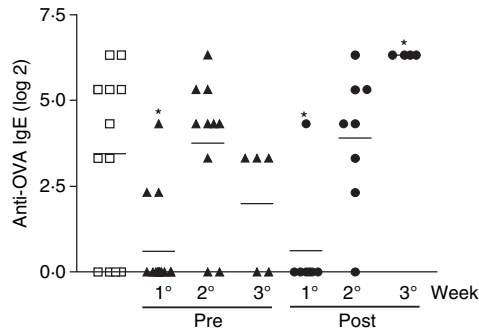


Figure 3. Effect of timing of ovalbumin (OVA) exposure in the prenatal or postnatal period on the neonatal immunoglobulin E (IgE) development. Female BALB/c mice were mated and orally administered 9.0 mg of OVA prenatally, divided into three doses in the first or second, or third week of the pregnancy or in the postnatal period, in the first or second, or third week of suckling (Protocol III). Three-day-old offspring were immunized with OVA, and IgE antibody production was evaluated by passive cutaneous anaphylaxis reaction. Horizontal line represents means. * $P \leq 0.05$ when compared with offspring from non-exposed mothers.

the cellular immune response of offspring immunized with OVA at 3 days was analysed. Prenatal exposure to a low antigen dose induced a decreased antigen-specific proliferative response, concomitant with reduced IL-4, IFN- γ and IL-10, compared with mice from non-exposed mothers (Fig. 4). The decrease in IFN- γ levels was reflected in the diminished anti-OVA IgG2a antibody production, but did not interfere with total IgG2a antibody levels. In addition, offspring from mothers exposed to a low antigen dose during pregnancy showed inhibition in total IgE levels (Fig. 4), whereas anti-OVA IgE antibody production remained unaltered (Fig. 2a). These findings demonstrate that a tolerogenic effect was established in offspring from mothers treated with low-dose antigen during pregnancy, as verified by the inhibition of total IgE levels, anti-OVA IgG1 and IgG2a antibody production, as well as by diminished antigen-proliferative response and cytokine production.

We next evaluated the effect of offspring immunization with an antigen heterologous to that used in the maternal exposure experiments. Offspring were immunized with HEL (hen egg lysozyme; Sigma) when 3 days old and their responses were compared with those of OVA-immunized offspring. Figure 4(h) shows that the proliferative response to anti-CD3 stimulation was significantly decreased in both offspring groups, i.e. HEL- and OVA-immunized offspring from exposed mothers compared with offspring from non-exposed mothers. In addition, the secretion of IFN- γ , IL-4 and IL-10 on anti-CD3 stimulus were significantly decreased (Fig. 4i–l, respectively).

Maternal TGF- β transference to offspring by breastfeeding

One of the regulatory factors involved in oral tolerance induction with low doses of allergen are cytokines, such as TGF- β .²⁹ Since we verified that milk from preconception allergen-immunized mothers contains high levels of TGF- β ,²⁴ the presence of this regulatory cytokine in milk from mothers exposed to a low-dose of OVA during pregnancy (Protocol I) or a high-dose during breastfeeding (Protocol II) was determined. Figure 5 indicates increased TGF- β levels in milk from mothers exposed to a low dose of OVA compared with non-exposed mothers. In parallel, an increase in total IgA antibody levels was detected. Low-dose exposure during pregnancy induced TGF- β transference during lactation that could be involved in the tolerance status of their progeny.

Prenatal CD4⁺ T-cell allergen sensitization

The effect of low-dose maternal exposure during pregnancy (1.5 mg, Protocol I) was further evaluated using OVA-specific TCR transgenic mothers (DO11.10) as a model. The kinetics of CD4⁺ T-cell cycle entry in non-immunized offspring (20 days old) was assessed by BrdU incorporation on OVA stimulation.

Figure 6 shows that up to 57% of the CD4⁺ T cells from the offspring of antigen-exposed mothers enter the S phase following 72 hr of antigen stimulation and about half that percentage enter the G0/G1 phase. Conversely, the opposite ratio was observed in Tg offspring from non-exposed mothers, in which the majority of cells were arrested at G0/G1. Following 96 hr of stimulation, a more pronounced number of CD4⁺ T cells reached S phase (up to 84%) in offspring from antigen-exposed mothers, whereas 40% of the cells remained in G0/G1 in the control group. In the absence of stimulation, both offspring groups displayed substantial proportions of CD4 cells in G0/G1 and minimal progression to S or G2/M.

Pups from antigen-exposed mothers showed a significant increase in IL-4 secretion levels that were detectable within 48 hr after OVA stimulation (Fig. 7). Although a considerable amount of IFN- γ was produced, similar production was seen in the control group. Furthermore, these offspring developed an increased frequency of splenic B220⁺ IgM⁺ cells, together with a decrease in absolute numbers of B220⁺ CD23⁺ cells. High levels of anti-OVA IgG antibody were detected in the serum of offspring from antigen-exposed mothers, compared with offspring from the control group (Fig. 7). Taken together, these findings demonstrate that feeding of a low antigen dose to the mother during pregnancy may induce *in utero* priming in Tg mice, evidence that antigen transfer to the fetus may occur.

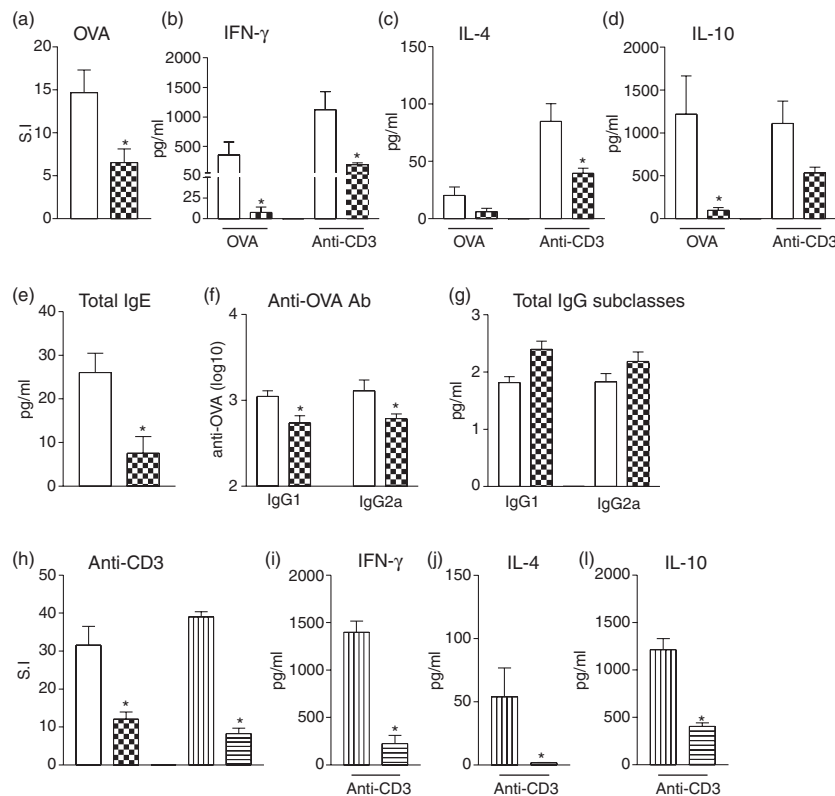


Figure 4. Maternal low-dose exposure during pregnancy induces inhibition of offspring cellular responsiveness. Three-day-old BALB/c mice from mothers exposed to ovalbumin (OVA; dotted bar) during prenatal period (1.5 mg, Protocol I) or from non-exposed mothers (open bar) were immunized with OVA or hen egg lysozyme (HEL). Proliferative spleen cells from OVA-immunized offspring (20 days old) in response to OVA are represented as the stimulation index (SI) (a); interferon- γ (IFN- γ) (b), interleukin-4 (IL-4) (c), and IL-10 (d) measurements in the supernatants of splenic cell culture after 24 hr of stimulation with anti-CD3 monoclonal antibody or 72 hr with OVA were detected by enzyme-linked immunosorbent assay (ELISA). Total IgE antibody (e) and anti-OVA IgG1 (f) and IgG2a antibody (g) with their respective total concentration levels were evaluated by means of ELISA. Proliferative spleen cell response of offspring immunized with HEL from non-exposed (vertical hatched bar) or exposed (horizontal hatched bar) mothers to anti-CD3 stimuli is represented as SI (h); IFN- γ (i), IL-4 (j), and IL-10 (l) measurements in the supernatants of splenic cell culture after 24 hr of stimulation with anti-CD3 monoclonal antibody by ELISA. Representative results from five to eight mice/per group are expressed as mean $\pm$ SEM. * $P \leq 0.05$ when compared with offspring from non-exposed mothers.

Discussion

Although the presence of allergen, either free or in complex with IgG, has been detected in human maternal fluids, the relationship between maternal exposure and the presence of aeroallergen-reactive T cells in cord blood remains unclear.^{16,17} Whether allergen transfer occurs pre- or postnatally and how these allergens might impact the developing immune responses of the infant and its clinical outcome remains uncertain. The fact that allergic symptoms usually appear early in life implies that the neonate is subjected to environmental and maternal factors, perhaps even at the fetal stage. The results of this study demonstrated that maternal mucosal allergen exposure during the pre- or postnatal period may lead to important repercussions on IgE development of the progeny.

We found that prenatal exposure led variously to tolerance or sensitization in the progeny depending on the

timing and amount of allergen administration. The offspring of mothers subjected to low allergen exposure during pregnancy developed a state of immunological unresponsiveness, reflected by the inhibition of the antigen-proliferative response and minimal Th1/Th2 cytokine production, as well as the inhibition of total IgE levels and specific IgG1 and IgG2a antibody levels. The effect of oral tolerance induction followed by mucosal administration of allergens or food antigens in adult mice is well known.^{28–32} One of the regulatory mechanism mediated in oral tolerance has been showed to be exerted by TGF- β ,³¹ in the prevention of allergy²⁹ and in airway remodelling. Exposure of lactating mice to OVA may cause the transfer of antigen to their progeny through breast milk, resulting in antigen-specific tolerance and prevention of allergen-induced lung pathology.³¹ The tolerance effect relied on the presence of TGF- β , was mediated by regulatory CD4⁺ T cells and was dependent on TGF- β signalling. In our model, mothers exposed to a

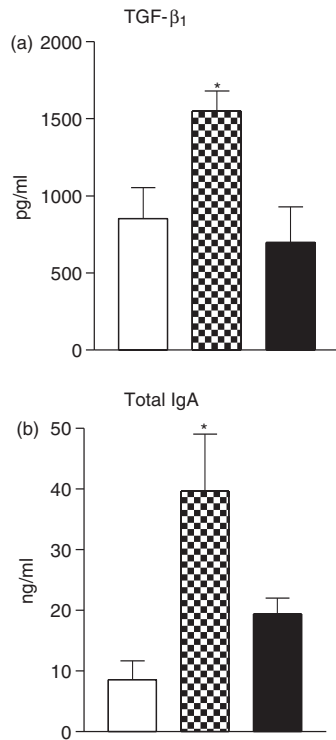


Figure 5. Transforming growth factor- β (TGF- β) levels in milk of mothers exposed to antigen during the prenatal or postnatal period. Female BALB/c mice received ovalbumin (OVA) by the oral route during gestation (1.5 mg, Protocol I, dotted bar) or in the first week of suckling (9.0 mg, Protocol II, solid bar). TGF- β_1 in individual milk samples ($n = 5$) (a) or total immunoglobulin A (IgA) levels (b) were assessed by enzyme-linked immunosorbent assay. Results are expressed as mean $\pm$ SEM. * $P \leq 0.05$ when compared with offspring from non-exposed mothers (open bar).

low-dose of OVA during pregnancy transfer increased amounts of TGF- β to their offspring via breastfeeding. The transference of TGF- β during lactation may reflect a potential maternal regulatory mechanism to avoid offspring allergen sensitization. Furthermore, stimulation of TGF- β production was not detected when mothers received a high-dose of OVA during the postnatal stage, correlating to a period of high susceptibility for IgE production. These findings may explain, at least partially, that the antigen-specific induction of TGF- β by mothers mediated downmodulation in the neonates, as verified by the suppressed IFN- γ and IL-4 secretion on anti-CD3 stimulation. Moreover, immunized offspring with HEL from mothers exposed to a low-dose of OVA also showed decreased proliferative responsiveness and cytokine production after anti-CD3 stimulation, which emphasizes that the bystander suppression effect was only induced by low-dose antigen administration during pregnancy.

We observed that increasing the amount of maternal antigen intake during pregnancy only led to transient IgE inhibition in their progeny; however, at infant ages, such exposure resulted in susceptibility to IgE production.

Although pregnancy could be a time for developing protective tolerance in the fetus, as verified by the low-dose protocol, at this time, high mucosal allergen exposure may break down its potent regulatory mechanism, favouring a progressive increase in IgE antibody levels.

Usually, allergic disease development occurs early in life so the effect of maternal allergen exposure on the development of the IgE response was evaluated in the neonatal period (at 3 days) and later, in infant mice (at 25 days). These periods in mice have been described as reflecting, to a large extent, the human immune maturation process observed in neonates and infants.^{4,33–35} The present results obtained for immunized neonates were consistent with findings involving cord blood studies that were unrelated to aeroallergen sensitization.¹⁷ Immune responses to inhalant allergens are commonly detected in cord blood in atopic and non-atopic infants,¹⁴ which are not necessarily associated with the development of allergic diseases.^{36,37} Infants at high risk for allergen sensitization display transient IgE production peaking at 6–12 months of age before returning to baseline, suggesting the onset of protective tolerance, in contrast with the progressive increase in IgE antibody levels that may occur in children up to 2 years of age.¹⁷ The current findings obtained for neonatal and infant mice confirmed that the amount of maternal allergen exposure during pregnancy influences the outcome of IgE response in offspring, where low-dose intake led to sustained IgE inhibition whereas high-dose resulted in the enhancement of IgE production.

A relation between allergen exposure of the mother during breastfeeding with progressive susceptibility for allergen sensitization according to post-birth age was observed. Maternal oral antigen administration provided antigen transfer across the mucosal barrier and transportation to lactating mammary glands, as demonstrated by the presence of OVA in milk proportional to maternal antigen intake. In humans, three routes of maternal allergen passage, transplacental, transamniotic and via breast milk have been demonstrated; the last one predominates as a source of allergen dose.³⁸ However, levels of OVA in breast milk have not been related to maternal diet intake or to atopic predisposition.⁹ Analysis of the present findings in mice shows that maternal allergen ingestion during breastfeeding facilitated the sensitization of suckling pups according to their immunological maturation.

It is less likely that prenatal allergen exposure involving low amounts of antigen exposure to the mother's mucosa crosses the maternal-fetal barrier, resulting in transamniotic or transplacental antigen transfer to the fetus. Consistent with this idea, this study failed to detect OVA in placental sera or amniotic fluid from the mothers. In an *ex vivo* model of transplacental transfer of nutritive and inhalant allergens, most of the allergens observed were accumulated in placental tissues, explaining the lack of a direct dose relationship between prenatal allergen

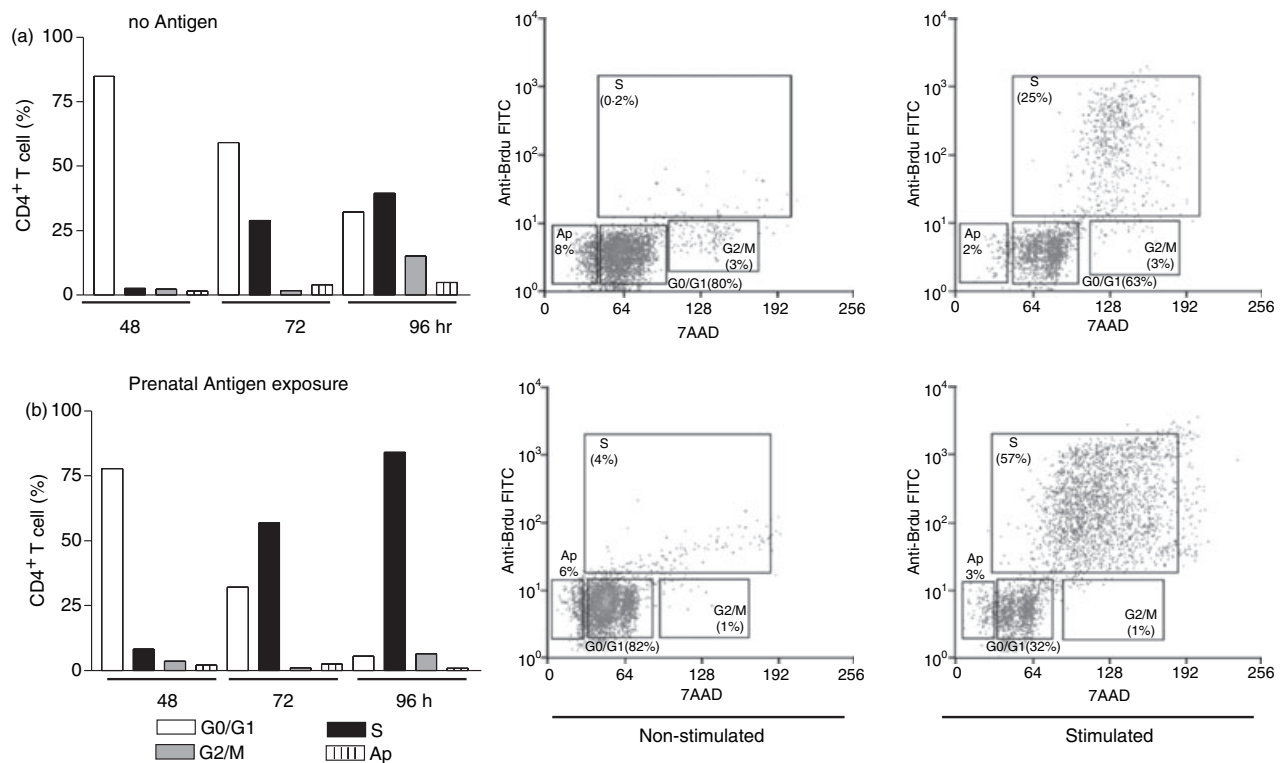


Figure 6. Prenatal ovalbumin (OVA) exposure induces activation of OVA-specific CD4⁺ T cells in the offspring of transgenic (Tg) DO11.10 mice. Female DO11.10 mice were mated and given OVA orally (1.5 mg) during the pregnancy (Protocol I). Splenic cells of offspring (20 days old) were pooled and cultured with OVA to evaluate the CD4⁺ cell cycle phase through bromodeoxyuridine (BrdU) incorporation. Spleen cells were stained with fluorescein isothiocyanate-labelled anti-BrdU monoclonal antibody, phycoerythrin-labelled anti-CD4 monoclonal antibody, and 7-amino-actinomycin D (7-AAD) for flow cytometry analysis. The percentage of gated CD4⁺ cells in the G0/G1, S, G2/M, and Ap (Apoptotic) stages of the cell cycle, represented by bars, were determined for offspring from non-exposed (a) or exposed (b) mothers and are representative of two experiments. A representative fluorescence-activated cell sorting analysis showing the percentage of stimulated cells following culture with OVA for 72 hr is shown. The percentages of cells in each phase of the cell cycle are shown in parentheses.

exposure and allergen-specific cellular reactivity in cord blood.¹² However, intravenous injection of 40 mg OVA in pregnant BALB/c mice has been reported to permit OVA transfer after 6 hr in maternal and fetal blood, as well as in the amniotic fluid.³⁹ It seems that diaplacental transfer of an allergen to the fetus depends on high amounts and on the route of allergen exposure to the pregnant mice.

To assess whether low amounts of allergen exposure (1.5 mg OVA) by the oral route in pregnant mice might be transferred to the fetus, T-cell activation was evaluated in the progeny of OVA-specific TCR Tg mice subsequent to low-dose antigen exposure during pregnancy. These offspring presented a pronounced progression of CD4⁺ T cells in the S phase of the cell cycle upon OVA stimulus, driving a Th2-skewed immune response, with important production of Th1 cytokines. Interestingly, this result indicates that the primed, OVA-specific T cells undergo rapid and more intense cycle entry. Indeed, primed T cells provide immunological help, through Th2-type cytokine production, to support the onset of antigen-specific antibody synthesis, as shown by the appearance of anti-

OVA IgG antibody in the offspring. However, IgG antibodies may also be a reflection of maternal antibody transfer following oral administration of antigen. Non-immunized offspring showed no alterations in total IgE levels and failed to produce IgE antibody with anaphylactic function following maternal exposure to 1.5 mg OVA (data not shown). It is possible that the generation of anaphylactic antibody requires continuous allergen exposure. Transgenic mice have been described as a potential food allergy model that develops specific IgE proportional to the length of the feeding period with a daily intake of 250 mg of OVA.⁴⁰ Moreover, the diminished number of B220⁺ CD23⁺ splenic cells observed in the Tg offspring of antigen-exposed dams may be related to allergy susceptibility. CD23, a low-affinity IgE receptor, is involved in negative regulation of IgE and antigen presentation.⁴¹ Our findings showed the occurrence of *in utero* allergen priming for the first time, as revealed by the generation of a substantial population of antigen-specific T cells early in life.

Conversely, in the naïve immune environment of non-Tg BALB/c mice, which have the same genetic

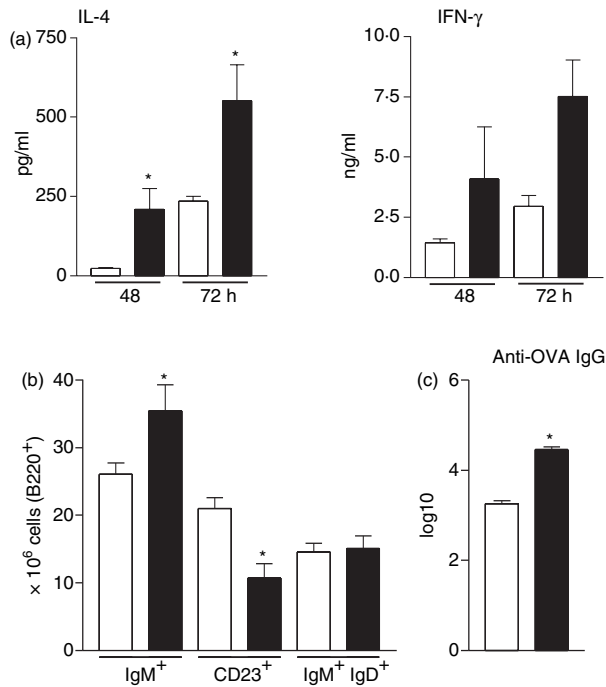


Figure 7. Cytokine production from the offspring of transgenic (Tg) DO11.10 mice subsequent to prenatal ovalbumin (OVA) exposure. Splenic cells from Tg offspring (20 days old) from mothers that were exposed (solid bar) to antigen during pregnancy (1.5 mg, Protocol I) or from non-exposed mothers (open bar) were stimulated with OVA (400 µg/ml). Cytokine measurements in the supernatant after 48 and 72 hr of stimulation were assessed by enzyme-linked immunosorbent assay (ELISA) (a); splenic B220⁺ IgM⁺ and B220⁺ CD23⁺ cells were evaluated by flow cytometry (b), and anti-OVA immunoglobulin G (IgG) antibody serum levels by ELISA (c). Results represent values from four to six mice per group expressed as mean ± SEM. * $P \leq 0.05$ when compared with offspring from non-exposed mothers.

background as the Tg mice, maternal prevention of allergy occurred following low-dose allergen exposure during gestation. Similar to non-atopic children, allergen unresponsiveness in the naïve neonatal immune system may suggest that a strong maternal regulatory mechanism exists, which acts to prevent early life sensitization. The acquisition of allergen-specific Th2-skewed immunity, such as occurred in the Tg mice, suggests that allergen exposure during pregnancy may lead to *in utero* antigen presentation and subsequent CD4⁺ T-cell activation. However, in high-dose antigen-exposed BALB/c mothers, two phases were verified in the progeny: an early transient tolerance and later, IgE sensitization.

Taken together, the results indicate that the amount and timing of prenatal antigen exposure determines either tolerance or sensitization in the progeny. Although gestation is a time for developing immunological tolerance in the fetus, high antigen exposure to the mother's mucosa may lead to offspring sensitization. In the perinatal period

or during suckling, the trend was towards the induction of susceptibility to IgE antibody production, irrespective of the maternal antigen dose. These results strengthen the idea that maternal mucosal allergen exposure during the pre- or postnatal period may affect the early onset of specific IgE sensitization.

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Disclosures

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