

ORIGINAL PAPER

Maternal tolerance achieved during pregnancy is transferred to the offspring via breast milk and persistently protects the offspring from allergic asthma

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Clinical and Experimental Allergy

Summary

Background Maternal, more than paternal, asthma is a risk factor for the development of asthma in children. Recently, epidemiologic studies have shown that environmental exposures during pregnancy might influence the development of childhood asthma and allergies.

Objective The aim of the present study was to investigate whether the induction of tolerance against a specific antigen during pregnancy prevents in the offspring the development of allergic asthma in response to this antigen.

Methods Balb/c mice were orally tolerized with ovalbumin (OVA) during pregnancy. The offspring of tolerized and naïve mothers were immunized with OVA at 6 weeks and 4 months of age and analysed in our murine asthma model.

Results While the offspring of naïve mice developed increased AHR, eosinophilic airway inflammation, T-helper type 2 cytokine production and high serum IgE levels in response to OVA sensitization, the offspring of tolerized mice were almost completely protected from asthma, even when immunized as late as 4 months after birth. Breastfeeding was crucial for protection because tolerance was only observed when the offspring were nursed by their own mothers and not when nursed by naïve wet-nurses. Allergen-specific IgG₁ antibodies were exclusively increased in the breast milk of tolerant mothers and serum of protected pups, indirectly supporting its important role in tolerance transfer from the mother to the offspring. Sensitization of the F1 generation from OVA-tolerized mothers with a heterologous allergen enhanced the immune response to this antigen.

Conclusion Our results demonstrate that mucosal allergen contact during pregnancy modifies the asthma and allergy risk of the offspring mediated via breast milk. This observation may suggest that the time window for primary prevention strategies starts even before early childhood during pregnancy.

Keywords asthma, pregnancy, primary prevention, tolerance

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Introduction

In recent years, a number of studies have shown that environmental exposures in the first 12 to 24 months of life might indeed determine the development of childhood asthma and allergies [1–3]. Therefore, primary prevention strategies might ideally start in early childhood. There is increasing evidence that the temporal sequence of events might even be traced back to prenatal exposures, namely exposures of the mother. The mother plays a special role because the uterine environment influences the development of the fetus with infectious agents, specific anti-

bodies and food antigens crossing the placenta during pregnancy and influencing the unborn child [4, 5].

Recently, it has been shown that maternal exposure to an environment rich in microbial compounds might protect against the development of atopic sensitization in the children [6]. The protective effect of microbial compounds was confined to up-regulation of receptors of the innate immune system [6] and to T-helper type 2 (Th2)-dependent Ig isotype expression that was allergen and switch stage specific [7]. Studies with human cell cultures demonstrated an allergen-specific immune response already at birth after stimulation of cord blood

mononuclear cells (CBMCs) with Der p1 or birch pollen [8]. Furthermore, the increased risk of atopic disease in the offspring of atopic mothers compared with those of atopic fathers suggests that pregnancy may be a critical time in determining subsequent disease development [9].

In murine models of asthma, it has recently been shown that airway exposure to endotoxin during pregnancy prevents the offspring from developing allergen-induced asthma in response to immunization [10], while prenatal exposure to stress enhances the susceptibility of the offspring towards airway inflammation [11]. Little is known about the effect of exposure to environmental allergens during pregnancy on the development of atopy and asthma in the offspring.

The aim of the present study was to investigate whether immunologic tolerance to a specific antigen that is induced in the mother during pregnancy influences the immune status of the offspring and protects the offspring from developing an asthma-like phenotype in response to the same or another allergen. We could demonstrate that indeed immunologic memory acquired during pregnancy by oral tolerization is transferred to the offspring via breast milk and transmits allergen-specific protection.

If maternal tolerance to harmless antigens like allergens acquired during pregnancy is transferred to the offspring and thereby modifies the asthma and allergy risk of the offspring, the appropriate time window to modulate immune responses and disease manifestation might already start during pregnancy.

Material and methods

Mice

Female BALB/cByJ mice (6–8 weeks of age) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and bred and maintained in our animal facility at the Martin Luther University in Halle/Saale (Germany). All animal experiments involved groups of four to six mice and were performed at least two times according to institutional and state guidelines. The Committee on Animal Welfare of Saxony-Anhalt approved the animal protocols used in this study.

Tolerance induction and immunization

For induction of oral tolerance, mice received 5 or 100 mg OVA (Sigma-Aldrich, Grade V, Steinheim, Germany; results from 100 mg OVA, 5 mg OVA produced comparable effects) or β -lactoglobuline (BLG, Sigma-Aldrich) by gastric intubation on 2 days (3 days apart) within the last trimester of pregnancy. Control mice received normal saline orally. Offspring mice (6–10 weeks or 4 months old) were immunized intraperitoneally (i.p.) with OVA (20 μ g) or BLG (10 μ g) adsorbed to 2 mg of an aqueous

solution of aluminium hydroxide and magnesium hydroxide (Alum, Perbio Science, Bonn, Germany) on days 0 and 14, followed by 20 μ g OVA in 40 μ L normal saline given intranasally (i.n.) on days 14–16 and 21–23. Control mice received Alum i.p. and normal saline i.n. One day after the last i.n. challenge (day 24), AHR was measured. Mice were killed on day 25. To verify the tolerization model 6–8-month-old ('mother') mice that received OVA orally during pregnancy were immunized with OVA i.p. on days 0 and 14 and challenged i.n. on days 14–16 and 21–23.

Measurement of airway responsiveness

Airway responsiveness was assessed by methacholine-induced airflow obstruction from conscious mice placed in a whole-body plethysmograph (model PLT UNR MS, EMKA TECHNOLOGIES, Paris, France) as described previously [12].

Collection of bronchoalveolar lavage (BAL) fluid

Animals were killed by CO₂ asphyxiation 1 day after the last airway hyperreactivity measurement. The trachea was cannulated, the left lung was tied off and the right lung was lavaged three times with 400 μ L NaCl 0.9%. Cells in the lavage fluid were counted using a haemocytometer, and BAL cell differentials were determined on slide preparations stained with May–Grünwald–Giemsa (Merck, Darmstadt, Germany) on blinded samples by an independent investigator. At least 200 cells were differentiated by light microscopy based on conventional morphologic criteria.

Cytokine production

Splenocytes (5×10^6 cells per well) were isolated and restimulated *in vitro* with 200 μ g/mL OVA in culture medium (RPMI medium supplemented with 10% FCS, 100 U/mL penicillin and 100 μ g/mL streptomycin) 1 day after airway function test. Cytokines (IL-4, IL-5, IL-10, IL-13 and IFN- γ) were measured in supernatants from restimulated spleen cells after 3 days using DuoSet[®] ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Lung histology and computer-based quantification of inflammation and mucus production

The left lung was fixed in 10% formalin and stained with haematoxylin & eosin (H&E, Merck) or with PAS (Sigma, Taufkirchen, Germany). For objective evaluation and quantification of the degree of pulmonary inflammation and mucus production, lung sections were scanned with a digital camera (visitron systems, Puchheim, Germany, 5

shots per lung) and analysed with HistoClick-Software based on morphometric image analysis developed in our laboratory. The degree of inflammation and mucus production is expressed by the number of pixels.

Immunoglobulin assay

OVA- or BLG-specific IgG₁ and IgE serum levels were measured by sandwich ELISA according to a standard protocol as described previously [13, 14]. Serum was taken on day 25 or as indicated in the figure legend. Briefly, 96-well microtitre plates (Nunc, Roskilde, Denmark) were coated overnight with 10 µg/mL OVA diluted in bicarbonate buffer (pH 9.6). After washing and blocking plates, samples were incubated for 2 h. Subsequently, 96-well plates were washed and HRP-conjugated goat-anti-mouse-IgE antibody (Bethyl Laboratories, Montgomery, TX, USA) was added. Tetramethylbenzidine was used as a substrate and the OD was determined at 450 nm using a Bio-Rad microplate reader (Bio-Rad, Munich, Germany). The titre was calculated by logarithmical regression as the reciprocal dilution of the sera, where the extinction was twofold the background extinction.

Milk was extracted from stomachs from 4-day-old neonates, diluted 1:2 with RPMI and centrifuged at 200 g. The top layer was discarded and the whey was collected and stored at -20 °C until Ig levels were measured as described above.

Statistical analysis

One-way ANOVA and Bonferroni's multiple comparison test were used to determine the statistical significance of differences between groups.

Results

Tolerization of Balb/c mice during pregnancy protects the offspring from developing an asthma-like phenotype in response to ovalbumin immunization

Oral application of OVA to BALB/c mice induces tolerance to the applied antigen and protects the animals from developing an asthma-like phenotype with AHR, eosinophilic airway inflammation, high IgE serum levels and Th2 cytokine production in response to subsequent OVA sensitization. This protective effect was long lasting as shown for mice at the age of 6–8 months (mothers of the investigated offspring, Figs 1a–e). To determine whether this state of tolerance may be transferred to the offspring during pregnancy, Balb/c mice were tolerized during the last trimester of pregnancy and the offspring was sensitized to OVA by systemic and intrapulmonary application of OVA at the age of 6–10 weeks. The development of an

asthma-like phenotype was investigated afterwards in our well-established murine asthma model [13, 14]. The offspring of mice that were tolerized during pregnancy were, to a large extent, protected from developing airway hyperreactivity in response to methacholine challenge in comparison with the offspring of naïve mice (Fig. 2a). Tolerization of Balb/c mice during pregnancy also significantly reduced the number of eosinophils in the BAL fluid of the offspring compared with the offspring of naïve mothers (Fig. 2b). Accordingly, histological analysis of H&E-stained lung sections isolated from the offspring of tolerant mothers showed substantially less inflammatory infiltrates in the airways compared with control animals (Fig. 2c). An objective, investigator-independent computer program based on morphometric image analysis of lung sections from at least nine animals per group confirmed these results and demonstrated significant differences in airway inflammation of the offspring of tolerized mothers compared with the offspring of naïve mothers (Fig. 2d). Similar results were observed for mucus production by histologic investigation of PAS-stained lung sections. Mucus production was markedly reduced in the offspring of tolerized mothers (Fig. 2c). As for lung inflammation, this effect could be verified by our investigator-independent computer-based analysis of the PAS-stained lung sections (Fig. 2e).

Another hallmark of allergic asthma is the production of allergen-specific IgE. OVA immunization of the offspring of tolerant mothers resulted in markedly reduced serum levels of OVA-specific IgE as compared with control mice (Fig. 2f).

To investigate the effect of maternal tolerization during pregnancy on cytokine production in the OVA-immunized offspring, spleen cells of the OVA-immunized offspring were isolated and restimulated *in vitro* with OVA for 3 days. Cytokine levels in cell culture supernatants showed that Th2-cytokines IL-4, IL-13, IL-5 and IL-10 as well as the Th1 cytokine IFN-γ were significantly reduced in the offspring of tolerant mothers (Fig. 2g).

Asthma prevention of the offspring by tolerization of Balb/c mice during pregnancy is long lasting and depends on breastfeeding

The clinical relevance of the tolerance transfer from mother to offspring depends on the persistence of the protective effect in the F1 generation. When we immunized the offspring of tolerized female mice 4 months after birth, the asthma phenotype including AHR, pulmonary inflammation, OVA-specific IgE and Th2 cytokine production was significantly reduced, demonstrating the long-lasting effect of tolerance transfer (Figs 3a–e).

Several studies demonstrate a critical role of breastfeeding in the maternal immunologic 'priming' of the offspring [15–17]. However, the data are not consistent

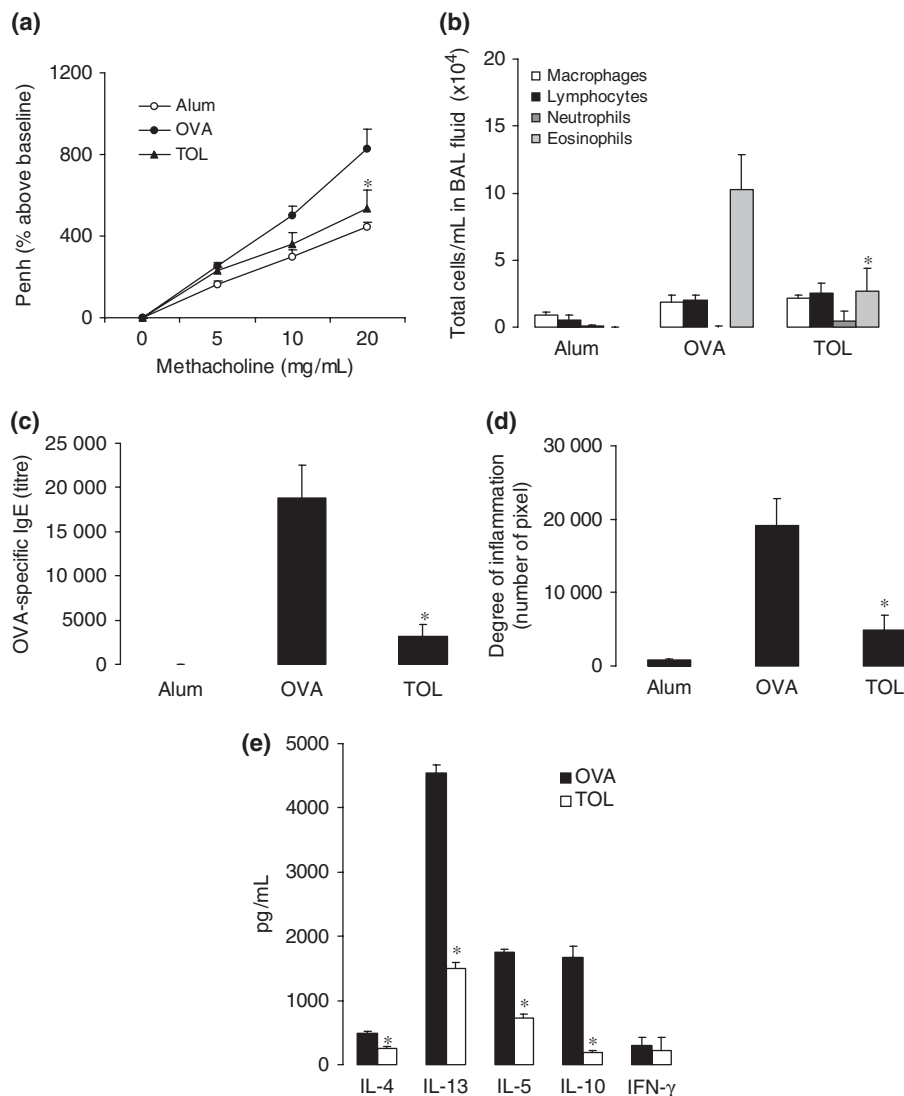


Fig. 1. Tolerization of Balb/c mice prevents the development of an asthma-like phenotype in response to the same antigen. Balb/c mice were tolerized to OVA orally (100 mg on 2 days, 3 days apart,) during pregnancy (last trimester). 6–8 months-old 'mother' mice were immunized intraperitoneally with OVA (20 μ g) on days 0 and 14 followed by 20 μ g OVA in 40 μ L normal saline given intranasally on days 14–16 and 21–23. AHR was measured on day 24. In contrast to naïve mothers, tolerized mothers showed significantly reduced (a) AHR, (b) total cell number in BAL fluid, (c) OVA-specific IgE serum levels, (d) airway inflammation, quantified by a computer-based image analyzing program and (e) cytokine production. Data are expressed as mean $\pm$ SEM, $n \geq 9$ animals per group; * $P < 0.05$, OVA-tolerized mothers vs. naïve mothers, one-way ANOVA and Bonferroni's multiple comparison test.

and there are also studies that show the contrary [18, 19]. To investigate the role of breastfeeding in the transfer of tolerance in our model, some of the neonates of tolerant mothers did not receive breast milk from their own mothers but were nursed immediately after birth by naïve wet-nurses. In the murine asthma model, these mice were not protected from the development of an asthma-like phenotype compared with their siblings that had received breast milk from their own tolerant mother. Our data demonstrate that in our model breast milk is crucial for the transfer of tolerance from the mother to the offspring (Figs 3a–e).

Maternal antibodies, in particular IgG antibodies but not IgE or IgM antibodies, are actively transferred via the placenta to the fetus and moreover via maternal milk to the neonate. It is known that they mediate maternal immunologic memory that is essential for the survival of the fetus and newborn [20].

To analyse the role of allergen-specific IgG antibodies, we measured OVA-specific IgG₁ levels in the serum of the mother, fetus, neonate and in the milk. We found that 5 days after the second oral application of OVA, the IgG₁ levels had increased (Fig. 4a). While we could not find OVA-specific IgG₁ in the fetus of tolerized mothers, the

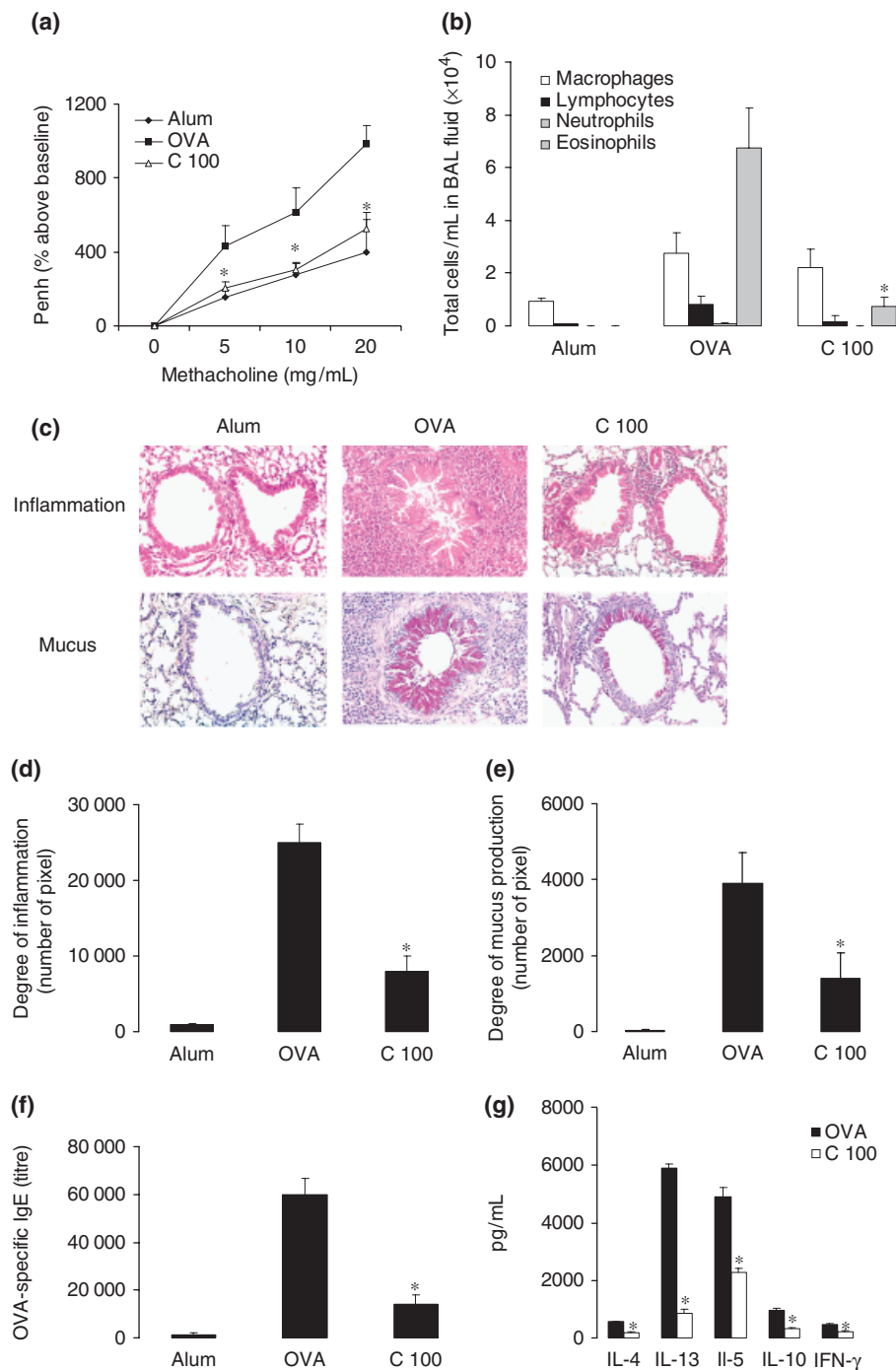


Fig. 2. Tolerization of Balb/c mice during pregnancy protects the offspring from developing an asthma-like phenotype in response to OVA immunization. Balb/c mice were tolerized to OVA during pregnancy by oral application of 100 mg OVA (C 100). The offspring was immunized with OVA at the age of 6–10 weeks. In contrast to the offspring of naïve mothers, the offspring of tolerized mothers showed significantly reduced (a) AHR, (b) total cell number in BAL fluid, (c) inflammation (H&E, $\times 200$) and mucus production (PAS, $\times 200$), (d, e) quantified by a computer-based image analyzing program, (f) reduced OVA-specific IgE serum levels and (g) cytokine production. Data are expressed as mean $\pm$ SEM, $n \geq 9$ animals per group; * $P < 0.05$, offspring from OVA-tolerized mothers vs. offspring from naïve mothers, one-way ANOVA and Bonferroni's multiple comparison test.

IgG₁ antibody response was enhanced in the offspring of tolerized mothers 1 week after birth. The OVA-specific IgG₁ could not be detected in the offspring of tolerized

mothers that were nursed by naïve wet-nurses. Moreover, elevated IgG₁ levels were detected in the breast milk of tolerized mothers but not of naïve mothers, suggesting

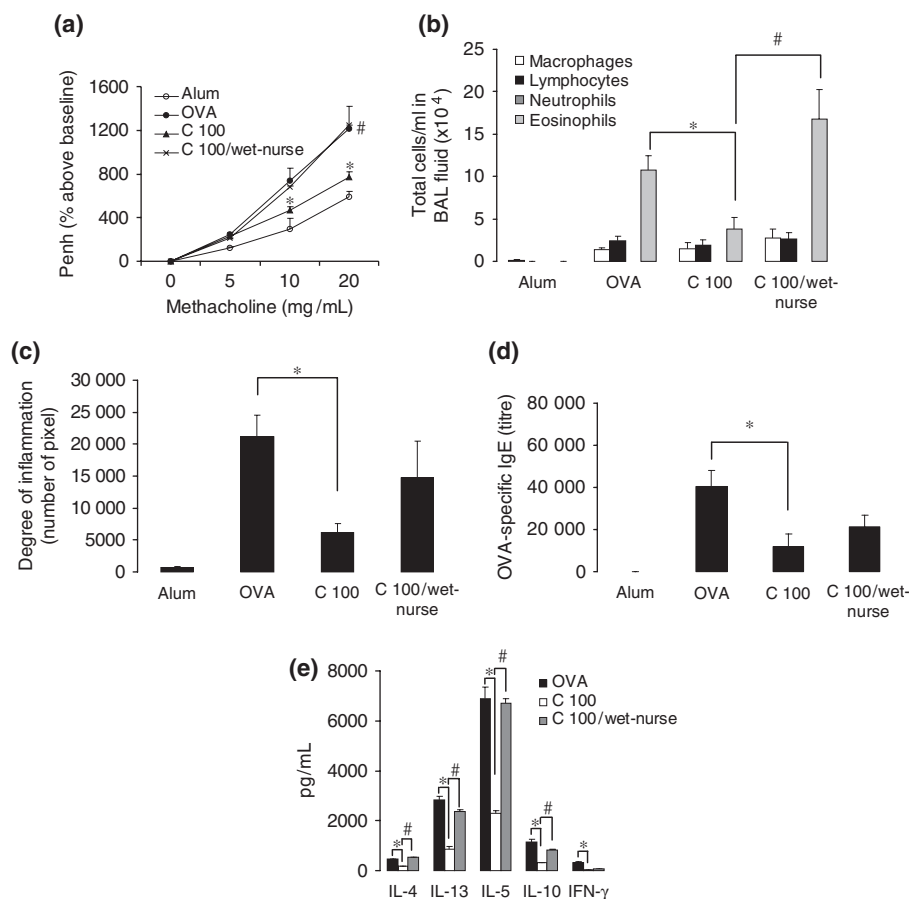


Fig. 3. Transfer of tolerance from the tolerized mother to the offspring is long-lasting and depends on breastfeeding. Balb/c mice were tolerized to OVA during pregnancy (C 100). Some neonates were given to naïve wet-nurses immediately after birth (C 100/wet-nurse). The offspring was immunized with OVA at the age of 4 months. (a) AHR, (b) total cell number in BAL fluid, (c) inflammation quantified by a computer-based image analyzing program (d) OVA-specific IgE serum levels and (e) cytokine production were significantly reduced even 4 months after tolerization. In contrast, the asthma-phenotype was not reduced when the offspring was nursed by a wet-nurse immediately after birth (a–e). Data are expressed as mean $\pm$ SEM, $n \geq 9$ animals per group; * $P < 0.05$, offspring from OVA-tolerized mothers vs. offspring from naïve mothers, # $P < 0.05$, offspring from OVA-tolerized mothers vs. offspring from OVA-tolerized mothers nursed by naïve mice, one-way ANOVA and Bonferroni's multiple comparison test.

that the allergen-specific IgG₁ is transferred to the newborn from the tolerized mother via breast milk (Fig. 4b).

Tolerization of pregnant mice resulted in antigen-specific immunoglobulin E and immunoglobulin G₁ responses in the offspring

To assess whether the transfer of maternal tolerance to the offspring is also possible with a different antigen, we tolerized mice with β -BLG and immunized the 6- to 8-week-old F1 generation from BLG-tolerized mothers with the same antigen. In accordance with tolerization with OVA, tolerization with BLG during pregnancy also resulted in reduced BLG-specific IgE and IgG₁ levels in response to sensitization with the homologue antigen BLG in the offspring (Table 1). To further analyse whether the transfer of maternal tolerance to the offspring is antigen-specific,

we immunized the 6- to 10-week-old F1 generation from OVA-tolerized mothers with the heterologous allergen β -BLG. In contrast to our observation with the offspring of OVA-tolerized mice that were sensitized to OVA, sensitization with the heterologous antigen BLG led to an increase of BLG-specific IgE and IgG₁ antibody production as compared with control animals. This finding demonstrates first that the tolerance transfer is allergen-specific and second that the protective effect of tolerization against one allergen during pregnancy might increase the immune response to other allergens in the offspring (Table 1).

Discussion

In the present study, we demonstrate in a murine asthma model that immune tolerance induced by oral antigen application in the last trimester of pregnancy can be transferred from the mother to the offspring. The

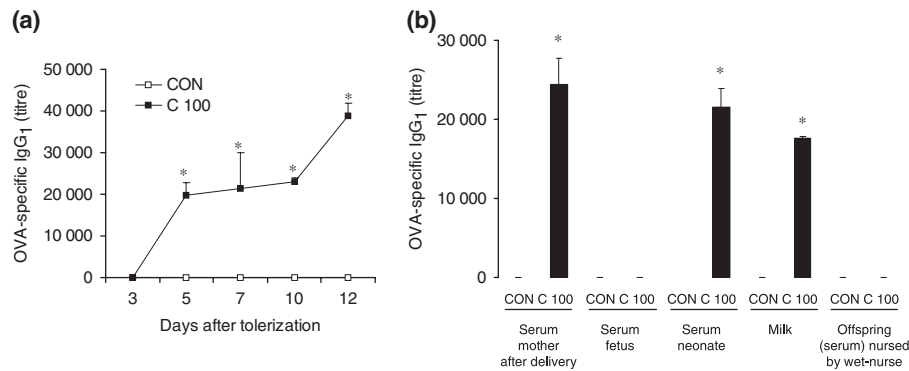


Fig. 4. Tolerance transfer is mediated by OVA-specific IgG₁. Balb/c mice were tolerized to OVA during pregnancy (C 100). Oral tolerization led to an increase of Ag-specific IgG₁, antibodies in the serum starting 5 days after the second OVA application (a). OVA-specific IgG₁ levels were increased in the serum of the mother (1 week after delivery) and neonate (1 week old) and in the milk but not in the serum of the mother and the fetus (1–3 days before delivery) or in the serum of the offspring nursed by naïve wet-nurse (b). Data are expressed as mean ± SEM, $n \geq 9$ animals per group; * $P < 0.05$, OVA-tolerized mothers or their fetus, neonate vs. naïve mothers or their fetus, neonate, one-way ANOVA and Bonferroni's multiple comparison test.

Table 1. Tolerance transfer is allergen specific

F1 generation from	BLG sensitization	
	IgE (titre)	IgG ₁ (titre)
Non-tolerized mothers	7013 ± 1820	9855 ± 275
BLG-tolerized mothers	2580 ± 1350	4624 ± 2007
Non-tolerized mothers	3461 ± 660	16 945 ± 2361
OVA-tolerized mothers	14 459 ± 4600*	76 806 ± 26 122*

Balb/c mice were tolerized to BLG during pregnancy (100 mg) and the offspring was immunized with BLG at the age of 6–8 weeks. BLG-specific IgE and IgG₁ levels were reduced in the offspring of BLG-tolerized mothers. In a further experiment Balb/c mice were tolerized to OVA during pregnancy (C 100) and the offspring was immunized with BLG at the age of 6 to 8 weeks. BLG-specific IgE and IgG₁ levels were increased in the offspring of OVA-tolerized mothers. Data are expressed as mean ± SEM, $n \geq 9$ animals per;

* $P < 0.05$, offspring from OVA-tolerized mothers vs. non-tolerized mothers, one-way ANOVA and Bonferroni's multiple comparison test. OVA, ovalbumin; BLG, β -lactoglobuline.

tolerance transfer was mediated via breast milk because it was only observed when the offspring were weaned by tolerant mice but not when they were weaned by naïve wet nurses. The preventive effect of tolerization for the next generation was long lasting and could still be observed 4 months after birth. Our data directly show that environmental influences like mucosal allergen contact during pregnancy have a significant and persistent impact on the immune response of the offspring and thereby support recent epidemiologic studies that demonstrated a critical role of the living environment on the immune status and allergy risk of the unborn child. These data may indicate that the immunologic window to modulate immune responses in childhood might open already during pregnancy so that the ideal time for primary prevention strategies might start already before birth.

So far it has not been fully understood how the maternal environment exerts its effect on the offspring. It is known that infectious agents, food antigens and specific antibodies can cross the placenta during pregnancy [4, 21]. It is possible that the fetal immune system interacts with a certain cytokine pattern revealed in the maternal organism [22] or with the antigens themselves [23]. Several human studies demonstrated an allergen-specific immune response directly after birth to a range of common allergens, both dietary and inhalant [8, 24–26]. Stimulation of umbilical CBMCs from antigen-exposed mothers with allergens such as Der p1, birch pollen or cow's milk induces T cell proliferation and cytokine production [8, 24–26]. This reactivity supports the contention that the fetal immune system attains a level of maturity enabling the initiation of antigen-specific immune responses before birth. However, in our study tolerance of the offspring did not seem to be actively acquired by the offspring themselves *in utero* because it was clearly dependent on breastfeeding by tolerized mothers and were not observed when the offspring of tolerized mothers was weaned by naïve wet nurses. These data demonstrate that in our model tolerance is transferred from the mother to the offspring via breast milk and that breast milk has a strong potential to modulate the immune response of the pup.

It is conceivable that not only protective but also 'harmful' immunity is transferred from the mother to the offspring. When in our model the offspring of OVA tolerized mothers were immunized with a heterologous antigen, the immune response to this antigen was clearly increased, demonstrating that the influence of the immune status of the mother for the child can be protective but also harmful. This observation suits to conflicting data of the many studies that investigate the role of breastfeeding in allergy prevention. While several studies demonstrated a protective role of breastfeeding in the maternal immunologic 'priming' of the offspring [15–17], others showed the contrary and

demonstrated that it could even be a risk factor [18, 19] for the development of asthma and atopy in children.

Next, we were interested in observing how breast milk transfers immunologic tolerance or memory from the mother to the offspring. In agreement with others [27], we did not find any differences in the cytokine content of the milk (data not shown) of tolerized mothers compared with the milk of naïve mothers so that a critical role of cytokine transfer was not supported by these measurements. However, we found significantly increased levels of allergen-specific IgG₁ in the serum (after delivery) as well as in the milk of tolerized mothers. Moreover, IgG₁ was increased in the serum of the neonates (1 week old) of the tolerized mothers weaned by their own mothers but not in the serum of the neonates of tolerized mothers weaned by naïve wet-nurses or in the serum of the neonate of naïve mothers. This finding indirectly supports the crucial role of IgG₁ antibodies in tolerance transfer. It seems that transfer of antibodies via breast milk provides passive protection not only against infections but also actively shapes childhood immunity and tolerance induction by providing the fetus and the infant with the mother's immunological experience [20]. In our study, IgG₁ was transferred via the breast milk and not via the placenta, probably due to the late time-point of tolerance induction in the third trimester of pregnancy so that IgG₁ was not generated by the pregnant mouse before birth in sufficient amounts. Nursing of naïve pups by tolerized mothers decreased the asthma-like phenotype of the offspring but did not completely and significantly protect the offspring from allergy development in response to OVA immunization (data not shown). Therefore, it is possible that factors other than IgG₁ in the milk like the antigen itself or regulatory T cells as shown by Verhasselt et al. [28] might be partly responsible for the observed protective effects. However, we could not find regulatory T cells in the milk of tolerized female mice (data not shown).

A protective effect of IgG antibodies has also been shown before in other different studies. In animal studies, it has been shown that allergen-specific IgG antibodies either induced in female rats by immunization [29] or injected into pregnant mice as pre-formed monoclonal antibodies [30] are transferred to the offspring and reverse a seemingly genetically based IgE high responder phenotype in an allergen-specific manner. Similar results were described in a study showing that the reduced antigen-specific IgE response in the offspring of immunized mice was mediated by diaplacental transfer of IgG₁ [31]. Antibody transfer through the placenta differs between humans and mice [32]. Human placenta actually allows the passage of IgA (and possibly also IgM and IgE) although at low levels, which makes a maternal-fetal interaction much more likely to occur in humans than in mice, where the pups receive antibodies primarily through suckling. It is therefore difficult to conclude from these data that

the tolerance transfer occurs through milk in humans, although the results presented here support that this is the case in mice. However, data from some human studies show some interesting parallel findings to our results. For instance, high cord blood IgG concentrations of antibodies to cat dander and the major allergen of birch pollen have been associated with fewer allergic symptoms in children during the first 8 years of life [33]. Moreover, children of mothers who received specific immunotherapy during pregnancy developed reduced allergic sensitization [34].

To test whether the transfer of tolerance is also possible with a different antigen, we tolerized mice with BLG during pregnancy and later immunized the offspring to this antigen. According to our data with OVA, the response of the offspring to BLG sensitization was significantly reduced compared with the offspring of naïve mice, demonstrating that the tolerance to BLG – like the tolerance to OVA – was also transferred from the mother to the offspring. However, when we immunized the offspring of OVA-tolerized mice with the heterologous antigen BLG, the immune response to BLG was increased significantly. These data support that the transfer of tolerance is possible with different antigens but also show that the immune response to different antigens might even be exacerbated so that immune tolerance strategies for pregnant woman – independent of other limiting risk factors that make such an approach not really feasible at the moment – might even carry the risk for sensitization to other allergens.

In addition to our data, other data support the concept that the immune status of the mother actively shapes childhood immunity and tolerance induction by providing the fetus and the infant with the mother's immunological experience. For example, recently in a murine asthma model it has been shown that prenatal and postnatal sustained LPS exposure suppressed allergic airway disease in the offspring [10].

In conclusion, our results demonstrate that maternal tolerance induced in mice during pregnancy is transferred to the F1 generation and prevents the long-lasting offspring from the development of allergic asthma in offspring response to this antigen. Breast milk plays a crucial role in tolerance transfer when tolerance is acquired in the last trimester of pregnancy.

Our data directly support recent epidemiologic findings that environmental influences, here mucosal allergen contact, during pregnancy modify the asthma and allergy risk of the offspring and support the idea that the appropriate time window to modulate immune responses and disease manifestation might start already before birth.

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