

Allergic Sensitization and Allergen Exposure during Pregnancy Favor the Development of Atopy in the Neonate

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Key Words

Allergen exposure, prenatal · Th-1/Th-2 · IFN- γ · Pregnancy

Abstract

Background: Several studies have considered that the in utero environment plays an important role in the onset of the allergic phenotype. We assessed whether allergic sensitization and allergen exposure during pregnancy favor the postnatal onset of allergy in the neonate. **Methods:** BALB/c mice were sensitized to ovalbumin (OVA) before mating followed by allergen aerosol exposure during pregnancy. T and B cell responses in offspring were followed up until day 60 postpartum. At the age of 4 weeks offspring were exposed to a heterologous antigen, β -lactoglobulin (BLG). **Results:** Pregnant mice developed immediate hypersensitivity responses and Th-2/Th-0 immunity following allergen aerosol exposure. At birth, T cells from offspring of nonsensitized BALB/c mice were characterized by an impaired IFN- γ production, which was lowered even further in offspring of OVA-sensitized BALB/c mice. Offspring of OVA-sensitized BALB/c

mice responded with immediate-type cutaneous hypersensitivity reactions to OVA which could be related to the pre- and postnatal transfer of maternal OVA-specific IgG1 antibodies. After exposure to BLG, offspring of OVA-sensitized BALB/c mice developed an accelerated Th-2-driven immune response compared to offspring from nonsensitized BALB/c mice as indicated by enhanced anti-BLG IgG1 antibody production and increased numbers of positive immediate-type cutaneous hypersensitivity reactions to BLG. **Conclusion:** Our data suggest that Th-2/Th-0 immunity present during pregnancy has a decisive impact on shaping the Th-1/Th-2 T cell profile in response to postnatal allergen exposure.

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Introduction

It is well known that the expression of the allergic phenotype depends on the genetic predisposition and environmental factors. Environmental factors may influence the differentiation of allergen-specific T cells into Th-2 cells by acting both before and after birth. Several studies

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Table 1. Cytokine production after P/I stimulation in offspring from nonsensitized and OVA-sensitized BALB/c mice

	Day postpartum	Offsprings from	
		PBS	OVA
IL-4, pg/ml	1	1,713 ± 448	1,558 ± 935
	28	1,451 ± 344	964 ± 539
IFN- γ , pg/ml	1	2,099 ± 853	382 ± 39*
	28	1,379 ± 933	995 ± 612

Offspring from nonsensitized (PBS) or OVA-sensitized (OVA) mothers were analyzed on days 1 and 28 postpartum. 2×10^6 mononuclear cells were cultured for 24 h in the presence of PMA (10 ng/ml) and ionomycin (1 μ g/ml) (P/I). Cytokine production in cell-free supernatants was measured by ELISA. Presented are mean $\pm$ SD from 5 mice in each group. * $p < 0.05$ vs. PBS.

have considered that the in utero environment plays an important role as an initial step towards the development of the allergic phenotype. This concept of in utero priming is based on several observations. A proliferative response of cord blood mononuclear cells to aero- and food-allergens is common, and occurs whether the mother exhibits an allergic phenotype or not [1, 2]. This immune response is mediated by fetal T cells, as opposed to maternally derived contaminants [3] and depends on antigen exposure of the mother beyond the first trimester of pregnancy [4, 5]. T cells in cord blood from normal neonates of non-allergic mothers were characterized by low levels of Th-1 immunity. Moreover, the ability to develop Th-1 immunity seems to be even further reduced in neonates at risk [6–8]. It was the aim of this study to develop a mouse model for studying molecular and cellular mechanisms that contribute to the pre- and postnatal development of Th-2 immune responses.

Materials and Methods

Protocol

Female BALB/c mice were sensitized by intraperitoneal injections of 10 μ g chicken ovalbumin (OVA; grade VI, Sigma Chemie, Deisenhofen, Germany) emulsified in 1.5 mg Al(OH)₃ (Inject Alum, Pierce, Rockford, Ill., USA) on days –28, –14 and –7 prior mating (day 1). During the gestation period mice were exposed to OVA aerosol (1% OVA diluted in PBS) every second day for 20 min each as described previously [9]. Age-matched control animals received PBS instead of OVA. Offspring from PBS- or OVA-sensitized mothers received at 4 weeks of age a single injection of 10 μ g β -lactoglobulin (BLG, Sigma Chemie) emulsified in 1.5 mg Al(OH)₃.

Assessment of Immediate Cutaneous Hypersensitivity Reactions and Determination of Allergen-Specific IgE, IgG1, IgG2a Antibody Titers

Intracutaneous skin testing and allergen-specific IgE, IgG1, IgG2a antibody titers were assessed as previously described [9].

Determination of IL-4 and IFN- γ

IL-4 and IFN- γ were measured in culture supernatants by ELISA as previously described [9]. Sensitivities were 10 pg/ml for IL-4 and 100 pg/ml for IFN- γ .

Flow-Cytometric Analysis of Cell Surface Marker and Intracellular Cytokine Expression

The distribution of T cell subpopulations and frequencies of IL-4- and IFN- γ -producing T cells were determined by flow cytometry (FACS) as previously described [10].

Statistical Analysis

Results are presented as mean values $\pm$ SD. The Mann-Whitney U test was used to determine the level of difference between animal groups.

Results and Discussion

Female BALB/c mice were sensitized to OVA before mating followed by allergen aerosol exposure during pregnancy. According to this protocol anti-OVA IgE, -IgG1 and to a lesser extent -IgG2a serum antibody titers ($p \leq 0.05$) were induced, which reflect the development of allergen-specific Th-2/Th-0 immunity. In contrast, following mitogen stimulation, no significant differences in the frequencies of IFN- γ - and IL-4-producing T cells were detected between allergen-sensitized and nonsensitized mice. In both groups between 25.8 and 27.6% of T cells produced IFN- γ and about 1–3% were positive for IL-4. Therefore, on the level of memory T cells, no imbalance in the Th-2/Th-1 cytokine profile could be detected at the time of delivery.

The cytokine profile (IL-4, IFN- γ) in splenic mononuclear cells from offspring was analyzed on the single cell level by intracellular staining on days 1–2 postpartum. In offspring from nonsensitized BALB/c mice, 4.1% of the Thy1+ cells could be restimulated to produce IFN- γ . In contrast, offspring from OVA-sensitized BALB/c mice showed a further reduction in the capacity of IFN- γ production (0.8%). However, no significant difference in the frequency of IL-4-producing T cells was detectable (<1%) between the groups. Analysis of cell culture supernatants confirmed the lowered levels of IFN- γ production at birth. This difference in IFN- γ production in mice from OVA-sensitized mothers diminished by day 28 postpartum (table 1). These data demonstrate that in offspring

from OVA-sensitized mothers, memory T cells show an impaired production of IFN- γ . This effect seems to be the result of maternal sensitization.

In parallel to T cell functions, we also analyzed the development of B cell responses. By week 4 offspring from OVA-sensitized mice produced significantly ($p < 0.01$) higher levels of total IgE antibodies as compared to offspring from nonsensitized mice. However, anti-OVA IgE could not be detected. Antibodies directed against OVA were only detectable among IgG subclasses which most likely resulted from pre- and postnatal passive transfer. Different patterns of diaplacental/transintestinal transfer were observed during pre- and postnatal maturation including a diaplacental plus transintestinal transfer IgG1 and an exclusive diaplacental transfer of IgG2a antibodies. The postnatal transfer is most likely being explained by antibody uptake from breast milk and active transport via FcRn receptor [11]. Based on the presence of anaphylactogenic IgG1 antibodies it was not surprising that all offspring from OVA-sensitized mothers responded with positive immediate cutaneous hypersensitivity responses.

Since maternal OVA sensitization had apparently a marked impact on the overall Th-1/Th-2 balance, we examined possible functional consequences by sensitizing the offspring to a heterologous allergen. Therefore offspring received a single injection of BLG adsorbed to Al(OH)₃ 28 days postpartum. The development of anti-BLG immunoglobulin production was followed in weekly intervals. Prior to BLG immunization, no anti-BLG antibodies were detectable in sera of any mice (fig. 1). After BLG immunization, both the frequency of responders as well as the levels of anti-BLG IgG1 titers gradually increased. In contrast, only in 2 of 15 offspring from nonsensitized mothers, anti-BLG antibody production was detectable at this time. After intradermal challenge with BLG, 5 of 6 offspring from OVA-sensitized mothers developed positive immediate cutaneous hypersensitivity reactions, whereas only 1 out of 6 offspring from PBS mothers responded with a positive skin test reaction ($p < 0.05$). This result indicates that in offspring from OVA-sensitized mothers both the qualitative and quantitative response to BLG is markedly augmented as compared to offspring derived from non-allergic mice.

These data demonstrate that prenatal allergen sensitization inhibits the normal level of Th-1 cytokine production in offspring and such a reduced Th-1-type capacity is not antigen-restricted. The reduced IFN- γ levels preceded the onset of atopic phenotype, suggesting an important

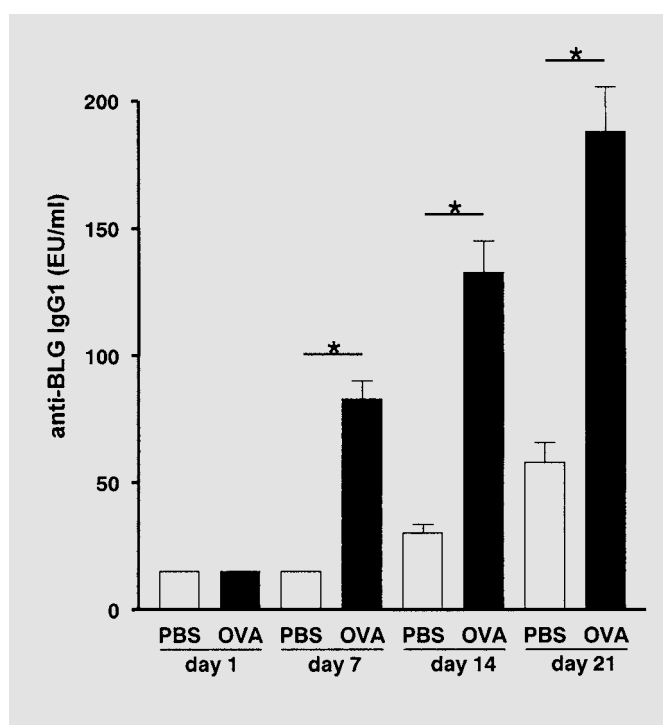


Fig. 1. Development of anti-BLG IgG1 antibody titers in BLG-sensitized offspring from non- and OVA-sensitized mothers. Offspring from PBS (□) or OVA-sensitized (■) mothers received a single injection of BLG as described in Materials and Methods. Allergen-specific IgG1 production was determined by ELISA in serum samples at the indicated time points after BLG immunization. * $p \leq 0.05$ between groups. EU = ELISA units.

factor for the development of atopy. These findings provide the basis for further studies aimed at delineating the mechanism responsible for these phenomena.

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