

Short paper

Impact of *in utero* Th2 immunity on T cell deviation and subsequent immediate-type hypersensitivity in the neonate

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It was the aim of this study to analyze the impact of maternal Th2 immune responses on onset and subsequent development of allergen-specific immunity and immediate-type hypersensitivity in early childhood. In a well characterized mouse model of Th2 immunity, BALB/c mice were sensitized to ovalbumin (OVA) before mating followed by allergen aerosol exposure during pregnancy. At the end of pregnancy mice developed allergen-specific Th2/Th0 immunity and immediate-type hypersensitivity responses to OVA. T cells from these newborns, when restimulated with PMA/ionomycin, demonstrated a lowered capacity to produce IFN- γ . To assess whether prenatal allergen exposure favors postnatal onset of a Th2-type immune response, these offspring were immunized to a novel antigen by a single injection of β -lactoglobulin (BLG). In contrast to offspring from non-sensitized mothers, offspring from OVA-sensitized mice showed both higher anti-BLG immunoglobulin titers and higher frequencies of immediate-type skin test responses. Our data suggest that Th2/Th0 immunity present during pregnancy has a decisive impact on shaping of the Th1/Th2 T cell profile in the neonate. Furthermore, this effect favors the development of Th2 immune responses, when mice are exposed to a novel antigen during early childhood.

Key words: Prenatal allergen exposure / Th1/Th2 / IFN- γ / Pregnancy

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1 Introduction

The development of Th2 skewed immunity against environmental antigens is recognized as a key event in the pathogenesis of allergic diseases. Genetic predisposition and environmental factors are undoubtedly important in the onset of the allergic phenotype. On the other hand, several studies have suggested that the *in utero* environment has an important impact on the onset of the allergic diseases later in life [1–5]. This concept of *in utero* priming is based on several observations. Already at birth, cord blood mononuclear cells (CBMC) proliferate to allergens [6, 7]. This allergen-specific immune response is mediated by fetal T cells, as opposed to maternally derived components [8]. Moreover, several studies have documented suppressed polyclonal IFN- γ responses by CBMC from neonates either at risk of atopy, or in those who later develop disease [3, 9–11].

[19762]

These observations lead to the hypothesis that maternal immunity represents an additional factor for early development of Th1/Th2 immunity in neonate. In a well-established mouse model of allergic sensitization, we asked, whether the maternal allergen-specific Th2 immune response, which is maintained during pregnancy, affects the Th1/Th2 balance in offspring. Furthermore, we analyzed the impact of maternal Th2 phenotype on the development of Th2 immune responses to novel allergens later in life.

2 Results

2.1 OVA-sensitization induces immediate-type hypersensitivity responses in pregnant BALB/c mice

Female BALB/c mice were sensitized to OVA before mating, followed by allergen aerosol exposure during pregnancy. This protocol induced anti-OVA IgE, anti-

Table 1. Development of Th2/Th0 immunity in pregnant PBS- and OVA-sensitized BALB/c mice

		BALB/c	
		PBS	OVA
<i>In vivo</i>	ICHS to OVA ^{a)}	0/6	10/10
	total IgE ^{b)} (ng/ml)	322 ± 171	4234 ± 1327
	anti-OVA IgE (EU/ml)	≤ 60	582 ± 232
	anti-OVA IgG1 (EU/ml)	≤ 20	5061 ± 1182
	anti-OVA IgG2a (EU/ml)	≤ 25	173 ± 93
<i>In vitro</i>	IL-4 ^{c)} (pg/ml)	≤ 30	417 ± 384*
	IL-5 (pg/ml)	≤ 30	962 ± 365*
	IFN-γ (pg/ml)	≤ 50	725 ± 647*

a) Intracutaneous skin testing was performed as described in Sect. 4.2. Indicated are numbers of mice that developed blue patches ≥ 3 mm in diameter after intradermal injection of OVA. All animals developed positive skin test responses after injection of Compound 48/80 and no reactions to PBS.

b) Allergen-specific serum antibody production was determined by ELISA. Blood from all animals was drawn 1 day after delivery. Presented are the means ± SD from 15 mice in the PBS group and 10 mice in the OVA group. *indicates statistical significance ($p \leq 0.05$).

c) IL-4, IL-5 and IFN-γ production in cell-free culture supernatants were measured by ELISA. Presented are the means ± SD from eight mice per study group. *indicates statistical significance ($p < 0.01$).

OVA IgG1 and, to a lesser extent, anti-OVA IgG2a serum antibody titers. Pregnant BALB/c mice developed immediate-type cutaneous hypersensitivity (ICHS) responses to OVA. In the presence of OVA, cultured splenic MNC produced significantly elevated levels of IL-4, IL-5 and IFN-γ ($p < 0.05$) (Table 1).

2.2 Reduced IFN-γ production in offspring from OVA-sensitized mothers

The level of Th1/Th2 immunity was analyzed in T cells from PBS- and OVA-sensitized mice and their respective neonates at the single cell level by intracellular cytokine staining after 6 h of stimulation with PMA/ionomycin, as well as in cell culture supernatants after 24 h of stimulation with PMA/ionomycin. At the time of delivery, mothers which were previously OVA-sensitized or PBS-sensitized showed a similar frequency of IFN-γ-secreting T cells (27.6 % vs. 25.8 %, respectively) while IL-4-secreting T-cells were only detectable in low frequencies

(0.1 % and 0.3 %) (Fig. 1). Compared to adult mice, neonates from PBS-sensitized BALB/c mice showed a lower frequency of IFN-γ-producing T cells (4.1 %) (Fig. 1). A further reduction in the frequency of IFN-γ-producing T cells (0.8 %) was detected in offspring from OVA-sensitized BALB/c mice (Fig. 1). The frequency of IL-4 production T cells was not increased in offspring from OVA-sensitized BALB/c (< 1 %) (Fig. 1). Analysis of cell culture supernatants confirmed the difference in the frequency of IFN-γ at birth. This difference in IFN-γ production in mice from OVA-sensitized mothers diminished by day 28 p.p. (Fig. 2).

2.3 Prenatal sensitization favors the development of immediate-type hypersensitivity in the neonate

In offspring from OVA-sensitized mothers anti-OVA IgG1 and IgG2a antibodies were transferred transplacentally during pregnancy and postnatally via the milk (data not shown). When these mice were tested for immediate type skin reactivity all offspring responded with positive immediate-type skin test reactions to OVA at least up to day 28 after birth (8 of 8 mice) (data not shown). To assess further whether the maternally driven immune response has any impact on the development of Th2

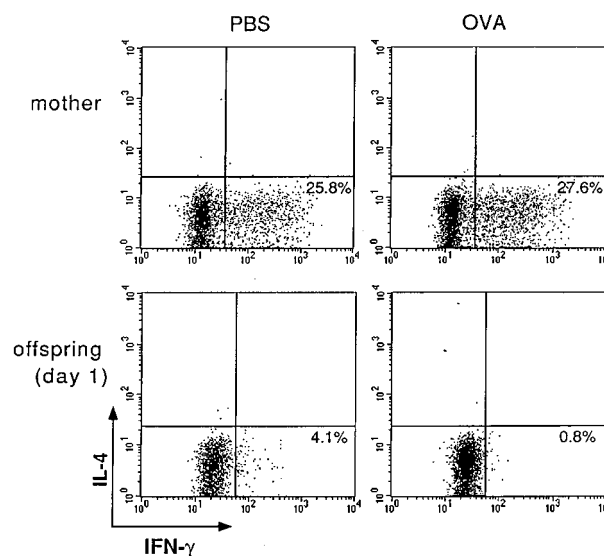


Fig. 1. Splenic MNC from newborns derived from PBS- (A) or OVA-sensitized mothers (B) and PBS- (C) OVA-sensitized offspring (D) were cultured for 6 h in the presence of PMA (10 ng/ml) and ionomycin 1 μg/ml). Cells were stained with antibodies directed against Thy-1, IFN-γ and IL-4 and analyzed by FACS technique. Shown are dot plots of IFN-γ⁺ (x-axis) and IL-4⁺ (y-axis) cells among Thy-1⁺ cells. Shown is one representative experiment out of three.

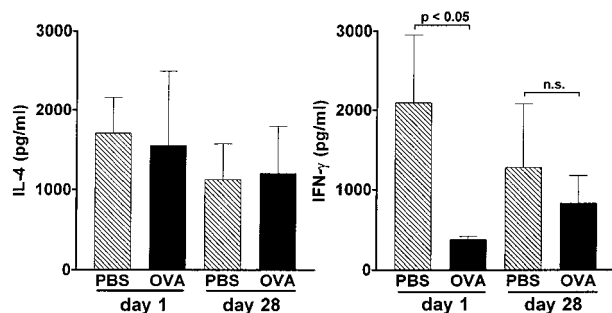


Fig. 2. Splenic lymphocytes were prepared from the same study groups as described in Fig. 1. PBMC (2.5×10^5 /well) were cultured for 24 h in the presence of PMA (10 ng/ml) and ionomycin (1 μ g/ml). At day 1 splenic MNC from littermates (6–10) of each mother were pooled. At day 28 splenic MNC from individual offspring of each mother were analyzed. Presented are the mean $\pm$ SD from for each group using six matings in each group. Statistical significance ($p \leq 0.05$) is indicated by (*).

immune responses to other allergens, young mice were immunized to a heterologous allergen with a single dose of β -lactoglobulin (BLG) at the age of 4 weeks. Prior to BLG immunization, no anti-BLG antibodies were detectable in sera of mice born from PBS- or OVA-sensitized mothers (Fig. 3). After BLG immunization, 10 of 15 offspring from OVA-sensitized mothers produced anti-BLG IgG1 antibodies within 3 weeks after immunization. In contrast, only in 2 of 15 offspring from PBS-sensitized mothers, anti-BLG antibody production was detectable at this time. In both study groups no anti-BLG IgE antibodies were detectable. After intradermal challenge with BLG, five of six offspring from OVA-sensitized mothers developed positive ICHS reactions, whereas only one out of six offspring from PBS-sensitized mothers responded with a positive skin-test reaction ($p < 0.05$). As a control, all animals developed positive ICHS reactions to compound 48/80, and no positive reaction was found to PBS. This result indicates, that in offspring from OVA-sensitized mothers both the qualitative and quantitative response to BLG is markedly enhanced as compared to offspring derived from non-allergic mice.

3 Discussion

Atopy in adults is associated with the long-term expression of allergen-specific Th2 immunity. It has been demonstrated, that CBMC from neonates with atopic family histories have a reduced capacity for secretion of Th1 cytokines (in particular IFN- γ) or exhibit an enhanced ability to produce Th2 cytokines (IL-4 and IL-5) [3, 5, 9–11]. These studies suggest that the onset of the allergic phenotype later on, may be primed *in utero* to some

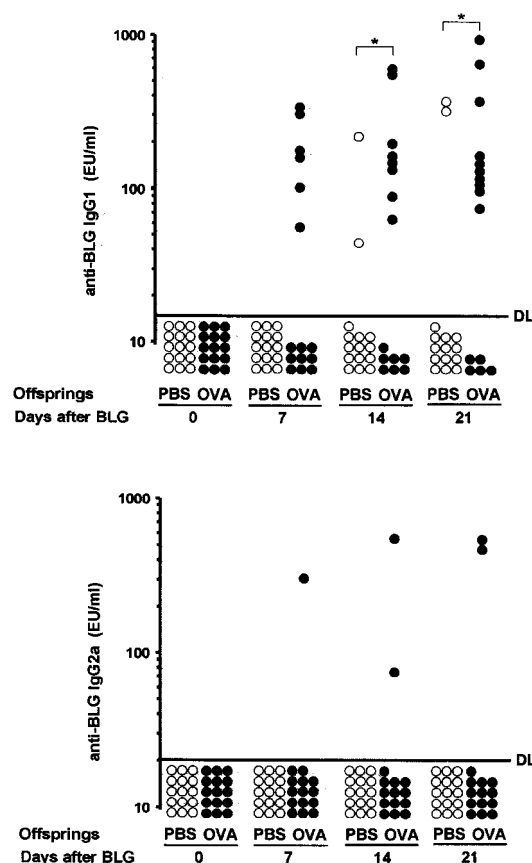


Fig. 3. Offspring from PBS- (white circles) or OVA-sensitized (black circles) mothers received a single injection of BLG as described in Sect. 4.1 Allergen-specific Ig production was determined by ELISA in serum samples at indicated time points after BLG immunization. * indicates significant differences ($p \leq 0.05$) between offspring from PBS- and OVA-sensitized mothers.

extend. Utilizing a well-defined model of Th2 immunity, we showed that offspring from OVA-sensitized mothers have a transient suppression of Th1 immunity and subsequently develop immediate-type hypersensitivity reactions after a single exposure to a heterologous allergen. These effects demonstrate the impact of maternal immunity for postnatal development of Th2 immune responses.

After OVA sensitization, pregnant mice development Th2/Th0 immunity to OVA as indicated by the presence of OVA-specific T cells which produced IL-4 and IL-5, and to a lesser extent IFN- γ . In addition, these animals produced high levels of anti-OVA IgE and IgG1 antibodies and developed ICHS reactions after intradermal injection of OVA. To assess whether the allergen-specific immune response was accompanied by an overall Th1/Th2 balance, IL-4 and IFN- γ production were examined

at the single-cell level by intracellular cytokine staining after restimulation with PMA/ionomycin for 6 h. This short-term restimulation minimizes the possibility of *de novo* T cell priming. In this system no significant difference in the frequencies of IL-4 and IFN- γ -producing T cells was detected between OVA- and PBS-sensitized mice. This result indicates that the development of the OVA-specific Th2 immunity at least in the spleen, is not associated with an overall Th2/Th0 imbalance. However, it should be emphasized that these data do not exclude a different cytokine pattern in the local environment of the feto-maternal interface. In fact it has been demonstrated that the immunological milieu at the feto-maternal interface is constitutively skewed towards a Th2 like profile [12, 13].

We next analyzed whether the allergen-specific Th2-like maternal immune response affects the immune response of the offspring. In offspring from non-sensitized mothers, an impaired capacity to produce IFN- γ compared to adults was detected at birth. This impaired capacity to produce IFN- γ reached adult-equivalent levels around day 28. This result in all likelihood reflects the lack of immunological experience in unborn and newborn animals. It is presently unclear to what extent this lower frequency of IFN- γ -expressing T cells in normal offspring is the result of maternal regulation. Our data are similar to observations in humans where CBMC after mitogen stimulation also produced less amounts of IFN- γ than their adult counterparts [10, 14].

Surprisingly, in offspring from OVA-sensitized mothers the frequency and the expression of IFN- γ was even further reduced. This effect is apparently related to the maternal allergen-specific Th2 immune response and has been initiated before birth. The mechanism that may contribute to this phenomenon include diaplacental transfer of low doses of allergen, either as free antigen or in form of IgG1 immune complexes. There is evidence that the ultra-low levels of antigen likely to cross the placenta are in a range that will preferentially stimulate differentiation of naive T cells into Th2 cells [15].

The present study suggests that the basis of the allergic phenotype later on in life is the failure to develop sufficient Th1 immunity at birth with the consequence of a persistent failure to suppress Th2 polarized memory against environmental antigens. Consistent with this general postulate, a retrospective study has demonstrated that expression of atopy in children was most common among those who had previously failed to develop Th1-dependent delayed tuberculin hypersensitivity in response to Bacillus Calmette-Guérin (BCG) vaccination during infancy [16]. In agreement with this result suppression of Th2 responses was observed during the

first year of life in children without atopic family history, but only in children with atopic family history these responses were consolidated and correlated to reduced neonatal IFN- γ production [4]. However, T cell clones from newborns with atopic parents exhibited an enhanced ability to produce Th2 cytokines compared to T cell clones from newborns with non-atopic parents. No differences were observed in the ability to produce in IFN- γ [5].

Our data demonstrate that prenatal allergen sensitization suppresses development of a systemic Th1 memory in the offspring. The reduced IFN- γ levels preceded the onset of an allergic phenotype, *i.e.* IgE-mediated allergy to environmental antigens. Nevertheless, at present it is unclear, whether the atopic predisposition in offspring from Th2 polarized mothers is due to the direct action of IFN- γ , or whether other regulatory events are responsible for this effect. Our findings support the hypothesis that delayed postnatal maturation of Th1 immunity induced in offspring of allergic mothers is crucial for the development of strongly polarized Th2 immunity to any antigen, and thus predisposes the offspring to develop allergies.

4 Materials and methods

4.1 Protocol of sensitization

Female BALB/c mice were sensitized by repeated intraperitoneal (*i.p.*) injections of 10 μ g chicken OVA (Grade VI, Sigma Chemie, Deisenhofen, Germany) emulsified in 1.5 mg Al(OH)₃ (Inject Alum Pierce, Rockford, IL) on days -28, -14 and -7 days prior mating (day 1). During the gestation period mice were exposed to OVA aerosol (1 % OVA diluted in PBS) every second day as previously described [17]. Age-matched control animals received *i.p.* injections of PBS and were exposed to PBS aerosol. Offspring from PBS- or OVA-sensitized mothers received at 4 weeks of age a single injection of 10 μ g BLG (Sigma) emulsified in 1.5 mg Al(OH)₃.

4.2 Assessment of ICHS reactions and determination of allergen-specific IgE, IgG1, IgG2a antibody titers

Intracutaneous skin testing was performed as previously described [17]. Allergen-specific IgE, IgG1, IgG2a antibody titers were measured by ELISA as previously described [17]. The anti-OVA IgE/IgG1/IgG2a and anti-BLG IgG1/IgG2a antibody titers of the samples were related to pooled standard sera that were generated in the laboratory.

4.3 Determination IL-4 and IFN- γ

IL-4 and IFN- γ were measured in culture supernatants by ELISA as previously described [17]. Sensitivities were 30 pg/ml for IL-4 and 50 pg/ml for IFN- γ .

4.4 Flow cytometric analysis of cell surface marker and intracellular cytokine expression

The distribution of T cell subpopulations was analyzed by flow cytometry. The following FITC-labeled monoclonal antibodies were used: rat anti-mouse -CD3 (clone 145-2C11), -CD4 (clone H 129.19), -CD8 (clone 53-6.7) and -CD19 (clone 1D3) and -Thy-1 (clone HO-13) (Pharmingen, Hamburg, Germany). To analyze intracellular cytokine expression, cells were fixed in formaldehyde (2 % diluted in PBS) and suspended in saponin (0.5 % diluted in PBS/0.5 % BSA/Azid). Frequencies of IL-4 and IFN- γ (rat α -IL-4, clone 11B11; rat α -IFN- γ , clone XMG1.2)-producing T-cells were determined after stimulation with PMA (10 ng/ml) and ionomycin (1 μ g/ml) for 6 h at 37 °C as previously described [18]. Background as determined by blocking of specific binding was below 0.5 %.

4.5 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.

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References

- Warner, J. A., Miles, E. A., Jones, A. C., Quint, D. J., Colwell, B. M. and Warner, J. O., Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin. Exp. Allergy* 1994. **24**: 423–430.
- Kondo, N., Kobayashi, Y., Shinoda, S., Takenaka, R., Teramoto, T., Kaneko, H., Fukao, T., Matsui, E., Kasahara, K. and Yokoyama, Y., Reduced interferon gamma production by antigen-stimulated cord blood mononuclear cells is a risk factor of allergic disorders-6-year follow-up study [see comments]. *Clin. Exp. Allergy* 1998. **28**: 1340–1344.
- Martinez, F. D., Stern, D. A., Wright, A. L., Holberg, C. J., Tausig, L. M. and Halonen, M., Association of interleukin-2 and interferon-gamma production by blood mononuclear cells in infancy with parental allergy skin tests and with subsequent development of atopy. *J. Allergy-Clin. Immunol.* 1995. **96**: 652–660.
- Prescott, S. L., Macaubas, C., Smallacombe, T., Holt, B. J., Sly, P. D. and Holt, P. G., Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999. **353**: 196–200.
- Piccinni, M. P., Beloni, L., Giannarini, L., Livi, C., Scarselli, G., Romagnani, S. and Maggi, E., Abnormal production of T helper 2 cytokines interleukin-4 and interleukin-5 by T cells from newborns with atopic parents. *Eur. J. Immunol.* 1996. **26**: 2293–2298.
- Kondo, N., Kobayashi, Y., Shinoda, S., Kasahara, K., Kameyama, T., Iwasa, S. and Orii, T., Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders. *Arch. Dis. Child* 1992. **67**: 1003–1007.
- Piccinni, M. P., Mecacci, F., Sampognaro, S., Manetti, R., Parronchi, P., Maggi, E. and Romagnani, S., Aeroallergen sensitization can occur during fetal life. *Int. Arch. Allergy. Immunol.* 1993. **102**: 301–303.
- Prescott, S. L., Macaubas, C., Holt, B. J., Smallacombe, T. B., Loh, R., Sly, P. D. and Holt, P. G., Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J. Immunol.* 1998. **160**: 4730–4737.
- Rinas, U., Horneff, G. and Wahn, V., Interferon-gamma production by cord-blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE-levels. *Pediatr. Allergy Immunol.* 1993. **4**: 60–64.
- Tang, M. L., Kemp, A. S., Thorburn, J. and Hill, D. J., Reduced interferon-gamma secretion in neonates and subsequent atopy. *Lancet* 1994. **344**: 983–985.
- Liao, S. Y., Liao, T. N., Chiang, B. L., Huang, M. S., Chen, C. C., Chou, C. C. and Hsieh, K. H., Decreased production of IFN gamma and increased production of IL-6 by cord blood mononuclear cells of newborns with a high risk of allergy. *Clin. Exp. Allergy* 1996. **26**: 397–405.
- Lin, H., Mosmann, T. R., Guilbert, L., Tuntipipat, S. and Wegmann, T. G., Synthesis of T helper 2-type cytokines at the maternal-fetal interface. *J. Immunol.* 1993. **151**: 4562–4573.
- Piccinni, M. P., Beloni, L., Livi, C., Maggi, E., Scarselli, G. and Romagnani, S., Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. *Nature Med.* 1998. **4**: 1020–1024.
- Wilson, C. B., Westall, J., Johnston, L., Lewis, D. B., Dower, S. K. and Alpert, A. R., Decreased production of interferon-gamma by human neonatal cells. Intrinsic and regulatory deficiencies. *J. Clin. Invest.* 1986. **77**: 860–867.
- Constant, S., Pfeiffer, C., Woodard, A., Pasqualini, T. and Bottomly, K., Extent of T cell receptor ligation can determine the functional differentiation of naive CD4+ T cells. *J. Exp. Med.* 1995. **182**: 1591–1596.
- Shirakawa, T., Enomoto, T., Shimazu, S. and Hopkin, J. M., The inverse association between tuberculin responses and atopic disorder. *Science* 1997. **275**: 77–79.
- Herz, U., Braun, A., Ruckert, R. and Renz, H., Various immunological phenotypes are associated with increased airway responsiveness. *Clin. Exp. Allergy* 1998. **28**: 625–634.
- Assenmacher, M., Scheffold, A., Schmitz, J., Segura Checa, J. A., Miltenyi, S. and Radbruch, A., Specific expression of surface interferon-gamma on interferon-gamma producing T cells from mouse and man. *Eur. J. Immunol.* 1996. **26**: 263–267.

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