

Modulation of the Induction of Lung and Airway Allergy in the Offspring of IFN- γ -Treated Mother Mice¹

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Recent studies have highlighted the influence of fetal/maternal interactions on the development of asthma. Because IFN- γ reduces Th2-mediated allergic responses, we assessed its capacity to modulate asthma in the offspring when injected into mothers during pregnancy. IFN- γ was injected in CD1 female mice on day 6.5 of gestation. Immediately after birth, male newborns were housed in cages with interchanged mothers: the offspring from IFN- γ -treated mothers were breastfed by normal mothers (IFN/nor), and those from normal mothers were breastfed by IFN- γ -treated (Nor/IFN) or normal mothers (Nor/nor). Immediately after weaning, the spleen cells from IFN/nor and Nor/IFN mice produced less IL-4 and more IFN- γ than Nor/nor mice when stimulated with Con A. At the age of 6–7 wk, mice were immunized with OVA on days 0 and 7. From day 14 to 16, they were exposed to aerosolized OVA. The bronchoalveolar lavage fluid from Nor/nor mice showed eosinophilia, a large number of these cells being present in perivascular and peribronchial regions of lung tissues. IFN/nor or Nor/IFN mice showed greatly reduced eosinophil numbers in bronchoalveolar lavage fluid. In addition, lung sections from IFN/nor, but not Nor/IFN mice showed almost normal histology. In OVA-sensitized IFN/nor and Nor/IFN mice, the production of IFN- γ , IL-4, and IL-5 by spleen cells was significantly reduced as compared with cells from the OVA-sensitized Nor/nor group. IgE and anaphylactic IgG1 were also reduced in plasma of IFN/nor mice. In conclusion, the presence of IFN- γ during pregnancy confers to the fetus a protection against allergenic provocations in the adult life. *The Journal of Immunology*, 2005, 175: 3554–3559.

Allergic diseases, including atopic asthma, are multifactorial, involving complex interactions between genes and the environment (1, 2). In asthma, pulmonary inflammation is characterized by edema, decreased mucociliary clearance, epithelial damage, increased bronchopulmonary responsiveness, and bronchoalveolar eosinophilia (3).

Recent studies have highlighted the possible influence of the in utero environment and fetal/maternal interactions on the development of atopy and asthma (4–6). Macaubas et al. (7) showed the relevance of immunological processes during early infancy, when the immature immune system is first directly exposed to environmental allergens, for the establishment of long-term patterns of responsiveness. They demonstrated that the prenatal cytokine environment, within which T cells develop, determines the degree of immune competence at birth and during early infancy.

There is compelling evidence that cytokines play an essential role in the maintenance of pregnancy by modulating the immune and endocrine systems (8). Placental tissue produces cytokines and hormones that are essential for regulating the fetal-maternal unit. Local dominance of Th2 and Th3 factors at the fetal-maternal in-

terface seems to be required to prevent fetal loss (9, 10). This includes a major role for IL-4 in dampening the local responses of maternal T cells against paternal Ags expressed on fetal cells (11). In contrast, Ashkar and Croy (12) showed that IFN- γ derived from uterine NK cell (uNK),⁴ the most abundant maternal lymphocyte population in the uterus (13, 14), contributes to the initiation of pregnancy-induced vascular modifications in the uterus, the persistence of decidual-artery remodeling, and the regulation of maturation and senescence of the uNK cell population. High concentrations of IL-4 and IFN- γ in the fetal circulation may thus indicate a healthy placental function, which, in turn, may positively affect maturation of immune competence in the developing fetus.

Maternal-derived T cells have previously been detected in the fetal/neonatal circulation (15–17). We hypothesized that regulatory cells induced by environmental factors may favor the modulation of the immune competence in the developing fetus. The investigations presented in this study were undertaken to assess whether exogenous IFN- γ administered during pregnancy affects the subsequent development of asthma in the offspring.

Materials and Methods

Animals

CD1 mice aged 3–4 mo were housed in the animal care facility at the Institute of Biomedical Sciences of the University of São Paulo. Females were caged overnight with males (1:1), and successful mating was checked daily according to the presence of a vaginal plug. The morning when the plug was found was designated as the first half day of pregnancy. Pregnant females received on gestation day 6.5 an i.p. injection of 100 IU of mouse recombinant IFN- γ (Sigma-Aldrich) in 0.1 M PBS (pH 7.2). Immediately

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Received for publication December 30, 2004. Accepted for publication June 22, 2005.

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¹ This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo.

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⁴ Abbreviations used in this paper: uNK, uterine NK cell; BALF, bronchoalveolar lavage fluid; PCA, passive cutaneous anaphylaxis; PAS, periodic acid-Schiff; BAL, bronchoalveolar lavage.

after birth (postnatal period), newborns were housed in cages with changed mothers: the offspring from IFN- γ -treated mothers were breastfed by normal mothers (IFN/nor), and the offspring from normal mothers were breastfed by IFN- γ -treated (Nor/IFN) or normal mothers (Nor/nor). Six- to 7-wk-old male CD1 mice were used for immunization, in accordance with the guidelines provided by the Brazilian College of Animal Experimentation, and were authorized by the Ethics Committee for Animal Research of the Institute of Biomedical Sciences (University of São Paulo).

Immunization and challenge protocol

On the first day of the experiments (day 0) and on day 7, two groups of male CD1 mice ($n = 6$) were actively immunized by i.p. injection of 10 μ g of OVA (grade V; Sigma-Aldrich), adsorbed to 1.6 mg of aluminum hydroxide (Al(OH)₃). From day 14 to day 16, the mice were exposed to aerosolized OVA (grade V, 1%) for 20 min in an adapted chamber. Immunized mice challenged with PBS were considered as the control group. The aerosol was produced by an ultrasonic nebulizer (US-800; ICEL) as described elsewhere (18). According to the specifications of the manufacturer, the output of the nebulizer was 2 ml/min, and the mean particle size was 3.2 μ m.

Treatment of mice with neutralizing Ab

Groups of mice received i.v. injections with rat anti-mouse IFN- γ mAb (XMG1.2; 1 mg/animal) or with a control rat IgG mAb (GL113; 1 mg/animal). One hour after Ab treatment, the animals were submitted to three consecutive aerosol challenges.

Bronchoalveolar lavage fluid (BALF) analysis

Forty-eight hours after the last challenge with aerosolized OVA, mice were killed with an overdose of chloral hydrate, and the tracheas were cannulated. The airway lumina was washed with 4 $\times$ 0.5 ml of HBSS (Invitrogen Life Technologies) + 10 mM EDTA. The resulting BALF were immediately centrifuged for 10 min at 800 rpm at 4°C. The supernatant was removed and kept at -70°C for further analysis. BALF cells were washed twice with HBSS containing 2% newborn calf serum (Sigma-Aldrich). Cell counts were performed using a hemocytometer, and cytocentrifuge slides were prepared, air-dried, fixed in methanol, and stained (Wright-Giemsa; Scientific Products). For differential cell counts, 300 leukocytes were enumerated and identified as mononuclear cells, neutrophils, or eosinophils, on the basis of staining and morphological characteristics.

Stimulation of spleen cells in vitro

Spleen cells were isolated and cultured in 24-well tissue culture plates at a final concentration of 10 or 6 $\times$ 10⁶ cells/ml in RPMI 1640 (Invitrogen Life Technologies) supplemented with 10% FBS, 1% L-glutamine (Sigma-Aldrich), and 1% penicillin/streptomycin. The cells were stimulated with OVA (500 μ g/ml) or Con A (5 μ g/ml). Levels of cytokines were assessed in cell culture supernatants harvested after 24 h (cultures of 10 $\times$ 10⁶ cells) or 72 h (cultures of 6 $\times$ 10⁶ cells).

Quantification of cytokines

Cytokines were measured in the plasma of mothers immediately after birth, and in the supernatants of spleen cell cultures 2 days after the last challenge with aerosolized OVA by a specific two-site sandwich ELISA, using the following mAb: for IL-2, JES6-IA12 and biotinylated JES6-5H4; for IL-4, BVD-1D11 and biotinylated BVD6-24G2; for IL-5, TRFK5 and biotinylated TRFK4; for IFN- γ , XMG 1.2 and biotinylated AN18; for IL-10, 2A5 and biotinylated SXC-1. Binding of biotinylated mAbs was detected using streptavidin-HRP complex (Amersham Biosciences) and ABTS (Sigma-Aldrich) in 0.1 M citrate buffer containing hydrogen peroxide. Samples were quantified by comparison with standard curves of recombinant mouse cytokines. Detection limits were 0.43 ng/ml for IL-2, 0.39 ng/ml for IFN- γ , 39 pg/ml for IL-4 or IL-5, and 0.156 ng/ml for IL-10.

Titration of IgG1, IgG2a, and IgE by ELISA

Blood samples from the offspring were obtained 2 days after the last challenge with OVA by retro-orbital bleeding. Plasma were tested for IgG1 or IgG2a Abs using OVA-coated 96-well plates and biotinylated goat anti-mouse IgG1 or IgG2a antiserum. The reactions were developed with streptavidin-HRP complex (Sigma-Aldrich), *o*-phenylenediamine, and H₂O₂, and the plates were read at 490 nm on an automated ELISA reader (Spectramax; Molecular Devices). The results were expressed as the mean $\pm$ SEM absorbance at 1/512 plasma dilution. An IgE-specific ELISA was used to quantitate total IgE Ab levels in plasma using matched Ab pairs (553413 (BD Pharmingen) and 1130-08 (Southern Biotechnology

Associates)), according to the manufacturer's instructions. Samples were quantified by comparison with a standard curve of IgE (0313ID; BD Pharmingen).

Passive cutaneous anaphylaxis (PCA)

The anaphylactic activity of IgG1 was evaluated by PCA reactions in mice as described by Ovary (19). Mice were previously shaved and received injections intradermally (50 μ l) with three serial dilutions of plasma (inactivated for 1 h at 56°C) in each side of the dorsal skin. After 2 h, they were challenged i.v. with 250 μ g of OVA + 0.25% of Evans blue solution. For IgE titration, PCA reactions were performed in rats using noninactivated plasma, according to Mota and Wong (20). All of the tests were made in triplicate, and PCA titers were expressed as the reciprocal of the highest dilution that gave a lesion of >5 mm in diameter. The detection threshold of the technique was established at 1/5 dilution.

Morphometric analysis of the lungs

H&E and periodic acid-Schiff (PAS)-stained tissue sections were examined with light microscopy, and morphometric measurements were performed using a Zeiss Axiophot microscope (Zeiss) at a magnification of $\times$ 100. For each group of six mice, four stained lung sections from each mouse were analyzed. The degree of peribronchial and perivascular inflammation was evaluated in all airway cuts using a subjective scale of 0, 1, 2, 3, and 4 corresponding to none, mild, moderate, marked, or severe inflammation, respectively. Four fields per lung tissue were categorized according to the abundance of PAS⁺ goblet cells and assigned numerical scores (0, <5% goblet cells; 1, 5–25%; 2, 25–50%; 3, 50–75%; and 4, >75%). The sum of the airway scores from each lung was divided by the number of airways examined for the histological goblet cell score (expressed as arbitrary units) (21). Statistical significance was calculated using the Wilcoxon test.

Statistical analysis

All values were expressed as mean $\pm$ SEM. Parametric data were evaluated using ANOVA, followed by the Tukey test for multiple comparisons. Nonparametric data were assessed using the Mann-Whitney *U* test. Differences were considered statistically significant at $p < 0.05$. The SPSS statistical package (release 8.0, standard version, 1997; SPSS) was used.

Results

Treatment of mothers with IFN- γ during pregnancy strongly reduces the production of Th2 cytokines by offspring cells

The basic protocol for these studies was applied to mice derived from control or from IFN- γ -treated mothers: the offspring from IFN- γ -treated mothers were breastfed by normal mothers (IFN/nor), and those from normal mothers were breastfed by IFN- γ -treated (Nor/IFN) or normal mothers (Nor/nor).

Spleen cells were prepared immediately after weaning (day 30 of life), and the levels of cytokines after in vitro stimulation with Con A were assessed by ELISA (Table I). Cells from Nor/nor mice produced IL-2, IL-4, IL-5, and IFN- γ . This cytokine profile in response to mitogen was altered in IFN/nor mice, who produced less IL-4 and IL-5. In contrast, they produced more IFN- γ . The same results were obtained in spleen cells from Nor/IFN mice, except for the levels of IL-5 that did not differ significantly from those found in Nor/nor mice. In the three groups, IL-2 production in response to Con A was similar, the amounts of IL-10 remaining below the detection threshold.

IFN- γ treatment during pregnancy protects offspring from allergic sensitization

To assess the effects of the administration of IFN- γ during pregnancy on the development of Th2 responses in the offspring, mice were immunized and challenged later on for three consecutive days with aerosolized OVA.

BALF of PBS-challenged mice of the three groups contained only a few macrophages, and no eosinophils were noted in the lung tissues (data not shown). The BALF from Nor/nor OVA-challenged mice contained a large number of eosinophils (Fig. 1A). In agreement, a large number of eosinophils accumulated predominantly in the perivascular and peribronchial regions of lung tissues

Table I. Synthesis of cytokines by spleen cells from Nor/nor, IFN/nor, or Nor/IFN mice immediately after weaning^a

	IL-2 (ng/ml)	IFN- γ (ng/ml)	IL-4 (pg/ml)	IL-5 (pg/ml)	IL-10 (ng/ml)
Nor/nor	10.1 $\pm$ 0.0	3.5 $\pm$ 1.6	71.0 $\pm$ 0.0	147.0 $\pm$ 2.7	<0.156
IFN/nor	12.0 $\pm$ 0.6	14.0 $\pm$ 0.1*	<39*	53.0 $\pm$ 1.9*	<0.156
Nor/IFN	12.3 $\pm$ 1.2	16.2 $\pm$ 1.7*	<39*	100.0 $\pm$ 4.6	<0.156

^a Spleen cells were cultured in 24-well tissue culture plates at a final concentration of 10 or 6 $\times$ 10⁶ cells/ml. The cells were stimulated with Con A (5 μ g/ml) for 24 or 72 h, respectively. Detection limits were 0.43 ng/ml for IL-2, 0.39 ng/ml for IFN- γ , 39 pg/mL for IL-4 or IL-5, and 0.156 ng/ml for IL-10. The results were expressed as mean $\pm$ SD of duplicate cultures (n = 6).

*, p < 0.05 compared with Nor/nor group.

(Fig. 1B). Compared with Nor/nor group, IFN/nor or Nor/IFN mice immunized and challenged with OVA showed reduced eosinophil numbers in the BALF (reduction of 97 and 73%, respectively) (Fig. 1A). Nor/IFN mice showed a significant lung inflammation with peribronchiolar and perivascular cell infiltrate, consisting of eosinophils, lymphocytes, and some neutrophils, like Nor/nor mice. In contrast, lung sections from IFN/nor mice showed almost normal histology, with marginal perivascular and peribronchiolar lymphocytic infiltrate (Fig. 1B). Goblet cell hyperplasia was observed in Nor/nor airways after OVA challenge, but it was absent in IFN/nor. Nor/IFN mice had significantly less PAS⁺ cells in airways than Nor/nor mice (Fig. 2).

We further analyzed the cytokine production by spleen cells from immunized mice 48 h after challenge with aerosolized OVA (Table II). After in vitro OVA stimulation, the amounts of IFN- γ and of Th2 cytokines (IL-4 and IL-5) in cell culture supernatants from IFN/nor cells were significantly reduced as compared with those from Nor/nor cells. Although the same was observed in Nor/IFN cells, the levels of IL-5 were 2.5-fold above those obtained in all of the supernatants from IFN/nor cells. These results show that the reduced capacity of naive IFN/nor mice to produce Th2 cytokine remained after OVA sensitization and that this effect may be related to the inhibition of eosinophil recruitment to the lungs.

The plasma content of anti-OVA Abs was also determined. Fig. 3A shows that anaphylactic IgG1 Abs were significantly reduced in plasma of IFN/nor mice, but not in Nor/IFN mice, compared with plasma of Nor/nor mice. Anti-OVA IgG1 Abs determined by ELISA were not significantly different in the three groups of mice (data not shown). In contrast, the IgG2a response was increased in IFN/nor and in Nor/IFN groups (Fig. 3B). In Fig. 3C, the total amount of IgE is shown in immune plasma from the three groups after inhaled allergen challenge. IFN/nor plasma contained less IgE than Nor/nor, and plasma from Nor/IFN mice contained even less. Similar results were obtained before challenge (data not shown).

Inhibition of airway eosinophilia does not correlate to plasma levels of IFN- γ

We tested whether the protection of the offspring against respiratory allergy following the administration of IFN- γ to their mothers resulted from the presence of active IFN- γ circulating in their blood. Fig. 4A shows that after breastfeeding (day 30 of life), mice from the Nor/IFN group had the highest levels of IFN- γ in plasma, probably as a result of its presence in the milk of the mothers at this time point. Before immunization (at day 45 of life), only IFN/nor mice showed reduced levels of plasma IFN- γ . After challenge, the plasma levels of IFN- γ declined back to basal levels in the three groups. In addition, immediately after delivery, IFN- γ -treated mothers and normal mothers had similar IFN- γ blood levels (7.42 $\pm$ 0.83 vs 5.8 $\pm$ 0.48 ng/ml, respectively) (data not shown). When all three groups of mice were treated with IFN- γ -

blocking Abs and subsequently submitted to OVA challenge, the treatment did not increase eosinophil recruitment into bronchoalveolar lavage (BAL) of IFN/nor or Nor/IFN mice compared with mice treated with isotype control Abs (Fig. 4B). Moreover, in the Nor/nor group, less eosinophils were found in BAL from mice treated with anti-IFN- γ Abs.

Discussion

The aim of this study was to investigate the impact of the IFN- γ administration during pregnancy on the development of allergy-related Th2 responses in the offspring. To this end, we used mice derived from IFN- γ -treated mothers in a model of sensitization and airway challenge with the allergen. To distinguish between the prenatal and the postnatal events, the offspring from IFN- γ -treated

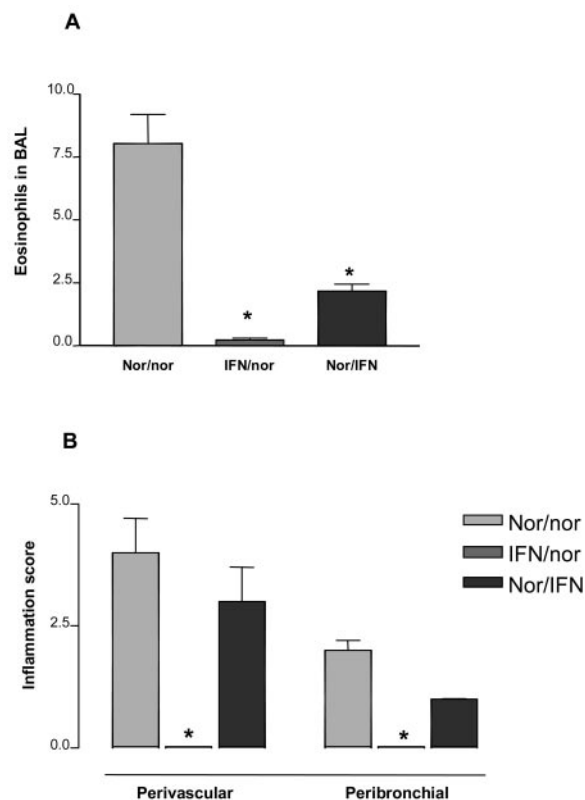


FIGURE 1. Effect of treatment of mothers with IFN- γ on lung eosinophilia induced by OVA in offspring. Mice immunized with OVA were challenged with aerosolized OVA for three consecutive days. Forty-eight hours after the last aerosol challenge, BALF was collected for eosinophil counts ($\times 10^5$; A), and stained lung tissues were scored for inflammation (0, 1, 2, 3, and 4 corresponding to none, mild, moderate, marked, or severe inflammation, respectively) (B). The results represent the mean $\pm$ SEM of six animals per group. *, p < 0.001 compared with Nor/nor group.

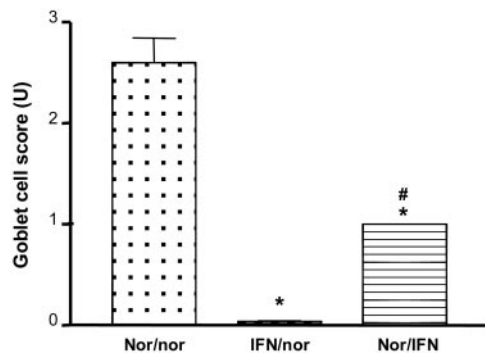


FIGURE 2. PAS-stained lung sections were examined at magnification $\times 100$. Twenty consecutive airways from PBS- and OVA-challenged mice were categorized according to the abundance of PAS⁺ goblet cells and assigned numerical scores (0, $<5\%$ goblet cells; 1, 5–25%; 2, 25–50%; 3, 50–75%; and 4, $>75\%$). The sum of the airway scores from each lung was divided by the number of airways examined for the histological goblet cell score (expressed as arbitrary units). *, $p < 0.001$ compared with Nor/nor group. #, $p < 0.05$ compared with IFN/nor group.

mothers were breastfed by normal mothers (IFN/nor), and offspring from normal mothers were breastfed either by IFN- γ -treated (Nor/IFN) or normal mothers (Nor/nor). This latter group was used as the positive control of allergic inflammation.

In the dose used here, IFN- γ has been reported to be abortifacient in matings of some mouse strains, even after a single treatment (22). However, the treatment of pregnant females on gestation day 6.5 with an i.p. injection of 100 IU of mouse recombinant IFN- γ did not elevate the resorption rates over PBS-treated pregnant female controls (data not shown). Thus, our model suggests that high doses of IFN- γ are compatible with healthy pregnancy. This may underscore the importance of other factors (genetic susceptibility and cytokines as TNF- α) in the induction of IFN- γ -mediated abortion.

In this study, we demonstrated that as a result of treatment of pregnant females with IFN- γ , the eosinophilic lung inflammation and the goblet cell hyperplasia that follow allergen challenge in the immunized offspring were profoundly suppressed. A striking decrease in eosinophil influx into airway lumen and lung sections in IFN/nor mice was seen with marginal perivascular and peribronchiolar lymphocytic infiltrate. The secretion of mucus was also absent in these mice. The production of Th2 cytokines in response to mitogen seen in spleen cells from naive Nor/nor mice was altered in IFN/nor mice, suggesting that the administration of IFN- γ to mothers during pregnancy reduces the capacity of splenic cells from infant mice to produce Th2 cytokines.

Moreover, the suppression of airway eosinophilia in sensitized IFN/nor mice after challenge was accompanied by the synthesis of reduced levels of IL-4 and IL-5 by splenic cells from these mice after OVA restimulation. The role of IL-4 in allergic sensitization

and eosinophil accumulation has been demonstrated in IL-4-deficient mice (23, 24). In addition, it is known that IL-4 induces the expression of adhesion molecules for eosinophils in the endothelium (25) and eotaxin synthesis by lung epithelial cells (26). IL-5 was shown to be the primary determinant of eosinophil differentiation, activation, and survival (27). Furthermore, IL-5 and eotaxin cooperate in the orchestration of eosinophil accumulation in tissues (28).

In addition to the marked reduction of eosinophils and Th2 cytokines in IFN/nor mice after OVA challenge, low titers of IgE and anaphylactic IgG1 Abs were observed in the plasma of these mice, although no difference in the IgG1 measured by ELISA was detected. IgE and anaphylactic IgG1 production are stimulated by IL-4 secreted by Th2 cells (29). However, IgG1 production via an IL-4-independent pathway has also been described (30, 31). Furthermore, the ability to make OVA-specific IgG1 and IgG2a responses (determined by ELISA) excludes the possibility of induction of full tolerance in baby mice.

IFN- γ suppresses proliferation of Th2 cells (29), inhibits IgE production (32), and promotes Th1 differentiation by inducing IL-12 receptor β_2 -chain expression on T cells (33). In addition, when administered before inhaled Ag challenge, IFN- γ reduces the number of CD4⁺ T cells in the respiratory tract and, consequently, the eosinophil recruitment into the mouse airways (34–36). Therefore, the suppression of all of the allergic parameters evaluated and, consequently, the protection of offspring against respiratory allergy following the administration of IFN- γ to their mothers might result from the presence of active IFN- γ circulating in their blood. This nevertheless seems unlikely, because neutralizing anti-IFN- γ Abs administered to offspring before challenge did not increase the eosinophil recruitment into the airways of IFN/nor or Nor/IFN mice, and they persistently showed less eosinophils in BAL compared with Nor/nor mice treated with the same anti-IFN- γ Abs.

Altogether, our data extend those previously published by Hamada et al. (37), showing that the offspring of asthmatic mother mice become more allergic, and this effect is mediated by IL-4. Treatment of baby mice with anti-IL-4 Abs did not abolish this enhancing effect, and anti-IFN- γ Abs did not change the suppressive effect in our model.

Furthermore, a comparison between the data obtained in IFN/nor and in Nor/IFN mice shows that the prenatal events induced by treating mother mice with IFN- γ are more effective in preventing the development of allergy in offspring than the postnatal events induced by breast milk throughout nursing. Thus, the eosinophilic inflammation in lung tissue of Nor/IFN mice did not differ significantly from Nor/nor mice, and the number of eosinophils in BALF was higher when compared with IFN/nor mice. These results correlated well with the greater amounts of IL-5 produced by Nor/IFN cells than by IFN/nor cells. Furthermore, production of mucus was detected in airways of Nor/IFN group differently from IFN/nor

Table II. Synthesis of cytokines by spleen cells from immunized Nor/nor, IFN/nor, or Nor/IFN mice after aerosol OVA challenge^a

	IL-2 (ng/ml)	IFN- γ (ng/ml)	IL-4 (pg/ml)	IL-5 (pg/ml)	IL-10 (ng/ml)
Nor/nor	<0.43	4.2 ± 0.2	56.3 ± 1.7	477.0 ± 15.8	2.0 ± 0.1
IFN/nor	<0.43	$<0.39^*$	$<39^*$	$85.3 \pm 4.6^*$	1.8 ± 0.1
Nor/IFN	<0.43	$1.0 \pm 0.2^*$	$<39^*$	$218.3 \pm 11.7^*$	1.2 ± 0.0

^a Spleen cells were cultured in 24-well tissue culture plates at a final concentration of 10 or 6×10^6 cells/ml. The cells were stimulated with OVA ($500 \mu\text{g/ml}$) for 24 or 72 h, respectively. Detection limits of cytokines are indicated in Table I. The results were expressed as mean $\pm$ SD of duplicate cultures ($n = 6$).

*, $p < 0.05$ compared with Nor/nor group.

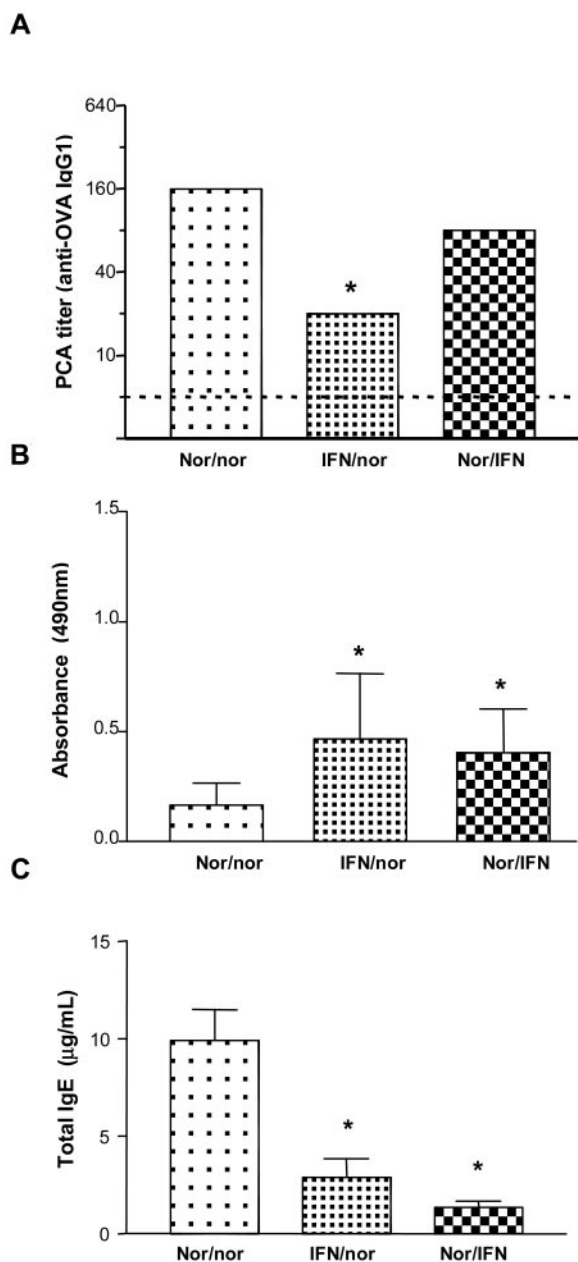


FIGURE 3. Effect of treatment of mothers with IFN- γ on OVA-specific anaphylactic IgG1 and IgG2a levels and IgE amounts in plasma from the offspring. Immunized mice were challenged with aerosolized OVA for three consecutive days. Forty-eight hours after the last aerosol, mice were bled for IgG1 determination by PCA (A). The PCA titer represents the highest dilution of pooled plasma that gave a positive reaction (diameter, >5 mm). The dashed line represents the detection threshold. Anti-OVA IgG2a was measured by ELISA (B). Total IgE was measured by sandwich ELISA (C). Each bar represents the mean $\pm$ SEM of six animals per group at 1/512 dilution of plasma. *, $p < 0.001$ compared with Nor/nor group.

group in which it was absent. Anaphylactic IgG1 Ab production in Nor/IFN mice was also similar to Nor/nor mice. In contrast, IgE Ab levels were mostly inhibited in Nor/IFN mice whose plasma levels of IFN- γ were significantly higher than the other groups of mice at weaning. This cytokine is known to antagonize the IgE-switching action of IL-4 on B cells (38).

The maternal-fetal relationship is not a simple induction of maternal tolerance to a semiallogeneic tissue, but consists of a series of intricate cytokine interactions governing selective immune reg-

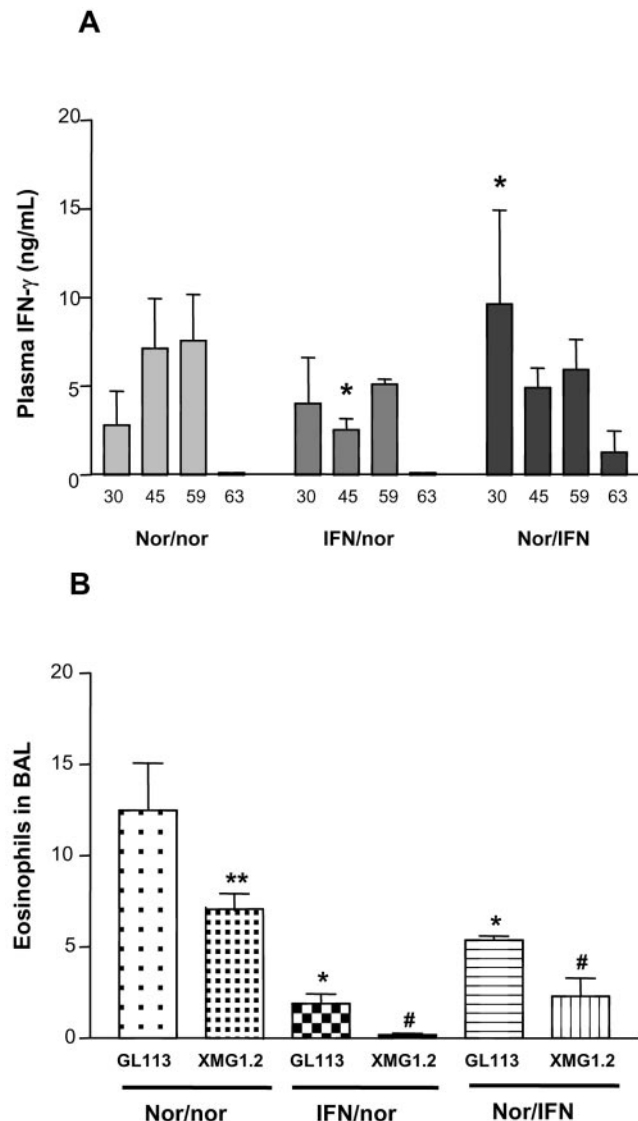


FIGURE 4. Evaluation of IFN- γ levels in the offspring. Plasma from offspring at different times (day 30, after weaning; day 45, before immunization; day 59, before the first aerosol OVA challenge; and day 63, 48 h after challenge) were collected for IFN- γ measurement by ELISA (A). Effect of IFN- γ neutralization on eosinophilia. OVA-sensitized offspring were treated with rat anti-mouse IFN- γ mAb (XMG 1.2) or isotype control mAb (GL113) before challenge. Eosinophil counts in BAL ($\times 10^5$) were obtained 48 h after the last aerosol challenge (B). Bars represent the mean $\pm$ SEM of six animals per group. *, $p < 0.05$ compared with Nor/nor GL113 group; #, $p < 0.05$ compared with Nor/nor XMG 1.2 group; **, $p < 0.05$ compared with the respective group of mice treated with GL113.

ulation and the control of the adhesion and vascularization processes during this dialogue (10, 39). The special immunosuppressive environment in the maternal-fetal interface is regulated by immunoinhibitory macrophages, immature myeloid dendritic cells, and regulatory T cells present among murine and human decidual, placental, and uterine cells (40–44). Uncommitted immature myeloid dendritic cells exposed to IFN- γ in peripheral tissues develop the capacity to produce IL-12p70 upon subsequent contact with naive T cells in lymph nodes (45). Macrophages activated by IFN- γ also produce IL-12 and induce strong Th1 responses (46). Therefore, IFN- γ administration during early pregnancy can modify the maternal immune response by acting on immature myeloid

dendritic cells, macrophages, uNK cells, and nonimmune cells present in placenta.

In conclusion, altered APC derived from treated mothers might cross the placenta or IFN- γ given to the mother reaches the fetus and changes the profile of its own APC, down-regulating the Th2 cells in benefit of antiallergic Th cells in adult life. Alternatively, the fetus might contain an unknown IFN- γ -target, which, when stimulated, confers a long-lasting protection against allergenic provocations in the adult. Mature T cells are probably not this target because they are undetectable in the fetus until around day 17. Although additional experiments focusing the phenotype and function of placental and fetal APCs are required to identify the precise mechanism of maternal transfer of the IFN- γ -mediated suppression of allergy to offspring, the model reported in this study strongly suggests that the allergic status of newborns can be influenced by the presence of IFN- γ inducers in the mother during pregnancy, independently of breastfeeding.

Disclosures

The authors have no financial conflict of interest.

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