

Transplacental priming of the human immune system with environmental allergens can occur early in gestation

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Background: Allergen-specific T cells play an important role in the allergic immune response to various environmental allergens. In vitro studies have shown that T-cell responses to these allergens do occur prenatally. Some allergens (milk proteins) appear to lead more often to fetal T-cell priming than others (house dust mite allergen, ovalbumin, and birch and grass pollen allergens).

Objective: We sought to determine the window of opportunity for prenatal T-cell priming with inhalant and nutritive allergens.

Methods: The T-cell reactivity of cord blood cells derived through cordocentesis from unborn (n = 62) and term babies (n = 114) in response to inhalant allergens (birch pollen major allergen, recombinant Bet v 1, and timothy grass major allergen, recombinant Phl p 1) was investigated.

Results: The results demonstrate that allergen-specific T-cell reactivity is as common in preterm as in term infants (Bet v 1, 8% and 5%, respectively; Phl p 1, 20% and 25%, respectively).

Conclusions: Our data support the hypothesis that differential handling of the allergenic proteins by the feto-placental barrier and possibly by antigen-presenting cells may directly modulate the ensuing T-cell immune response. (*J Allergy Clin Immunol* 2000;106:530-6.)

Key words: Cord blood cells, allergen, T-cell proliferation, diaplacental transfer, sensitization

Clonal expansion of antigen-specific T lymphocytes represents the cellular basis of memory.¹ In atopic subjects the selective expression and expansion of T_H2-skewed immunity to particular soluble protein antigens (allergens) present in the normal environment is regarded as the primary cause of allergic inflammation.² The precise window of opportunity for sensitization is not clear, but it is generally believed to occur postnatally and to be related to the first exposure with environmental allergens.³ Early postnatal exposure to high levels of allergen,

Abbreviations used

CBMC: Cord blood mononuclear cell

SI: Stimulation index

exemplified by birth during the pollen season, increases the risk for subsequent expression of allergic reactivity to the particular allergen.⁴

The occurrence of prenatal T-cell priming and its possible effect on later allergic status has been the subject of many rather contradictory articles.⁵⁻¹⁵ The immunologic mechanisms involved in this process are not clear. They may involve allergen or peptide transfer through the placenta. Alternatively, stimulation of fetal allergen-specific T cells by diaplacentally transferred anti-idiotypic antibodies is conceivable.¹⁶

The opinion discussed so far lead to the conclusion that T-cell priming with antigens appears to be a physiologic mechanism that trains the immune system.¹²⁻¹⁴ However, T_H1- and T_H2-related cytokine analysis reveals that decreased IFN- γ expression and enhanced proliferation in response to mitogen or allergen in atopy-prone individuals may be associated with subsequent allergy development.^{17,18} Another hypothesis discussed in a recent study favored a universal skewing of initial fetal-postnatal T-cell responses toward the T_H2 cytokine profile.^{12,19}

By studying umbilical cord mononuclear cells of term newborns, we have demonstrated that prenatal priming with allergens occurs and is more pronounced when the pregnant mother comes in contact with allergen (through inhalation or ingestion) within the first 6 months of gestation.¹⁴ Jones et al⁵ proposed the cut-off level at greater than 22 weeks' gestation for possible T-cell priming with inhalant and nutritive allergens.

The aim of the present study was to evaluate at what gestational age prenatal priming is predominantly initiated. Umbilical cord blood cells derived from cordocentesis were obtained for this purpose and stimulated with seasonal allergens, and the results were compared with those of stimulation experiments performed with cord blood cells derived from term newborns.

METHODS

Subjects

Two groups of cord blood samples were collected: human umbilical cord blood (n = 64) was drawn on heparin and collected by means of cordocentesis from unborn babies (n = 62) and term new-

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borns ($n = 114$). The number of cord blood samples exceeded the number of babies by two because two consecutive cord blood samples were obtained from two unborn babies. Detailed results of the term newborns have been published previously.¹⁴ For the present investigation, the data are used for direct comparison with data obtained from unborn babies. Cordocentesis was performed for the following reasons: vitium cordis, fetal tumor mass, diaphragmatic hernia, Rh incompatibility, oligohydramnios, rubeola-embryopathy, hydrocephalus, and gastroschisis. The samples were taken randomly and independently of the clinical history of the family concerning allergy. Clinical history concerning type I allergy was established by interviewing the parents. Furthermore, parents were questioned as to whether they had been in the area of Vienna during the complete ongoing pregnancy. This study had the approval of the institutional review board. For blood analysis, informed consent was obtained from the patient's guardian.

Antigens

The recombinant major birch pollen allergen Bet v 1 (*Betula verrucosa*) and the recombinant timothy grass allergen Phl p 1 (*Phleum pratense*) were purchased from Biomay (Linz, Austria). The recombinant nonfusion proteins Bet v 1 and Phl p 1 were expressed by using the pKK 223-3 expression vector, the promoter of which is regulated by the lac repressor and can be induced by adding isopropyl-beta-D-thiogalactoside to the medium.²⁰

Grass pollen exposure periods and birch-hazel-alder pollen exposure periods in 1995 and 1996 were registered from the Austrian Pollen Flight Registry.

Cell preparation and T-cell proliferation

Cord blood mononuclear cells (CBMCs) were separated from heparinized blood by using density gradient centrifugation and cultured at a concentration of 10^5 cells per 200 μ L in cell culture medium supplemented with 1% human serum, 2 mmol/L glutamine, and 50 mg/mL gentamicin. The cells were incubated at 37°C in a humidified atmosphere with 5% CO₂ for 7 days in the presence or absence of recombinant birch pollen major allergen Bet v 1 (0.5 and 2.0 μ g/200 μ L) and recombinant timothy grass allergen Phl p 1 (0.5 and 2.0 μ g/200 μ L).¹⁴ IL-2 at 20 IU/mL was used as a positive control, and medium alone was used as a negative control. After 6 days of culture, tritiated thymidine (Amersham; 0.5 μ Ci per well) was added, and the incorporated radioactivity (in counts per minute) was measured by using scintillation counting after 16 hours. Proliferation was expressed as the stimulation index (SI) of a geometric mean of triplicates.²¹ According to the literature, an SI of 2 was considered positive. SI was determined by using the following equation:

$$SI = \frac{\text{cpm in cultures stimulated with allergen}}{\text{cpm in unstimulated cultures.}}$$

Cord blood volumes between 0.4 and 1.0 mL were collected. A total number of 1.1×10^6 and 7.5×10^6 CBMCs were assigned for proliferation assays.

Statistical analysis

Pairwise comparisons between groups were carried out by using the Kruskal-Wallis nonparametric *U* test.

RESULTS

Cord blood cells of different gestational ages display high IL-2 reactivity

CBMC responses to IL-2 (20 IU/mL) are shown in Table I. All probes showed positive proliferative responses to IL-

2 in an SI range from 1.7 to 180 (Table I). IL-2 reactivity in cord blood cells was independent of the gestational age of the conceptus at the time of cordocentesis. We found no correlation between a fetal or maternal infectious disease and high IL-2 reactivity in cord blood. Data were used for correlative analysis with IL-2 reactivity but were excluded for the allergen-specific reactivity measurements.

Lymphoproliferative responses of cord blood cells to environmental allergens

CBMC responses to recombinant allergens (rBet v 1 and rPhl p 1) are shown in Table II. Of the 62 individuals, 64 cord blood measurements were performed; in two patients consecutive cord blood samples at different gestational ages could be obtained. Six (9%) of 62 cord blood samples with Bet v 1 and 15 (23%) of 62 cord blood samples with Phl p 1 displayed T-cell proliferation considered as positive, with tritiated thymidine incorporation levels in stimulated cultures greater than 2-fold of background levels and greater than 1000 cpm above background levels.

To analyze whether gestational age may influence positive proliferative responses, we divided the group of 62 samples into subgroups according to gestational age as follows: less than 19 weeks ($n = 1$); 20 to 22 weeks ($n = 10$); 23 to 25 weeks ($n = 7$); 26 to 28 weeks ($n = 10$); 29 to 31 weeks ($n = 17$); 32 to 34 weeks ($n = 12$); 35 to 37 weeks ($n = 4$); and greater than 38 weeks ($n = 1$). As shown in Table II, there was no predominant association between gestational age and response to either of the two tested allergens (no significant difference). This indicates that maternal anti-idiotypic antibodies of IgG antibody isotype that may mediate antigen-specific fetal T-cell responses are unlikely to be responsible for the allergen-specific reactivity of cord blood cells.

T-cell priming with inhalant allergens occurs early in gestation

To elucidate the gestational age favorable for T-cell priming with inhalant allergens, we investigated cord blood samples (from term and preterm babies) collected over 1 year and stimulated CBMCs with rBet v 1 and rPhl p 1 allergen. The results are shown in Table III. Taking all samples from different gestational ages together and comparing their allergen-specific proliferative responses with those achieved in the 114 samples from term babies, we found comparable amounts of positive responses. Eight percent versus 5% reacted with rBet v 1 (not significant), and 20% versus 25% reacted with rPhl p 1 (not significant) in the groups of preterm and term babies, respectively. These findings lead us to assume that the window of opportunity for prenatal priming must be early in gestation, possibly even before a gestational age of 20 weeks.

Influence of maternal allergen exposure on fetal T-cell priming with the same allergen

To study the direct effect of allergen exposure and proliferative response to the same allergen, we mapped the proliferative responses according to gestational age

TABLE I. Patient data concerning gestational age at blood taking, indication for cordocentesis, and proliferative responses of cord blood cells to inhalant allergens at two concentrations (mean of triplicate cultures)

Patient No.	Gestational age (wk)	Indication for cell cordocentesis	Cell numbers (in millions)	Stimulation index in response to:					IL-2	Month of conception
				Birch pollen		Grass pollen				
				A	B	A	B			
1	35	Tumor	4	0.5	1.5	1.1	1.3	50	May 1995	
2	33	Diaphragmatic hernia	2.1	0.8	0.6	1	1.9	6.2	June 1995	
3	27	Rh incompatibility	4	0.7	0.7	0.6	1.5	11.9	July 1995	
4	26	Fetal hydrops	3.6	0.8	0.7	0.3	1.6	19.8	July 1995	
5	32	Intrauterine growth restriction	6.4	1.2	0.8	1.4	0.9	69.9	July 1995	
6	35	Congestive heart disease	3	1.1	9.6	1.2	1.3	180.8	July 1995	
7a	23	Abdominal mass	2.1	1	0.8	1.1	0.9	7.2	August 1995	
7b	40	Abdominal mass	4	0.8	1.3	0.9	1.3	10.9	August 1995	
8	28	Rh incompatibility	2.5	2	0	0.9	0.8	8.8	August 1995	
9	29	Pulmonary cyst	3.8	0.6	0.4	0.5	1.4	60.5	August 1995	
10	31	Congestive heart disease	3.1	0.5	0.5	0.5	0.5	21.6	August 1995	
11	33	Congestive heart disease	3.8	2.4	0.8	1.4	4.1	50	August 1995	
12	20	Rh incompatibility	2.9	1.8	0.4	1	1.3	8.9	September 1995	
13	22	Congestive heart disease	3.2	2.6	0.7	2.7	3.7	22.9	September 1995	
14	21	Rubella	6	1.2	0.3	0.8	0.8	53.1	September 1995	
15	30	Intrauterine growth restriction	2.1	0.4	1.1	0.9	2	9.9	September 1995	
16	31	Intrauterine growth restriction	3.2	1.4	1.4	1.2	2.7	20.6	September 1995	
17	33	Intrauterine growth restriction	3.8	0.9	0.8	0.9	0.9	66.5	September 1995	
18	34	Renal cyst	3.5	0.6	0.5	1.1	4.4	65.6	September 1995	
19	36	Thrombocytopenia	1.7	1.2	1.1	0.8	0	2	September 1995	
20	31	Diaphragmatic hernia	3.5	0.8	0.7	0.6	1.5	33.6	October 1995	
21	25	Polyhydramnios	3.5	1.2	3.9	3.6	4.6	44	October 1995	
22	32	Hydrocephalus	7.5	0.4	0.6	1	0.9	9.5	October 1995	
23	22	Single umbilical artery	4	0.6	0.6	0.5	2.5	63	November 1995	
24	25	Polyhydramnios	6.2	1.2	1.4	1.1	1.8	108.7	November 1995	
25	25	Congestive heart disease	2.6	0.6	1	0.7	0.7	15.7	November 1995	
26	22	Congestive heart disease	1.7	0.4	1.4	0.9	0.7	7.6	November 1995	
27	26	Hydrocephalus	3.2	0.9	0.7	2.3	2.9	28.4	November 1995	
28	30	Congestive heart disease	2.5	0.6	1.1	0.5	1.6	10	November 1995	
29a	27	Rh incompatibility	2.2	0.9	0.9	1.1	1.1	1.7	November 1995	
29b	29	Rh incompatibility	1.1	1.1	0.8	1.1	1.1	2.7	November 1995	
30	27	Congestive heart disease	1.9	0.9	0.8	1	1.1	4.7	November 1995	
31	34	Polyhydramnios	1.7	1.6	2.8	5.3	4.6	46.5	November 1995	
32	34	Hydrocephalus	1.3	0.7	0.2	0.5	0.4	10.1	November 1995	
33	30	Polyhydramnios	1.8	0.6	0.8	1.2	1.2	6.9	November 1995	
34	29	Oligohydramnios	1.9	0.6	0.6	0.9	1.5	6.8	November 1995	
35	32	Intrauterine growth restriction	2	0.5	1.1	1.9	0.9	49.1	November 1995	
36	37	Diaphragmatic hernia	3.4	0.8	1.3	1	1.8	14.7	November 1995	
37	35	Intrauterine growth restriction	2.4	0.8	0.7	1.1	1.3	26.2	November 1995	
38	30	Dysmorphism	7	1.2	1.1	0.7	1	124.9	November 1995	
39	30	Hydrocephalus	3.5	0.5	0.6	0.5	1.6	11.4	December 1995	
40	29	Intrauterine growth restriction	3.7	0.8	0.6	1	0.6	87.8	December 1995	
41	19	Rubella	3.2	1.4	0.6	1	1.7	58.4	January 1996	
42	21	Brain cyst	2.7	1	0.9	3.3	1	112.6	January 1996	
43	22	Renal cyst	3.2	0.8	0.9	1.1	1.3	12.8	January 1996	
44	22	Hydronephrosis	2.8	1.3	1.6	2.7	1.5	50.2	January 1996	
45	28	Hydrocephalus	2.9	0.6	0.4	1.3	1.1	5.7	January 1996	
46	26	Ascites	4.2	0.2	0.6	1.2	0.8	8.5	January 1996	
47	29	Dysmorphism	3.5	0.9	0.7	0.8	0.8	86.5	January 1996	
48	28	Oligohydramnios	2.3	1	1.1	0.7	1.1	5.6	January 1996	
49	29	Intrauterine growth restriction	2.1	1	1.1	0.8	1.1	67.3	January 1996	
50	29	Cleft jaw	3.6	0.6	0.2	0.3	0.4	19.1	January 1996	
51	34	Corpus callosum agenesis	2.4	1.5	3	1.4	1.3	83	February 1996	
52	24	Rh incompatibility	1.5	1.2	0.7	0.7	3.9	34.5	February 1996	
53	22	Rh incompatibility	1.8	1.4	1.3	1.6	2.5	66.4	March 1996	
54	28	Congestive heart disease	2.5	1.1	1	1	1.3	31.6	March 1996	
55	34	Gastroschisis	2.8	0.9	1.2	1	1	3	March 1996	
56	32	Hydrothorax	1.7	0.8	0.8	0.8	0.9	25.2	April 1996	
57	31	Duodenal stenoses	2.5	0.8	0.9	0.8	1.2	27.1	April 1996	
58	25	Chondrodystrophy	1.9	1	1.3	2.1	1.9	55.6	June 1996	
60	31	Holoprosencephaly	1.7	1	0.7	2.2	1	15.2	August 1996	
61	20	Rh incompatibility	2.9	0.8	1.2	0.7	1.4	16.7	October 1996	
62	38	Rh incompatibility	3.6	0.7	0.5	1	1.2	18.7	October 1996	

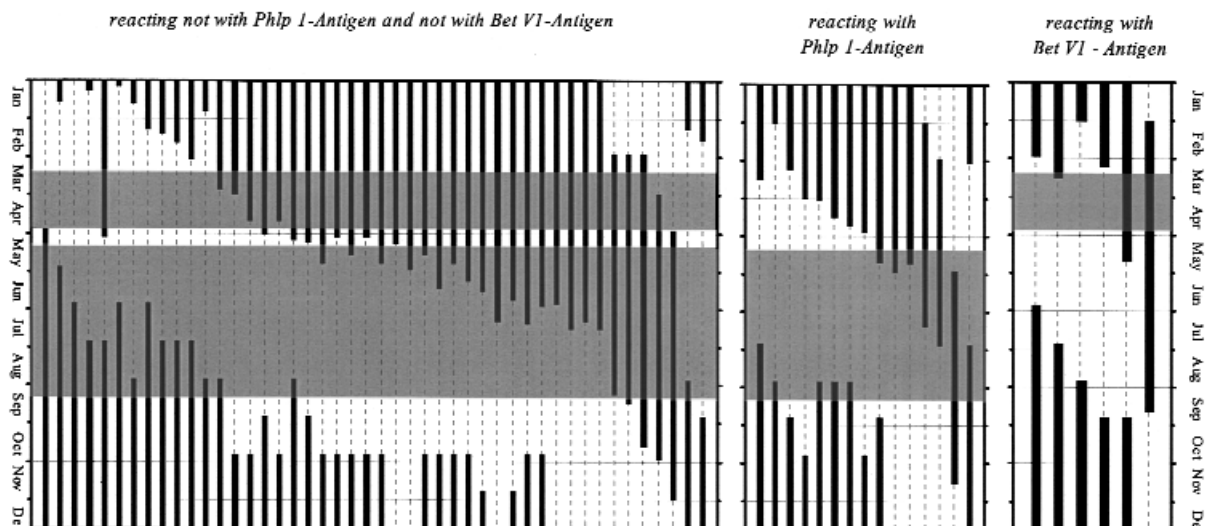


FIG 1. T-cell priming to inhalant allergen is established early in gestation and does not directly correlate with maternal allergen exposure. For all 62 cord blood samples, the total life span is plotted as a *bar*. The maternal allergen exposure is expressed as the *hatched field*. If a bar crosses a hatched field, then the unborn baby was potentially exposed to the respective allergen during the ongoing gestation. **A**, Six cord blood samples responding to Bet v 1; **B**, 15 Phl p 1 responders; **C**, sum of 45 nonresponders.

TABLE II. Proliferative response of cord blood cells (cordocentesis) to inhalant allergens according to gestational age

		Responder					
Gestation		Bet v 1		Phl p 1		Nonresponder	
No. per group	Age (wk)	n	SI	n	SI	n	Median (range)
1	<19	None		None		1	1.0 (0.6-1.4)
10	20-22	1	2.6	4	2.7, 3.7, 2.5, 2.5	5	0.9 (0.3-1.8)
7	23-25	1	3.9	4	3.9, 4.6, 3.9, 2.1	4	1.0 (0.6-1.4)
10	26-28	None		1	2.9	10	0.8 (0.0-1.9)
17	29-31	None		2	2.7, 2.2	15	0.7 (0.2-1.2)
12	32-34	3	2.4, 2.8, 3.0	4	4.1, 4.4, 4.6, 5.3	5	0.8 (0.2-1.2)
4	35-37	1	9.6	None		4	1.0 (0.5-1.5)
1	>38	None		None		1	1.1 (0.8-1.3)
66		6	4.05 (2.4-9.6)	15	3.6 (2.0-5.3)	45	0.8 (0.0-1.9)
100%		9%		23%		68%	

Sixty-two cord blood samples were analyzed. In 4 samples double positivity was observed, explaining how the sum of patients with Bet v 1-positive results plus patients with Phl p 1-positive results plus nonresponders is 66.

and potential maternal allergen exposure. The results are expressed in Fig 1. Each horizontal bar represent the time from conception until cord blood analysis by cordocentesis. The hatched areas represent the time of potential maternal allergen exposure as determined by the national pollen flight registry. When the horizontal bar and the hatched area are overlapping, allergen exposure during gestation may have occurred. For some of the samples displaying positive proliferative response to one or the other allergen, allergen exposure was likely during pregnancy (rBet v 1, 3/6; rPhl p 1, 12/15). For the samples declared as nonreactive, in a majority of the patients, an exposure with one or the other allergen was likely (rBet v 1, 32/45; rPhl p 1, 37/45). These data show that maternal allergen exposure may only partly explain a corresponding ensuing

TABLE III. Cord blood cell proliferation in response to inhalant allergens according to gestational age

	Bet v 1	Phl p 1
Preterm (gestational age 20-37 wk)	8%	20%
Term (gestational age 38-40 wk)	5%	25%

For cordocentesis, 62 consecutive cord blood samples from unborn (preterm) babies and 114 consecutive cord blood samples from term babies were drawn and tested for lymphoproliferative responsiveness to recombinant allergens from birch pollen (Bet v 1) and from timothy grass pollen (Phl p 1) at a concentration of 0.5 and 2 µg/mL. The results are expressed as the percentage of positive responses by means of an SI greater than 2.

fetal, allergen-specific, T-cell reactivity. In contrast, most of the fetuses exposed to allergen through maternal allergen exposure do not respond with detectable allergen-specific T-cell reactivity.

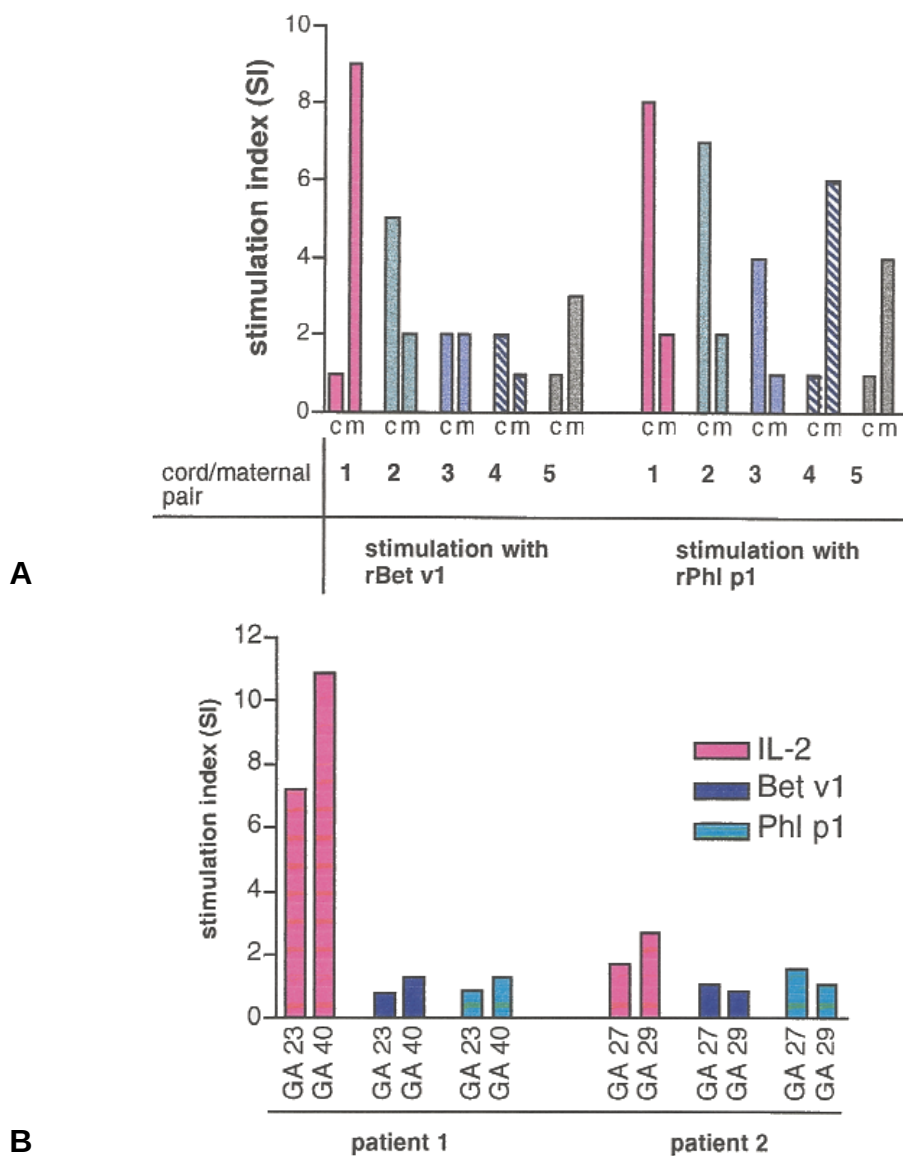


FIG 2. Cord blood allergen-specific T-cell reactivity is specific and is not the result of maternal T-cell contamination. Cord blood and maternal blood samples ($n = 5$; 5 colors for 5 different cord-maternal pairs) have been stimulated with rBet v 1 and rPhl p 1, and the consecutive SI is expressed (**A**). From the same individual, repeated cordocentesis has been analyzed, and the values of two sample pairs are shown for gestational ages of 23 and 40 weeks and gestational ages of 27 and 29 weeks, respectively (**B**). Data are expressed as SIs.

Fetal T-cell priming with allergens is specific and not caused by maternal blood contamination

Contamination of fetal blood with maternal blood has been attributed to influence proliferative responses of cord blood cells. To test this hypothesis, we performed two different experiments. First, maternal and fetal blood pairs were investigated simultaneously for allergen stimulation. As shown in Fig 2, A, some of the maternal samples displayed higher proliferative responses to allergen stimulation than the corresponding fetal sample. In other pairs, fetal cell reactivity was higher than maternal pro-

liferative response. Second, we tested consecutive cord blood samples from the same individual with the recombinant allergens. In the described cases (Fig 2, B) IL-2 and allergen reactivity were reproducible. In summary, these findings confirm that proliferative responses of cord blood cells to inhalant allergens are reproducible and possibly confined to fetal cells and not to maternal blood contamination.

DISCUSSION

Allergen-specific T-cell proliferation in cord blood cells has been demonstrated repeatedly for different

allergens.⁵⁻¹⁵ A line of evidence now supports the hypothesis that fetal T cells are exposed to maternally derived allergens during gestation. These allergens may be ingested or inhaled by the mother.¹⁴ The point in time at which allergen exposure takes place in the course of a pregnancy is less clear.^{5,14} The present study suggests that it is early in gestation, presumably around week 20.

Our experimental data clearly show that fetal T-cell priming with inhalant allergens occurs predominantly early in pregnancy, likely before the gestational age of 20 weeks. This conclusion is based on the fact that lymphoproliferative reactivity to inhalant allergens (rBet v 1 and rPhl p 1) is comparable in cells derived from cord blood samples of term newborns and of unborn (preterm) babies with a gestational age from 20 to 37 weeks (Table III). Moreover, it appears that fetal T-cell priming with inhalant allergens can occur before or around the gestational age of 20 weeks and that later in gestation, additional allergen-specific T-cell priming decreases.

Prenatal T-cell reactivity against various environmental allergens occurs, but the definite value of this in respect to the development of allergic disease is unclear. Initial exposure to allergens systemically during the early gestational period is thought to engage small numbers of immature professional antigen-presenting cells. Indeed, in a variety of experimental systems, tolerance to the antigen develops in neonatal experimental animals thus immunized, characterized by an apparent failure to recognize whether it is presented to them subsequently during adulthood.^{22,23} This cellular network is poorly developed at birth and matures relatively slowly postnatally; during the preweaning period, the airway dendritic cells exhibit low MHC class II expression and poor antigen-presenting cell function and are refractory to inflammatory stimuli compared with similar cells in adults.^{3,24,25} Thus the initial T-cell stimulation with nutritive and inhalant allergens may induce T-cell proliferation and limited cell activation. Allergen-driven cytokines, presumably of the T_H2 -skewed type, are produced at low levels.¹² Rechallenge of these primed T cells leads to activation and immune deviation, as previously described.^{22,23}

The present data may also be interpreted as the result of an allergen-driven T-cell response that is regulated by the T cell itself. T-cell recognition of allergens may occur systemically early in gestation, and the initial T-cell response (in terms of activation quality and cytokine skewing toward T_H1 or T_H2) may not be altered throughout the gestational period. In addition, allergen exposure outside the window of opportunity would lead to non-recognition of the allergen or to induction of allergen-specific hyporesponsiveness. Support for this hypothesis is given by the observation that most of the individuals (unborn babies) who have undergone prenatal exposure do not mount a detectable T-cell response to the allergen to which they have been exposed (Fig 1). Nevertheless, a truly naive state of fetal T cells is also possible, reflecting a lack of transfer of maternally exposed allergens. Furthermore, the T-cell priming rate to allergens is slightly higher around the gestational age of 20 weeks than

around the gestational age of 40 weeks. This fact could be explained by increasing hyporesponsiveness on rechallenge during increasing gestational age. Hence, factors in addition to allergen exposure and window of opportunity influence the outcome of this immune response. To understand and study the mechanism operative in prenatal T-cell priming, it appears necessary to know how the allergen or allergenic peptides are transferred through the feto-placental membrane and at what concentration the allergen is present in fetal circulation.

For some pathogens or antigens, prenatal exposure has been shown to lead to immunologic sensitization in human newborns.²⁶ Offspring of mothers infected with mumps or with toxoplasmosis during pregnancy have antigen-specific antibody responses and memory T cells.^{27,28} Immunization of pregnant mothers with tetanus toxoid, streptococcal, or meningococcal vaccines also induces antibody responses in neonates that persist into childhood.²⁹ Thus the almost universal induction of an immune response to infectious or pathogenic antigens compared with barely occurring responses to allergenic proteins can be interpreted as follows. A large number of pathogen-specific precursor T cells might be stimulated and activated prenatally by a strong antigenic (infectious) stimulus, which, compared with allergenic proteins, could involve different models of antigen presentation both at initial priming and during the ensuing antigen-driven T-cell selection process. On the other hand, the absence of substantial allergen-specific T-cell response in cord blood cells does not rule out T-cell responses at levels below the current assay detection limits.

Stimulation of cord blood cells with β -lactoglobulin is associated with substantial proliferative responses in most of the samples compared with limited cord blood cell reactivity in response to ovalbumin. A preferential handling of the allergen β -lactoglobulin may be responsible for this difference in T-cell reactivity in cord blood cells.^{12,30,31}

Privileged diaplacental passage of particular allergens may explain why T-cell responsiveness to one allergen is more pronounced or more often detected than the response rate to other allergens. In fact, we and others have shown that T-cell proliferative response to β -lactoglobulin is very common, as detected in 95% to 100% of the cord blood samples measured.^{7,13,31} For other allergens (eg, the perennial allergen house dust mite), stimulation of cord blood cells does not lead to a universal response in all individuals.³¹ For seasonal allergens, lymphoproliferative response is rather uncommon, as demonstrated by the high number of mothers potentially exposed to that particular allergen and low corresponding reactivity rates in cord blood cells (Fig 1). These findings would support the idea of privileged handling of particular allergens by the placental membrane.

In summary, two concepts will have to be studied in more detail in the future to elucidate the clinical effect of lymphoproliferative responsiveness and cytokine skewing toward a T_H2 profile in cord blood cells to common environmental allergens. First, allergen transfer through the

placenta appears to occur. Preferential handling of particular allergens by the materno-fetal barrier would explain many of the open questions arising from the present literature. Second, assuming that allergen is delivered to the fetal circulation in sufficient amounts to induce a T_H2-skewed immune response, why is the ensuing immune reaction not followed by a clinical allergic response? The subclinical fetal allergen-driven immune response may reflect the immaturity of the fetal immune system, the components of which remain to be studied in more detail.

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