

Early expression of I ϵ , CD23 (Fc ϵ RII), IL-4R α , and IgE in the human fetus

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Background: A major predictor of childhood atopy is the concentration of IgE in the cord blood, but whether the source of cord blood IgE is maternal or fetal remains unclear.

Objective: We sought to determine the pattern of in situ IgE production during ontogeny.

Methods: Ninety-seven fetal, 142 natal, and 96 childhood samples were analyzed by using reverse transcription PCR for transcription of VDJC ϵ , I ϵ , and CD23. Thirty-eight fetal liver samples were analyzed for the *IL4RA* genotype.

Results: IL-4R α , CD23a, CD23b, and sterile I ϵ transcripts were present as early as 8 weeks' gestation. VDJC ϵ transcripts were found in second-trimester fetal liver and third-trimester cord blood, although they were rare. VDJC ϵ transcripts were more common in the blood of children 9 months and older. Sequence analysis suggested that fetal VDJC ϵ was the product of selection. All fetal livers actively transcribing I ϵ , VDJC ϵ , and IL-4R α contained at least one copy of the atopy-associated *IL4RA**A1902G polymorphism.

Conclusion: The human fetus contains B cells that are primed to undergo IgE class switching from the earliest stages of ontogeny and can produce endogenous IgE by 20 weeks' gestation. However, IgE-producing cells are rare until 9 months after birth. (J Allergy Clin Immunol 2000;106:911-7.)

Key words: IgE, immunoglobulin variable region, CD23, IL-4 receptor, receptors Fc, spleen, liver, cord blood, gut, mesentery, fetus, human

In countries where helminthic infections are rare, elevated cord blood IgE is the best predictor of future atopic disease.¹ However, in neonates with IgE directed against maternal helminthic antigens, the result is sensitization to these parasites.² Activation of IgE in utero could thus be a two-edged sword: deleterious in an environment where innocuous allergens become primary antigens but protective in one where parasites are common.

The source of in utero IgE remains unclear. Although IgE synthesis can be induced in cultures of fetal tissue of 11 weeks' gestation,³ spontaneous production of IgE in fresh fetal tissue has not yet been observed.^{4,5} Thus it has

Abbreviation used

RT: Reverse transcription

been proposed that prenatal IgE is maternally derived, diffusing across the placenta into the amnion, where it is swallowed by the fetus.⁶ Given that the presence of neonatal IgE is linked to future sensitization or atopy, it may be that the inability to detect endogenous IgE reflects the relative insensitivity of the serologic methods used to examine fetal tissues.

If IgE is produced in utero instead of, or in addition to, being passively acquired, fetuses must contain B cells that express IL-4 or IL-13 receptors and have activated I ϵ transcription,⁷⁻¹¹ as well as cognate, activated T_H2 T cells, and an inciting antigen.^{12,13} The low-affinity Fc ϵ receptor (Fc ϵ RII or CD23) further regulates IgE production in class-switched B cells.¹³ CD23 exists in two isoforms, CD23a and CD23b.¹² CD23a is constitutively expressed on mature activated B cells,^{13,14} whereas expression of CD23b is induced by IL-4 or IL-13.^{12,13}

To determine whether endogenous production of fetal IgE can occur, a sensitive, molecular, reverse transcription (RT) PCR technique was used to sample fetal tissues and infant blood for VDJC ϵ and I ϵ transcripts. IL-4R α , CD23a, and CD23b transcripts were also evaluated.¹²⁻¹⁵ I ϵ , IL-4R α , and CD23 transcripts were detected as early as 8 weeks' gestation, with I ϵ more common among individuals with the atopy-associated *IL4RA**A1902G allele.¹⁵ Rare VDJC ϵ -expressing cells were found in second-trimester tissues and third-trimester cord blood. Their presence indicates that the fetus can produce IgE in situ.

METHODS

Source of tissues and cells

We obtained cord blood from infants of 24 to 40 weeks' gestation and peripheral blood from an adult with X-linked agammaglobulinemia. The acquisition of fetal liver, spleen, mesentery, and gut of 8 to 28 weeks' gestation and peripheral blood of children aged 2 to 18 months was previously described.¹⁶ RNAs from the IgE-producing U266 cell line¹⁷ and from pokeweed mitogen-stimulated PBMCs (the gift of Dr Hiromi Kubagawa) were used as controls. All protocols were approved by the Institutional Review Board of the University of Alabama at Birmingham.

Analysis of VDJC μ , VDJC ϵ , I ϵ , CD23a, CD23b, and IL-4R α transcripts

Oligo (dT)₁₂₋₁₈ primed complementary (c)DNA was generated from total RNA, as previously described.¹⁶ Control synthesis reac-

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tions for each cDNA preparation were performed without reverse transcriptase to test for contamination and for β -actin to assess genomic DNA contamination.¹⁶

VDJ μ and VDJ ϵ transcripts were amplified in a seminested dual 50-L reaction by using 1 μ L of each cDNA sample for first-round PCR and 2 μ L of the PCR product for the second-round PCR. PCR conditions were as follows: 0.2 mmol/L deoxyribonucleoside triphosphates, 1 μ mol/L of each primer, 2 mmol/L $MgSO_4$, and 2.5 U of *Pwo* DNA polymerase in the manufacturer's PCR buffer (Boehringer Mannheim). Only one round of amplification was used for CD23a, CD23b, and IL-4R α cDNA. The PCR amplification protocol for VDJ μ , VDJ ϵ , I ϵ , CD23a, and CD23b was as follows: 30 cycles of denaturation at 94°C, primer annealing at 56°C, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. The amplification protocol for IL-4R α was as follows: initial denaturation at 95°C for 5 minutes, 30 cycles of denaturation at 95°C for 30 seconds, primer annealing at 56°C for 45 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 5 minutes.

Primer sequences are listed in Table I. Primers used for VDJ μ amplification were as follows: first round, HSJ 12 and HSJ-14; second round, HSJ 12 and H-14. Primers for VDJ ϵ ¹⁸ were as follows: first round, HSJ-12 and HSJ-13; second round, HSJ12 and HSJ-3. Primers for I ϵ transcripts were as follows: first round, IEP-2 and IEP-3; second round, IEP-2 and IEP-4. Primers for IL-4R α transcripts were as follows: IL-4Rf and IL-4Rr. Primers for CD23a were CD23AF and CD23AR, and primers for CD23b were CD23BF and CD23BR.¹⁹ Except for IL-4R α , where the genomic organization is not listed in Genbank, each set of primers amplify exons that are known to be separated by at least one intron.

Amplified VDJ ϵ and I ϵ cDNA were detected by Southern analysis with ³²P end-labeled HSJ-2. Each CD23a and CD23b product was digested with *Bst*EII, yielding 87 and 131 base pair products, respectively, that bound ³²P-radiolabeled primer CD23I. Blotted filters underwent autoradiography, followed by phosphorimager analysis with MacBas V2X software (Fuji Photo Film Co, LTD). HCDR3 spectrotyping (length distribution) analysis of VDJ μ and VDJ ϵ was performed as previously described.¹⁶ Selected I ϵ , VDJ ϵ , and VDJ μ PCR products and all IL-4R α cDNA products were double strand sequenced by using standard protocols.²⁰

RESULTS

Detection of VDJ ϵ transcripts in fetal liver and cord blood

A primer derived from Framework 3 (FR3) sequence that is conserved in all V_H¹⁶ was used with nested C ϵ C_H1 primers for the RT-PCR amplification of VDJ ϵ transcripts. PCR amplification parameters were optimized by using oligo d(T) primed cDNA from the IgE-producing myeloma cell line U266.¹⁷ Assay sensitivity was evaluated by mixing serial 10-fold dilutions of U266 cDNA with PBMC cDNA from a patient with X-linked agammaglobulinemia who did not express IgE. In ethidium bromide-stained agarose gels, a band of the appropriate size could be detected at less than one cell equivalent. By Southern blot analysis, a VDJ ϵ product of the appropriate size could still be detected at the equivalent of a 1 in 10,000 dilution of RNA from a single cell.

VDJ ϵ transcripts indicative of endogenous IgE production were detected in fetal tissue and cord blood. Of 97 fetal samples from 62 individual fetuses (Table II), VDJ ϵ transcripts were detected in two fetal livers of 20 and 21 weeks' gestation. Of 133 samples (Table II) of cord blood

mononuclear cells from preterm and term neonates of 23 to 40 weeks' gestation, VDJ ϵ transcripts were detected in two samples from infants of 31 and 33 weeks' gestation.

To test for maternal contamination, HCDR3 spectrotyping analysis¹⁶ was performed on VDJ μ transcripts from the 4 VDJ ϵ -containing samples. Average lengths were 13.0 (8416, 20 weeks), 14.0 (EUAB-5, 21 weeks), 14.2 (S113-KM, 31 weeks), and 14.8 (S119-TW, 33 weeks), respectively. This distribution was typical for fetal tissues of the same age and differed from the pattern seen in adults, arguing against maternal contamination. As a further test, 5 μ HCDR3 sequences were obtained at random from each of the two fetal livers. D_H and J_H use and HCDR3 length distribution were typical for the fetal liver, with a preference for D7-27 (DQ52, 4/10 samples), a paucity of J_H6 (0/10 samples), an average length of 12.8 $\pm$ 4.0 codons, and 1 of 10 sequences out of frame.¹⁶

To facilitate statistical analysis, fetal tissues and cord blood samples were grouped by trimester, and peripheral blood samples were grouped into those derived from infants 2 months of age and from infants ranging in age from 6 to 18 months (Table II). These groupings are based on previously reported patterns of VDJ μ repertoire diversification during ontogeny.¹⁶ These include the change in D_H and J_H gene segment use that occurs between the first and second trimester, the increase in distribution of HCDR3 lengths that occurs during the third trimester, the immature HCDR3 length distribution observed at 2 months in spite of exposure to external antigen, and the achievement of an adult pattern of HCDR3 lengths at 6 months after birth.

Although VDJ ϵ transcripts were detected in second- and third-trimester samples, they were rare until 9 months after birth (Table II, Fig 1). Because of sample size, the difference in prevalence of VDJ ϵ transcripts between the second-trimester fetal liver and children aged 6 to 18 months did not reach statistical significance. However, the difference in the prevalence in these transcripts between children aged 6 to 18 months and third-trimester cord blood was significant at a *P* value of less than .01 (Fisher exact test, two-tailed).

Composition of fetal ϵ HCDR3 differs from fetal μ , as well as adult μ and ϵ

HCDR3 spectrotyping analysis of the VDJ ϵ cDNAs revealed 4 bands in the 20-week liver sample and 1 band in each of the 3 remaining fetal and natal samples, each of which was sequenced and analyzed (Fig 2).

To provide a standard of comparison, Genbank was searched for ϵ sequences, and 78 were found (also AF110479, AF110484-87, AF110490, AF110491, HSMT11IGE-HSMT17IGE, HSMT21IGE-HSMT27IGE, and HSIGEVH4).²¹⁻²⁶ Analysis of D_H and J_H use in their HCDR3 intervals revealed a pattern typical for adult μ , including an absence of D7 gene segments and common use of J_H6.^{16,27} However, two sequences contained D-D fusions, and the D_H was inverted in 6 sequences. The average HCDR3 interval length was 14.9 $\pm$ 3.6 codons, which was significantly shorter than adult μ (17.7 $\pm$ 0.5 codons).¹⁶

TABLE I. Primers used for amplification of VDJC μ , VDJC ϵ , I ϵ , CD23a, CD23b, and IL-4R α

Name	Source	Sequence (5'→3')
BAF	β -Actin exon 4	GCGGGAAATCGTGCGTGACATT
BAF	β -Actin exon 6	GTGGACTTGGGAGAGGACTGGG
HSJ-12	V H exon 2	CGCAAGCTTACAC (TGA) GC (CT) G
HSJ-14	C μ exon 1	TATCCGACGGGGAATTCTCAC
H-14	C μ exon 1	AATTCTCACAGGAGACGAG
HSJ-13	C ϵ exon 1	GAAGGTTTGTGTGTCGACCCA
HSJ-3	C ϵ exon 1	TGTTTTTGCAGCAGCGGGTCA
HSJ-2	C ϵ exon 1	CGGATGGGCTCTGTGTGGAGG
IEP-2	I ϵ exon 1	CCACGGTTACTGATCATCTG
IEP-3	C ϵ exon 1	GTCTGTGGACGATGGAGTGT
IEP-4	C ϵ exon 1	GGCTGGTAAGGTCAATAGTTG
CD23AF	CD23a exon 2	ATGGAGGAAGGTCAATATTC
CD23AR	CD23a exon 3	TCCAGCTGTTTTAGACTCTG
CD23BF	CD23b exon 1	CAGAGGCCAAATAGAACAGG
CD23BR	CD23b exon 2	CACAGGAGAAGCAGAGTCAG
CD23I	CD23 exon 2/3	CCCACGCTGCAACACCGCC
IL-4Rf	IL-4R α exon 12?	CAGACACCTGGAGGAAGTAG
IL-4Rr	IL-4R α exon 12?	CTGGCAAGCAGGCTTGAGAA

TABLE II. Prevalence of ϵ and CD23 transcripts during ontogeny

	Tissue	n*	VDJC ϵ	I ϵ	CD23a	CD23b
First trimester	Liver	19	0 (0%)	4 (21%)	18 (95%)	17 (80%)
	Spleen	20	0 (0%)	6 (30%)	17 (85%)	16 (80%)
	Mesentery	11	0 (0%)	3 (27%)	11 (100%)	11 (100%)
	Gut	11	0 (0%)	1 (9%)	11 (100%)	10 (91%)
Second trimester	Liver	18	2 (11%)	3 (17%)	15 (83%)	13 (72%)
	Cord blood	9	0 (0%)	2 (22%)	6 (67%)	7 (78%)
	Spleen	14	0 (0%)	8 (57%)	14 (100%)	12 (86%)
	Mesentery	1	0 (0%)	0 (0%)	1 (100%)	1 (100%)
	Gut	1	0 (0%)	1 (100%)	1 (100%)	1 (100%)
Third trimester	Liver	1	0 (0%)	1 (100%)	1 (100%)	1 (100%)
	Cord blood	133	2 (2%)	19 (14%)	120 (90%)	77 (58%)
	Spleen	1	0 (0%)	1 (100%)	1 (100%)	1 (100%)
2 mo	Blood	16	0 (0%)	1 (6%)	16 (100%)	11 (69%)
6-18 mo	Blood	80	10 (13%)	35 (44%)	78 (98%)	69 (86%)

To perform a statistical analysis of transcript prevalence, samples were grouped by tissue and by age into the first trimester (8-13 weeks' gestation), the second trimester (14-26 weeks' gestation), the third trimester (27-40 weeks' gestation), 2 months after birth, and 6 to 18 months after birth. The number and percentage of samples in which VDJC ϵ , I ϵ , CD23a, and CD23b transcripts could be detected are indicated in their respective columns.

*Number of samples of the indicated tissue examined at the designated ages.

The average length of the 5 fetal liver ϵ HCDR3 intervals was 12.6 ± 4.1 codons or similar to their companion μ (12.8 ± 4.0 codons) but shorter than adult ϵ (14.9 ± 3.6 codons). The two ϵ sequences from cord blood were longer, 21 and 22 codons, respectively. Of the 5 VDJC ϵ transcripts from fetal liver, 3 used J $H3$ and 3 used J $H4$, which is typical for second-trimester μ but rare for adult μ and ϵ . Both of the cord blood ϵ transcripts used J $H6$, a gene segment commonly used in adult μ and ϵ . None of the fetal transcripts used D7-27. One fetal liver transcript contained an inverted D1-14 gene segment, and the two samples from cord blood contained D-D fusions that are rare at any age in μ but were more common in adult ϵ . The differences between in utero ϵ HCDR3 sequences and both fetal and adult μ HCDR3 sequences suggest that the fetal ϵ HCDR3 sequences were the product of selection, an observation supported by the presence of apparent somatic mutations (Fig 2).

I ϵ and CD23 transcripts can be detected as early as 8 weeks' gestation

Activation of the C ϵ locus requires sterile I ϵ transcription.⁷ Of the four 8-week fetal liver samples, two contained I ϵ transcripts, which was confirmed by sequencing. Although the numbers were insufficient to allow statistical analysis, to better visualize the pattern of I ϵ transcription, the prevalence of the sterile transcripts was plotted as a function of age in 4-week intervals for fetal samples and, after birth, at 2- and then at 3-month intervals between 6 and 18 months (Fig 1). This analysis revealed an increased prevalence of sterile I ϵ transcripts in the spleen and blood of older children. This pattern was confirmed when the samples were analyzed by trimester to enable statistical comparison (Table II). The difference in the prevalence of the transcripts in the second-trimester spleen was significant for second-trimester

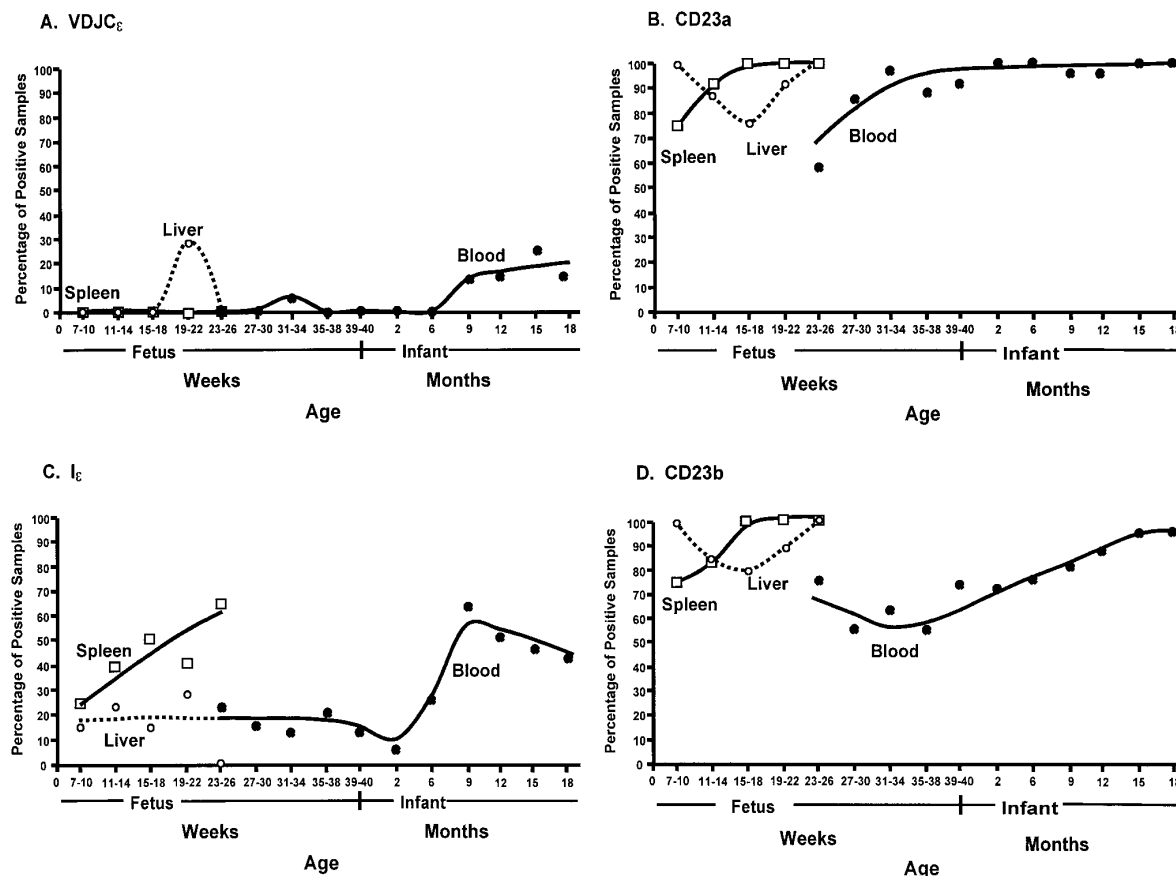


FIG 1. The proportion of liver, splenic, and blood samples containing ϵ and CD23 transcripts as a function of age in 4-week intervals for fetal samples and after birth at 2- and 3-month intervals between 6 and 18 months. **A**, VDJC ϵ transcripts; **B**, CD23a transcripts; **C**, sterile I ϵ transcripts; **D**, CD23b transcripts. Note that unlike Table II, where samples are grouped by trimester, grouping by age in weeks and months does not allow a statistical analysis because of limitations in the number of samples obtained.

liver ($P = .03$, Fisher exact test, two-tailed), third-trimester cord blood ($P < .001$), and the blood of 2-month-old infants ($P < .01$). Similarly, the difference in prevalence in transcripts between the blood of infants of 6 to 18 months of age was significant for second-trimester liver ($P = .04$) and third-trimester blood ($P < .001$) and the blood of 2-month-old infants ($P < .01$). There was no significant difference in prevalence for sterile I ϵ transcripts between the second-trimester spleen and the blood of infants aged 6 to 18 months.

CD23a, which is constitutively expressed in mature activated B cells, was highly prevalent throughout ontogeny (Table II, Fig 1). CD23b, which is induced by IL-4 or IL-13, was highly prevalent in first- and second-trimester tissues, but its prevalence declined in third-trimester blood, only to rise after birth. The decrease in prevalence in third-trimester blood was statistically significant when compared with the first-trimester liver ($P = .01$, Fisher exact test, two-tailed) and second-trimester spleen ($P = .05$) and the blood of infants aged 6 to 18 months ($P < .001$).

IL-4R α transcript expression and prevalence of the IL4RA*A1902G allele

Although the numbers were insufficient to achieve statistical significance, all of the first- and second-trimester samples positive for I ϵ or VDJC ϵ for which IL-4R α sequence could be obtained demonstrated the presence of at least one IL4RA*A1902G allele ($P = .1$). RT-PCR-amplified IL-4R α cDNA transcripts were obtained from 30 of the 38 fetal livers (Table III), including three of five 8-week samples. The prevalence of the IL4RA*A1902G polymorphism was comparable with that of the wild-type allele (27 and 33 chromosomes, respectively). One of the 30 samples contained a previously undescribed allele²⁸ containing a G to A transition at position 1910 that produces a conservative amino acid substitution of isoleucine for valine at position 579.

DISCUSSION

Cells generating IgE were detected in fetuses as young as 20 weeks' gestation, demonstrating that IgE can be

20 Week Fetal Liver

8416-2 TGT GCAGAAGA TATGGGGGGGA GGTTCGGG GGGAT GCTTTTGATATC TGG
iD1-14 JH3
8416-4 TGT GCGAG GTATTCCTCCTTTCTGGCCTCC TTGACTAC TGG
? JH4
8416-16 TGT GCAAGAG GAAGGGGGGTGGGC GCTTTTGATATC TGG
? JH3
8416-9 TGT GCGAG TGGCCC CTTTGACTAC TGG
? JH4

21 Week Fetal Liver

EUAB-5 TGT GCGAGAGA TC GGA ATTACGATATTTTACTGGTTATTATAA T GCTTTTGATATC TGG
D3-9 JH3

31 Week Cord Blood

S113-KM TGT GCGAGAGA GTCTCCTCGATCTA ATAGTGGCTACGATT CAGATGACTATTATtATTAC ATGGACGTC TGG
D5-12 D3-16 JH6

33 Week Cord Blood

S119-TW TGT GCGAAA AGTGACCC TTTTGGAGTGAAtATTATA AC TTTGACT ACTCGTACACTT TGGACGTC TGG
D3-3 D3-9 JH6

FIG 2. HCDR3 intervals from fetal and neonatal VDJCε transcripts. Shown are the nucleotide sequences for codons 92 (TGT) through 103 (TGG) of the variable domain. D_H and J_H identity is shown below the sequences. The error correcting *Pwo* DNA polymerase was used for PCR amplification of these transcripts. Nucleotides shown as lower case letters diverge from germline sequence and likely represent somatic mutations.

TABLE III. *IL4RA* gene transcription in fetal liver samples

Age	Iε		VDJCε	CD23a	CD23b	<i>IL4RA</i> genotype at nucleotide 1902			
	±	n*				AA	AG	GC	ND†
First trimester	+	4	0	4	3	0	1	1	2
	–	15	0	14	14	3	5	1	5
Second trimester	+	3	2	3	3	0	2	1	0
	–	15	0	12	10	6	5	3	1
Third trimester	+	1	0	1	1	1	0	0	0

Samples were grouped by age as noted in Table II, and within each age, they were divided into those samples where Iε transcripts were detected (+) and those where they were not (–). The number of samples in which VDJCε, CD23a, and CD23b transcripts could be detected are indicated in their respective columns. The distribution of *IL4RA* A and G genotypes for position 1902, as identified by sequence analysis of IL-4Rα transcripts, is indicated in the final 4 columns.

*Number of samples with Iε transcripts within each sorted group.

†ND indicates that no IL-4Rα transcripts were detected.

produced endogenously by the fetus. However, these cells appear to be extremely rare. In contrast, sterile Iε transcripts were detected in a number of fetal and neonatal samples and were fairly common in the second-trimester fetal spleen. Thus many fetuses appear to contain B cells that are primed and ready to initiate a class switch to IgE as early as 8 weeks' gestation.

Why then are IgE-bearing cells so rare? Ultimately, the switch to IgE requires an antigen-dependent CD40:CD40L interaction between an activated B cell and an activated cognate T cell. Recent studies have demonstrated that T cells reactive with common allergen proteins can be detected in amniotic fluid.²⁹ Although these cells exist in the fetus, their numbers are likely to be small. Therefore the rarity of in utero IgE in developed countries could reflect the low likelihood of an encounter between an allergen, a primed B cell, and its cognate T cell. Moreover, the secondary lymphoid organs where this interaction occurs may not be fully mature,³⁰⁻³³ reducing the likelihood of IgE class switching.

Further support for the view that IgE production in utero represents a rare antigen-selected event derives from an analysis of the sequence of the fetal and cord blood VDJCε transcripts. These sequences were not representative of the preimmune fetal IgM repertoire.³⁴ It is possible that the rarity of fetal B cells bearing the antigen-binding structures required to optimally bind allergens may also play a role in limiting IgE production in utero.

Alternatively, it is possible that the influx of maternal IgG, which is significant at 20 to 21 weeks' gestation,³⁵ serves to neutralize external antigens that enter the fetus, including those that enter through fetal swallowing of amniotic fluid, altering induction and selection of the fetal IgE repertoire. Support for this hypothesis can be found in studies showing that administration of intravenous gammaglobulin inhibits IgE production.³⁶

Sterile Iε and CD23b transcripts, markers of B-cell activation in a T_H2 response, were common in the second-trimester spleen but rare in third-trimester cord blood. Indeed, the prevalence of sterile Iε and CD23b transcripts

in blood did not match the prevalence observed in the second-trimester spleen until 6 months after birth. The high prevalence of these transcripts in the spleen may reflect increasing maturity of this tissue in the second trimester, which contains many of the basic cells and structures necessary to initiate an immune response, including, by 22 weeks' gestation, B-cell follicles.³⁰⁻³³ The relative paucity of activated B cells in third trimester cord blood could also reflect limited circulation of the more mature B cells found in the spleen or suppression of B-cell activity as a result of exposure to maternal IgG. Regardless, extrapolation of lymphocyte function in the cord blood to the function of lymphocytes in the primary and secondary lymphoid tissues should be viewed with caution.

The correlation between the presence of at least one *IL4RA**A1902G-bearing allele, which has been associated with a high risk for atopy, and early transcription of *Iε* and *VDJCE* was absolute, although the numbers were insufficient to achieve statistical significance. It is possible that the presence of this allele lowers the threshold for T_H2 -influenced activation of B cells and increases the likelihood of activation of the *Cε* locus and class switching. The prevalence of the *IL4RA**A1902G allele in the study population (27/60 chromosomes [48%]) is two to three times greater than previously published estimates of 16% to 21%.³⁷ This could reflect a difference in the frequency of the two alleles in two separate populations. Unfortunately, because of patient confidentiality issues, access to the racial or ethnic derivation of the individual samples was not possible.

Our findings indicate that the IgH ϵ locus in the fetus is more active than previously appreciated. Fetuses as young as 8 weeks' gestation, all of which had inherited *IL4RA**A1902G alleles, were actively transcribing the *Cε* locus, and two second-trimester fetuses were endogenously producing IgE. The antigen specificity of fetal IgE and the relationship between early endogenous production of IgE and atopy remain open questions.

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