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IgA Response in Preterm Neonates Shows Little Evidence of Antigen-Driven Selection

Tobias Rogosch,* Sebastian Kerzel,* Katharina Hoß,* Gabriele Hoersch,* Cosima Zemlin,† Matthias Heckmann,‡ Claudia Berek,§ Harry W. Schroeder, Jr.,¶,*# Rolf F. Maier,* and Michael Zemlin*

After birth, contact to environmental Ags induces the production of IgA, which represents a first line of defense for the neonate. We sought to characterize the maturation of the repertoire of IgA H chain transcripts in circulating blood B cells during human ontogeny. We found that IgA H chain transcripts were present in cord blood as early as 27 wk of gestation and that the restrictions of the primary Ab repertoire (IgM) persisted in the IgA repertoire. Thus, B cells harboring more “mature” VH regions were not preferred for class switch to IgA. Preterm and term neonates expressed a unique IgA repertoire, which was characterized by short CDR-H3 regions and very low numbers of somatic mutations. During the first postnatal months, these restrictions were slowly released. Preterm birth did not measurably accelerate the maturation of the IgA repertoire. At a postconceptional age of 60 wk, somatic mutation frequency of IgA H chain transcripts reached 25% of the adult values but still showed little evidence of Ag-driven selection. These results indicate that similar to IgG, the IgA repertoire expands in a controlled manner after birth. Thus, the IgA repertoire of the newborn has distinctive characteristics that differ from the adult IgA repertoire. These observations might explain the lower affinity and specificity of neonatal IgA Abs, which could contribute to a higher susceptibility to infections and altered responses to vaccinations, but might also prevent the development of autoimmune and allergic diseases. The Journal of Immunology, 2012, 189: 5449–5456.

IgA production in mice is very low in the uninfected fetus and is stimulated after birth by exposure to commensal microorganisms and food Ags in the gut (3). Notably in mice, IgA production is particularly upregulated during weaning (4, 5). Whereas isolated lymphoid follicles, as inductive sites for B cell activation and expansion, develop after birth in mice in response to the microflora, they are already present in humans at birth (6). In humans, serum-IgA concentration increases during childhood and reaches adult levels during the second decade of life (7). Large amounts of IgA are secreted onto mucosal surfaces and by exocrine glands, including the mammary gland. Breast-fed neonates take up high amounts of IgA through their mothers’ milk, allowing a passive protection of the intestinal mucosa while the infant gradually establishes its own IgA production.

Hitherto, IgA production during human ontogeny has only been examined quantitatively (serum levels), but not qualitatively (characteristics of Ag binding sites) (7). Previous analyses of VH, DH, and JH gene utilization, N nucleotides, and somatic mutations of IgH gene transcripts have shown that the diversification of the primary (IgM) and the secondary (IgG) Ab repertoires are strictly regulated during ontogeny (8–12). Several observations in mice suggest that in contrast to IgG, the IgA repertoire might not predominantly reflect a focused Ag-driven selection but rather a diffuse, less selected production that might be directed against redundant epitopes of commensal microorganisms (13, 14). Moreover, normal serum IgA levels in mice can even be produced in the absence of organized secondary lymphoid structures such as Peyer’s patches and mesenteric lymph nodes (15). Study of the ontogeny of IgA production in human is important because, although sharing many similarities, the regulation of IgA production differs between mouse and humans in several important aspects (6). We postulated that a systematic analysis of the human IgA repertoire during ontogeny might clarify if the circulating IgA repertoire underlies different selective pressures than those of the other isotypes.

In this study, we have analyzed IgA transcripts from cord blood and from peripheral blood of preterm and term neonates during the first 6 mo of life, using adult blood samples as a comparison. We found that the IgA repertoire diversifies slowly after birth. Because of short CDR-H3 regions and very low numbers of somatic mutations, the immature IgA repertoire distinctively differs from the adult IgA repertoire. These characteristics may explain the low Ag affinity and polyreactivity of neonatal IgA Abs (16) and contribute to the altered pattern of Ag reactivity that characterizes the very young (17).
Materials and Methods

Patient samples

Blood samples were collected from both extremely preterm neonates (25–30 wk of gestation, n = 15) and from term neonates (39–42 wk of gestation, n = 14) at birth (cord blood) or at a postnatal age ranging from 1 to 35 wk (venous blood) and from healthy adults (n = 7). Postnatal samples were collected during routine blood tests, which were most frequently performed for the control of blood gases, serum electrolytes, or bilirubin. In each case, the blood for required clinical tests was collected first. Subsequently, 0.2 ml of EDTA blood was collected in a separate tube for this research project. The analysis of the IgA repertoire shown in this study is part of a project to describe IgM, IgG, and IgA repertoires during ontogeny. Each blood sample originates from a different individual. Cord blood was collected from umbilical cord arteries after thorough cleaning to avoid cross-contamination with maternal blood. The numbers of blood samples and sequences of IgA transcripts from each sample are given in Table I. All subjects were white. Gestational age was calculated from the first day of the last menstrual period and confirmed by early ultrasound and by clinical examination. Postconceptional age was calculated as gestational age plus postnatal age. The study was conducted in accordance with the guidelines of the World Medical Association’s Declaration of Helsinki. The institutional review boards of the Free University of Berlin and of Philipps University Marburg approved the study protocol. The written consent of parents and adult donors was obtained.

Preparation of RNA and RT-PCR

Erythrocytes were lysed and leukocytes were recovered by centrifugation. Total RNA was isolated using the QIAamp RNA Blood Mini-Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. A combination of primers for the framework region (FR) 1 of all human VH gene families (12) was used together with a consensus antisense primer specific for the first exon of the \( \alpha_{2}\) C region (Table II) (18). RT-PCR amplifications were carried out in a total volume of 50 μl containing 5 μl RNA eluate, 2.5 mM MgCl\(_2\), 7.5 U recombinant RNase inhibitor, and 0.66 μM of each forward and reverse primer using a OneStep RT-PCR kit (Qiagen). The following program was performed on a thermocycler (Sensoquest, Karlsruhe, Germany): 30 min at 50°C, 15 min at 94°C followed by 40 cycles using a cycle profile of 1 min at 94°C, 1 min at 66°C, and 1 min at 72°C, followed by a final extension of 10 min at 72°C. As a control for RNA quality, GAPDH transcripts were amplified from each sample using humGAPDH-1 and humGAPDH-2 primers (Table II). PCR products were gel purified, and DNA was extracted with QIAquick gel extraction kit (Qiagen).

Cloning of PCR products

Ligation and transfection were performed using standard protocols according to the manufacturer’s instructions (TOPO-TA cloning kit; Invitrogen, Karlsruhe, Germany).

Sequence analysis

After the transformed cells had grown on agar plates, 20–35 clones from each subject were randomly selected. Plasmid DNA was extracted, linearized, and sequenced using an ABI capillary sequencer. Gene segments were aligned to germline gene segments using the ImMunoGeneTics database with VQUEST (19). A minimum of six nonmutated nucleotides with at least two nonmutated nucleotides at each end were required to identify a Diversity (D) gene (20). The CDR-H3 was defined to include the conserved tryptophan (W118) of FR-H4. To analyze the patterns of somatic mutations in the CDR regions, a Diversity (D) gene was identified by VQUEST. The CDR-H3 was defined to include all N nucleotides in the CDR-H3 region.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad, La Jolla, CA) and SPSS 17.0 (SPSS, Chicago, IL). Normality distribution was examined with the Shapiro–Wilk test. Differences between populations were assessed by a two-tailed Student t test for normally distributed data or a Mann–Whitney U test for non-normally distributed data, respectively. For categorical data, a \( \chi^2 \) test with post hoc analysis was applied as described by Collis et al. (24). A \( p \leq 0.05 \) was accepted as significant. Means are given with SE.

Results

To compare the age-related and environmental influences on the postnatal development of the IgA repertoire in preterm and term neonates, we analyzed a total of 752 functional IgA transcripts. Of these transcripts, 663 (88%) were unique, including 235 from 15 preterm neonates, 276 from 14 term neonates, and 150 from 7 adult venous blood samples. This analysis includes 129 sequences from 8 blood samples that had previously been studied for the presence of homology-directed recombination (Table I) (18).

The IgA H chain repertoire of preterm neonates retained the characteristics of fetal IgM H chain variable regions, including short N(D)N regions and overrepresentation of D\(_{H}\)-7-27.

In preterm neonates, the N(D)N length increased during the period corresponding to the third trimester of gestation by 0.17 nucleotides per week (\( r = 0.538, p < 0.0001 \)) (Fig. 1). At a postconceptional age of >50 wk, the N(D)N length was similar in preterm and term neonates and had reached adult N(D)N length. Thus, N(D)N length increase was similar after premature birth and during intratuberine development. The increase in N(D)N length during ontogeny was mainly due to increasing numbers of N nucleotides that were added at the D\(_{H}-\)J\(_{H}\) junction by 0.11 nucleotides per week (\( r = 0.756, p < 0.001 \)).

The frequency of the D\(_{H}-\)7-27, the most J\(_{H}\) proximal D\(_{H}\) gene segment, undergoes great changes during ontogeny in humans and in mice (reviewed in Ref. 25). We found that D\(_{H}-\)7-27 was more frequently used in IgA transcripts from preterm (9.8 ± 2.3%) than from term neonate blood B cells (2.5 ± 1.0%, \( p < 0.05 \)) or adults (0.01 ± 0.01%, \( p < 0.001 \), Fisher exact test) (Fig. 2). Use of the other VH, D\(_{H}\), and J\(_{H}\) genes in IgA transcripts was similar. Briefly, in comparison with the frequency expected from the number of germline genes, the V\(_{H}^{4}\) and V\(_{H}^{6}\) families as well as the J\(_{H}^{4}\) genes were overrepresented, whereas the V\(_{H}^{3}\) family was underrepresented in all groups of IgA transcripts studied (data not shown). In all three groups, the V\(_{H}^{6}-1\) gene segment was used most frequently, followed by V\(_{H}^{4}-59\) (data not shown). In summary, the V\(_{H}^{6}-1\) gene utilization was similar in IgA transcripts as in previously published IgM and IgG transcripts.

The somatic mutation frequency rises slowly in preterm neonates

In neonates, the somatic mutation frequency within CDR-H1 to FR-H3 (number of mismatches to the most homologous VH gene per 1000 nucleotides) increased during the time period studied by 0.35% (preterm, \( r = 0.678, p < 0.0001 \)) and 0.44% (term, \( r = 0.731, p < 0.0001 \)) per week, respectively (Fig. 3). At a postconceptional age of ~60 wk, the somatic mutation frequency was similar in preterm (17.7%) and term (15.9%) neonates, but remained markedly below the somatic mutation frequency seen in adults (ranging from 54.4 to 97.5%, median 71.9%). The fraction of unmutated IgA transcripts was 42% in preterm neonates, 39% in term neonates (n.s.), and 3% in adults (\( p < 0.0001 \) versus preterm and term neonates, respectively, two-sided \( \chi^2 \) test).

We found a close correlation between somatic mutation frequency of IgA transcripts and of previously published IgG transcripts in 15 blood samples from the same neonates that were studied for both isotypes (\( r = 0.5234, p < 0.05 \)) (Fig. 4) (12), whereas the somatic mutation frequency remained very low in IgM transcripts throughout ontogeny.

To exclude significant biasing by Taq polymerase error, we counted the mismatches within the 17 nucleotides of the C region.
IgA H chain transcripts of neonates display a very low degree of Ag selection.

To evaluate whether IgA sequences demonstrated signs of Ag selection, we analyzed the distribution of replacement and silent mutations between FRs and CDR as described previously (23). A previously described binomial distribution method (22) was used to determine the 95% confidence limits for the random enrichment of replacement mutations in the CDRs (26). These confidence limits are depicted as gray areas in Fig. 5. A data point falling outside this gray-shaded area represents a sequence that has a high proportion of replacement mutations in the CDR. The probability that such a sequence has accumulated as many replacement mutations in the CDRs by mere random mutation is <0.05. Therefore, an allocation above the upper confidence limit was considered indicative of Ag-driven selection.

According to this definition, 34% of the IgA transcripts of adults showed signs of Ag selection. Notably, the picture was strikingly different in neonates: The percentage of IgA sequences with signs of Ag selection was 2.1% (p < 0.001 versus adults) in preterm neonates and 1.7% (p < 0.001, two-sided χ² test versus adults) in term neonates (Fig. 5). Some neonatal sequences with few somatic mutations fulfill the mathematical criteria for Ag selection, although the biological significance of these findings is uncertain. Thus it is possible that our approach overestimates the number of Ag-selected sequences in neonates.

The clonal diversity of each blood sample did not differ between the three groups, ranging from 71 to 100% in preterm neonates (median 87%), 50 to 100% in term neonates (median 91%), and 71 to 100% in adults (median 89%) (Table I).

Discussion

To our knowledge, in this study, we present the first ontogenetic analysis of the IgA repertoire in the perinatal period. We found that IgA H chain transcripts were present in cord blood as early as 27 wk of gestation and that known restrictions of the primary Ab repertoire (IgM) persisted in the IgA repertoire. Preterm and term
neonates possessed a unique IgA repertoire characterized by short CDR-H3 regions, biased DH gene usage, and very few somatic mutations. Thus, the IgA repertoire of the newborn is distinctly different from the adult IgA repertoire. During the first postnatal months, these restrictions were slowly released, and the IgA H chain transcripts contained increasing evidence of somatic hypermutation that reflects Ag exposure. Preterm birth did not significantly accelerate the maturation of the IgA repertoire.

It has been suggested that the perinatal period is a window of opportunity for imprinting the B cell repertoire toward or against diseases of a dysbalanced immune system, such as allergies and autoimmune diseases (27, 28). These diseases represent misled Ag-driven secondary Ab reactions (26, 29). Thus studying the ontogeny of secondary Ab repertoires could lead to a better understanding of the pathogenesis of many immune diseases and could help to identify key periods where manipulation of the repertoire might prevent diseases. We and others have demonstrated in previous studies that analyses of Ig transcripts can give valuable insights into the selective pressures acting on B cells during the recruitment into various B cell subpopulations and during physiologic and pathologic immune responses [(30, 31); for a review, see Ref. 32]. Comparison of the postnatal development of preterm neonates and term neonates represents a unique model that permits distinction between Ag-induced and maturity-induced mechanisms in the immune system.

Birth initiates the transition from the intrauterine germ-free environment to the extrauterine confrontation with microbial and food Ags. This stimulus has a profound influence on the maturation of mucosa-associated lymphatic tissues (e.g., Peyer’s patches) and other lymphoid tissues (7, 33, 34). Neonatal mice that were bred under germ-free conditions and fed an Ag-free diet failed to produce secondary Ab repertoires and were highly susceptible to infections when exposed to microbes later in life (35). The preterm neonates of our study were exposed to dietary Ags (formula milk or breast milk) from the first day of life, and skin-to-skin contact was encouraged from the first week of life, enabling the colonization with commensals approximately 3–4 mo earlier than in a term neonate. Despite this massive exposure to foreign Ags, the IgA H chain transcripts produced by the premature infants did not show more evidence of Ag-driven selection when they reached due date than those of term neonates of the same postconceptional age. This supports the view that many of the maturational steps involved in the ontogeny of the Ab repertoire are genetically regulated and are triggered independent of Ag exposure.

Most of the preterm neonates had at least one infection during the observed episode. Similar to previously published IgG sequences of mucosa-associated lymphatic tissues (e.g., Peyer’s patches) and other lymphoid tissues (7, 33, 34). Neonatal mice that were bred under germ-free conditions and fed an Ag-free diet failed to produce secondary Ab repertoires and were highly susceptible to infections when exposed to microbes later in life (35). The preterm neonates of our study were exposed to dietary Ags (formula milk or breast milk) from the first day of life, and skin-to-skin contact was encouraged from the first week of life, enabling the colonization with commensals approximately 3–4 mo earlier than in a term neonate. Despite this massive exposure to foreign Ags, the IgA H chain transcripts produced by the premature infants did not show more evidence of Ag-driven selection when they reached due date than those of term neonates of the same postconceptional age. This supports the view that many of the maturational steps involved in the ontogeny of the Ab repertoire are genetically regulated and are triggered independent of Ag exposure.

Most of the preterm neonates had at least one infection during the observed episode. Similar to previously published IgG sequences

![Figure 1](https://example.com/fig1.png)

**FIGURE 1.** N(D)N length of IgA transcripts. Each data point represents the mean of one blood sample (see Table I). N(D)N length in preterm infants increased with postconceptional age ($r = 0.538, p < 0.0001$). Near term, the N(D)N length in preterm and term neonates reached adult levels. The polynomial nonlinear best-fit curve is shown for IgA sequences. Adult data are shown as mean, quartiles, and Tukey whiskers.

![Figure 2](https://example.com/fig2.png)

**FIGURE 2.** Usage of DH gene families in IgA transcripts. The single member of the DH7 gene family, DH7-27, was overrepresented in IgA transcripts from preterm neonates, and its frequency decreased during ontogeny.

![Figure 3](https://example.com/fig3.png)

**FIGURE 3.** Average somatic mutation frequency of IgA transcripts. Each data point represents the mean of one blood sample (see Table I). Somatic mutation frequency increased with postconceptional age (preterm, $r = 0.678, p < 0.0001$; term, $r = 0.687, p < 0.0001$). At a postconceptional age of ~60 wk, the somatic mutation frequency was similar in preterm and term neonates. The polynomial nonlinear best-fit curve is shown for IgA sequences. Adult data are shown as mean, quartiles, and Tukey whiskers.

![Figure 4](https://example.com/fig4.png)

**FIGURE 4.** Correlation between the somatic mutation frequencies of IgA and IgG transcripts from the same blood samples. IgG transcripts had been obtained in a previous study (12) from the same 15 blood samples that were used to analyze IgA transcripts ($r = 0.5234, p < 0.05$, excluding adult samples).
from the same blood samples, extensive statistical comparisons did not reveal differences between children with or without a history of infection (data not shown; Ref. 12). This is not surprising because compared with the continuous stimulation by nonpathogenic microbiota or dietary AgS, an episode of infection is a rare and time-limited antigenic exposure.

Our study provides molecular evidence that class switch recombination to IgA during intrauterine life is occurring without the high level of somatic mutations normally observed during adult Ag-driven selection. The diversity of IgA transcripts, which can be interpreted as a rough estimate for the clonal diversity, reached similar levels in extremely preterm neonates as in adults. Notably, both class switch recombination and somatic hypermutation are dependent on the enzymatic activity of activation-induced deaminase, but the different processes are supported by different domains of the enzyme (36). This separation is in harmony with the observation that although specific Ab responses, including IgA, can be elicited by intrauterine infection or vaccination (37, 38), neonatal Ab responses are characterized by lower affinity and shorter half-life than in the mature organism (17).

After term, the somatic mutation frequency increased by ∼0.7‰ per week, both in term and preterm neonates. Even at a postconceptual age of 60 wk, equivalent to 5 mo of age after term, the somatic mutation frequencies in preterm and term neonates were only approximately a quarter of the adult level, and only very few somatic mutations fulfilled the criteria of Ag-driven selection (21, 22). The lower number of somatic mutations in IgA transcripts from neonates could reflect either a lower activity of the enzyme activation-induced deaminase, which normally introduces ∼1 somatic mutation per 1000 nucleotides and cell division into the IgH gene (39) and/or that the cells in neonates could have undergone fewer cell divisions. Alternatively, the low number of somatic mutations might reflect a bias in neonates toward T cell–independent IgA formation, as in mice, nonmutated IgA sequences appear to be generated through T cell–independent mechanisms, whereas mutated IgA sequences require the presence of T cell–mediated signals (40, 41). One can hypothesize that T cell help—and thus production of mutated IgA sequences—might be restricted during early ontogeny; for example, due to the decreased expression of costimulatory receptors (CD40, CD80, CD86) (reviewed in Ref. 17).

In adults, IgA H chain transcripts from peripheral blood contained as many somatic mutations as IgA H chain transcripts from tonsils (42) or from intestinal plasma cells (43). Moreover, the somatic mutations within the IgA transcripts from adult peripheral blood showed all known characteristics of the Ag-driven selection that have been observed in both IgG and IgE repertoires as well (12, 26). The somatic mutation frequencies of IgA and IgG transcripts from the same blood samples correlated, indicating that comparable mechanisms may regulate the number of somatic mutations in IgG and IgA. This argues against the assumption that the IgA repertoire in circulating blood lymphocytes would reflect a rather diffuse, less selected Ab production. However, this hypothesis had been put forth for murine gut plasmablasts, which often arise from low-affinity, polyreactive, IgM-expressing B1 cells (14). It must be considered that important aspects of the human and murine IgA production differ. For example, phenotypic differences have recently been discovered between human and murine B1 cells (44), and, in contrast to mice, human peritoneal cells probably do not contribute significantly to the IgA secretion of the gut (45).

We found that regarding D4 utilization and CDR-H3 length, the IgA repertoire of neonates retained the same restrictions as the primary (IgM) repertoire (11, 12). Thus, class switch to IgA did not favor B cells with more “mature” VH regions. This could indicate that the main characteristics of the secondary Ig repertoire are already predetermined during early B cell development prior to exogenous Ag contact. This finding is in line with observations in mouse models in which a bias had been introduced into the primary Ab repertoire by gene targeting the IgH Diversity gene locus (32, 46–48). In agreement with our findings in the human neonatal IgM and IgA repertoires, somatic selection during class switch and affinity maturation was also insufficient to correct a bias within the primary IgM repertoire in these gene-targeted mice. Notably, Kolar et al. (49) have shown that the differences in CDR-H3 length and D4 utilization were reduced in the IgM repertoires when NOD/SCID/β2m−/− mice were reconstituted with human fetal or adult lymphocyte progenitors. This indicates that the observed restrictions during early ontogeny are not exclusively caused by the B cell progenitors themselves but also by yet unknown factors of the environment (49).

Similar to IgM and IgG, the length of the CDR-H3 regions significantly increased by more than five nucleotides from extremely preterm neonates to adults. The paramount cause was the increased addition of nontemplate N nucleotides between the rearranged Variable, Diversity, and Joining gene segments. In theory, adding six random nucleotides to CDR-H3 would increase the potential diversity by 206 = 400 (two random amino acids out of 20 amino acids added). Similar to the fetus, TdT-deficient mice produce very short CDR-H3 regions due to the absence of N nucleotides. Notably, TdT-deficient mice fill their peripheral lymphocyte pool more rapidly than wild-type mice (50, 51), but their secondary responses to NP19-CGG and to lysozyme are weaker (52). In analogy, short CDR-H3 regions might enable human fetuses and neonates rapidly to establish their B cell populations despite the costs of lower Ag affinity of the Ab repertoire, which appears to also persist in the secondary IgA and IgG repertoires.

A further constraint of neonatal IgA transcripts was the predominant use of the Dh7-27 gene segment similar to IgM and IgG transcripts (11, 12). Dh7-27 is the shortest Dh gene segment and the only one of the 27 human Dh gene segments that does not predominantly encode for neutral-hydrophilic amino acids. On the basis of these differences in length and amino acid composition of the CDR-H3 loops, it can be assumed that not only the diversity but also the structural repertoire of Ag binding sites must differ between preterm neonates, term neonates, and adults.

The structure of the Ag binding site has strong influence on its function. It is highly probable that the differing characteristics of IgA transcripts during ontogeny are associated with differing preferences for Ag binding, as for example, anti-hapten Abs usually have shorter CDR-H3 loops than anti-DNA Abs (24, 53). The Ag
binding site typically forms a groove when the CDR-H3 loop is shorter than 14 aa, whereas longer CDR-H3 loops protrude into the solvent (53, 54). According to this differentiation, the frequency of Ag binding grooves would decrease from 55.3% in preterm neonates to 38.1% in term neonates and would subsequently remain stable (40.5% in adults) (preterm versus term, \( p < 0.001 \); preterm versus adults, \( p < 0.005 \)). Taken together, because similar restrictions characterize the CDR-H3 repertoires of neonatal IgM, IgG, and IgA, it is possible that similar restrictions also apply to the Ag binding properties.

One limitation of our study is that due to low sample volumes, we have analyzed IgA transcripts from whole blood and not from sorted B cell subpopulations. Because longitudinal samples were unavailable for this study, each blood sample originates from a different individual, and we cannot measure the intraindividual development. It is possible that the observed differences during ontogeny partially arise from differing relations of B cell subpopulations [e.g., plasmablasts/plasma cells and memory B cells, and newly defined B1 cells (44, 55)] or differing routes of IgA formations (T cell dependent/independent) (41). Moreover, differing selective forces could act upon the membrane bound versus secretory IgA, which cannot be distinguished with our experimental approach. The observed differences during ontogeny are probably not due to differing frequency of IgA1- and IgA2-producing cells, as in harmony with Schauer et al. (56), we found similar IgA1 to IgA2 ratios of \( \sim 6.5:1 \) in preterm neonates, term neonates, and adult

**FIGURE 5.** Ag selection of IgA transcripts. Inference of Ag selection in IgA transcripts from preterm neonates (A), term neonates (B), and adults (C). Shown is the ratio of replacement mutations in CDR-H1 and CDR-H2 \( (R_{\text{CDR}}) \) to the total number of mutations in the V region \( (M_V) \) plotted against \( M_V \). Numbers of sequences are written in the dots; the sizes of the dots increase with the number of sequences with the same parameters. The shaded area represents the 95% confidence limits for the probability of random mutations. A data point falling outside these confidence limits represents a sequence that has a high proportion of replacement mutations in the CDR. The probability that these mutations occurred randomly is \( p < 0.05 \). Two percent of preterm and term neonate IgA transcripts exhibited statistical signs of Ag selection in comparison with 34% of adult IgA transcripts \( (p < 0.05, \text{two-tailed } \chi^2 \text{ test}, \text{respectively}) \).
blood samples using quantitative PCR (data not shown). Feeding with breast milk versus formula milk has significant impact on the maturation of the IgA repertoire in both B-1 and B-2 cell-derived gut plasmablasts. J. Immunol. 174: 435–445.


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The authors have no financial conflicts of interest.

Disclosures

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23. Lanning, D., P. Sethupathi, K. J. Rhee, S. K. Zhai, and K. L. Knight. 2000. Characterization of the ability to form Abs against various Ags (59). We found that the IgA repertoires of preterm and term neonates are subject to the same restrictions as the IgM and IgG repertoires and that signs of Ag-driven selection accumulated very slowly. Thus, regarding D_{H} utilization, CDR-H3 length, and somatic mutations, the diversification of the IgM, IgA, and IgG repertoire depends predominantly on maturation and less on Ag contact. The time of maturation for the IgA repertoire in the neonate obviously exceeds the time of passive IgA transfer through mother’s milk by far. From a teleological view, it is highly unlikely that the strictly regulated diversification of the fetal and neonatal Ab repertoire would represent only immaturity per se. Instead, the current view is that the altered immune status of the fetus and neonate, including Ag binding properties of the IgM, IgA, and IgG repertoire, may be beneficial to establish tolerance to self-antigens or maternal Ags and may contribute to the prevention of autoimmune and allergic diseases (59). However, the preterm neonate might be highly susceptible to infections and less responsive to vaccination because its immune system is still at the stage of tolerance induction instead of self-defense.

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