Delayed B-cell maturation and attenuated vaccine responses in infants exposed to B-cell depleting therapies *in utero*

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Clinical Implications

In utero exposure to anti–cluster of differentiation 20 monoclonal antibodies can result in transient B-cell depletion, delayed B-cell maturation, decreased immunoglobulin production, and inadequate vaccine responses in the infant. These children require immunological follow-up and personalized vaccine schedules.

Pregnant women may require B-cell depleting therapies for various reasons, yet data on their safety and impact on infants' immune system remain limited.[1,2] Rituximab (RTX), an IgG_1 anti–cluster of differentiation 20 (anti-CD20) monoclonal antibody, can cross the placental barrier. RTX treatment during pregnancy or in close proximity to conception has been shown to induce B-cell depletion in infants (reviewed by Das et al[3]), but comprehensive details on their B-cell reconstitution are infrequently reported. Both the American College of Rheumatology and the British Society of Rheumatology recommend discontinuing RTX at conception but suggest considering its use during pregnancy in cases of severe maternal disease.[1,2] The sole current recommendation for infants exposed in the third trimester advises withholding live vaccines until 6 months of age, primarily based on expert opinion.[2] Obinutuzumab (OBI), a newer anti-CD20 monoclonal IgG_1, exhibits enhanced B-cell depletion *in vitro*.[4] Although its current indications are for hematologic malignancies, the similarity to RTX makes it likely that its use will expand to autoimmune diseases. To date, no information is available on the effects of *in utero* exposure to OBI.

In January 2019, Switzerland introduced universal newborn screening (NBS), quantifying both T-cell receptor excision circles and kappa-deleting recombination excision circles (KREC), thus allowing for the identification of not only severe T- but also B-cell deficiencies.[5] Here, we describe the natural course of B-cell reconstitution in children with abnormal KREC levels in their NBS after *in utero* exposure to anti-CD20 therapies.

The Cantonal Ethics Commission of Zurich approved this study (2022-01029). Parents or legal guardians gave informed consent per protocol.

Between January 2019 and October 2023, 9 of approximately 420,000 tested newborns were identified with low KREC levels and a history of *in utero* exposure to anti-CD20 therapies (Table I). In 2 cases, preterm labor was induced: one due to concern for maternal thrombotic thrombocytopenic purpura affecting the fetus and the other due to a preference for limiting fetal exposure to chemotherapy. Maternal IgG levels were available in 3 cases: 7.6 g/L in P3, 12.2 g/L in P5, and 2.4 g/L in P9. Immunological follow-up had been arranged for 2 newborns before NBS results were available.

Clinical examination showed normal findings in all children. In the first measurement (median age 31 days), 5 infants had unmeasurable or severely decreased CD19+ B cells (Figure 1). Children exposed to chemotherapy in addition to RTX during pregnancy tended to have lower B-cell counts at 1.5 to 3 months of age (Table I, 4 infants in each group, $P = .11$). B cells normalized in all children (median age 3, maximum 7 months). On detection of B cells, 4 of 7 patients with available data had no switched memory B cells (Figure E1, available in this article's Online Repository at www.jaci-inpractice.org). In addition, transient moderate neutropenia was observed in 3 children (minimal absolute neutrophile count 0.63×10^9/L).

All infants showed delayed production of IgM and IgA. IgM remained below the detection limit in 5 of 6 children with measurements available between 1 and 2 months of age, but normalized by a median age of 3 months (range: 2-7 months). IgG levels were reduced in the initial measurement in 1 child (Figure E2, available in this article's Online Repository at www.jaci-inpractice.org, P7) but fell below the age-appropriate reference range in 7 patients during follow-up. Spontaneous recovery to normal IgG levels was observed in 4 of these infants by a median age of 7 months (range: 5-18 months). Two children had reduced IgG levels at the age 7 months but did not undergo further follow-up. One child (P1) received immunoglobulin replacement therapy from ages 4 to 5 months.

Eight infants received 2 doses of the hexavalent DTPa-HepB-IPV+Hib (diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated polio virus, and *Haemophilus influenzae* type b) and PCV13 (13-valent pneumococcal conjugate) vaccine at ages 2 and 4 months. In 4 children, additional third doses of both vaccines were administered at 6 months of age without measuring vaccine antibody levels ("blindly adjusted"). Four children had inadequately low or unmeasurable levels of specific antibodies to vaccine antigens after the first or second dose and therefore received additional third doses of the hexavalent vaccine, PCV13, or both vaccines (Table I). By 9 months of age, in addition to normal T-cell numbers, vaccine-specific antibody levels similar to normal children[6,7] or increased levels compared with baseline in at least one of the tested vaccine antibodies were present in all 8 patients with available data. Therefore, MMR(V) (measles, mumps, rubella, and varicella) vaccine was recommended, in accordance with the Swiss national immunization schedule. All 3 children with follow-up data beyond the administration of MMR(V) experienced no complications. None of the 9 infants experienced severe or unusual infections.

This case series represents the first comprehensive and prospective longitudinal account, starting from birth, detailing the development of the infant immune system after *in utero* exposure to anti-CD20 monoclonal antibodies. In addition, it offers the first description of immunological findings in neonates after *in utero* exposure to OBI.

TABLE I. Overview of infant and maternal characteristics

Infant	Sex GA (w) BW (g)	Anti-CD20 indication in mother	Anti-CD20 No. of doses Timing	Other maternal medication or therapies	KREC/TREC (copies/punch, normal range: >3/>5)	Peripheral blood CD19+ cells at age 1.5-3 mo ($\times 10^9$/L, normal range: 0.3-2.0)	Ig levels at age 0-6 mo	Specific IgG antibodies to vaccine antigens	Vaccination schedule	Neutropenia Min ANC ($\times 10^9$/L, normal range: 1.0 to 9.0) Duration	Last FU (mo)
P1	F 36 6/7 2830	Diffuse large B-cell lymphoma	RTX 2 WG 27-36	CHOP	1/189	0.00	IgG ↓, IgM ↓, IgA ↓	Not measured	Delayed post-IVIG, further details n/a	No	7
P2	M 36 4/7 3460	Diffuse large B-cell lymphoma	RTX 4 WG 28-36	CHOP	2/43	0.12	IgG ↓, IgM ↓, IgA ↓	Anti-T and anti-Hib in adequate range after 3 doses	Blindly adjusted*: MMRV recommended at 9 and 12 mo	No	7
P3	F 36 6/7 2700	Diffuse large B-cell lymphoma	RTX 1 WG 35	CHOP	0/80	0.00	IgG ↓, IgM N, IgA ↓	No increase in anti-T, anti-Hib, or anti-PC after 1 dose Clear increase in anti-PC after third dose, but not for anti-T or anti-Hib	Adjusted due to low responses†: +1 DTPa-HepB-IPV+Hib +1 PCV13 MMRV at 9 and 12 mo	Yes 0.69 Age 2-18 mo‡	18
P4	M 37 0/7 2540	Hodgkin lymphoma	RTX 4 WG 21-30	CHOP	0/99	1.21	IgG ↓, IgM N, IgA ↓	Anti-T and anti-PC in adequate range after 3 doses	Blindly adjusted*: MMRV recommended at 9 and 12 mo	No	9
P5	F 37 2/7 2690	Kell-mediated HDFN	RTX 5 WG 23-35	Immunoadsorption WG 23-35, IVIG after each cycle	0/134	0.74	IgG N, IgM N, IgA N	Anti-T and anti-Hib in adequate range after 2 doses, anti-PC low Clear increase in anti-PC after third dose	Adjusted due to low responses†: +1 PCV13 MMR at 9 and 12 mo	Yes 0.63 Age 2-5 mo‡	14
P6	M 38 0/7 3310	Rhesus-mediated HDFN	RTX 6 WG 15-33	Immunoadsorption WG 15-33, IVIG after each cycle	0/80	2.81	IgG ↓, IgM N, IgA ↓	Anti-T and anti-Hib in adequate range after 3 doses	Blindly adjusted*: MMR recommended at 9 and 12 mo	No	7
P7	F 33 0/7 1280	TTP, recurrence at week 26	RTX 4 WG 30-33	Plasmapheresis WG 26-33, prednisolone WG 26-33, caplacizumab WG 33	0/32	2.64	IgG ↓, IgM N, IgA N	Anti-T, anti-Hib, and anti-PC in adequate range after 3 doses	Blindly adjusted*: MMRV recommended at 9 and 12 mo	Yes 0.89 Age 6-8 mo‡	8
P8	F 40 4/7 3150	Neuromyelitis optica	RTX 2 WG 22-24	Plasmapheresis WG 22, methylprednisolone pulses WG 19	0/280	0.64	IgG ↓, IgM ↓, IgA ↓	Anti-PC and anti-Hib in adequate range after 2 doses, anti-T low Clear increase in anti-T after third dose	Adjusted due to low responses†: +1 DTPa-HepB-IPV+Hib MMRV recommended at 9 and 12 mo	No	8
P9	F 39 0/7 3110	Follicular B-cell lymphoma	OBI for 2.5 y until 6 mo preconception	Venetoclax, dexamethasone until 6 mo preconception	1/116	2.07	IgG N, IgM N, IgA ↓	Anti-PC in adequate range after 2 doses, anti-T and anti-Hib low Further measurements refused	Adjusted due to low responses†: +1 DTPa-HepB-IPV+Hib MMR at 9 and 12 mo	No	36

↓, Below reference range; *ANC*, absolute neutrophile count; *BW*, birth weight; *CD*, cluster of differentiation; *CHOP*, cyclophosphamide, hydroxydaunorubicin, oncovin (vincristine), and prednisone; *D*, diphtheria; *F*, female; *FU*, follow-up; *GA*, gestational age; *HBV*, hepatitis B virus; *HDFN*, hemolytic disease of the fetus and newborn; *Hib*, *Haemophilus influenzae* type b; *IPV*, inactivated poliovirus; *KREC*, kappa-deleting recombination excision circles; *M*, male; *MMR*, measles, mumps, and rubella; *MMRV*, measles, mumps, rubella, and varicella; *n/a*, not available; *OBI*, obinutuzumab; *Pa*, acellular pertussis; *PC*, pneumococcus; *PCV13*, 13-valent pneumococcal conjugate vaccine; *RTX*, rituximab; *T*, tetanus; *TTP*, thrombotic thrombocytopenic purpura; *TREC*, T-cell receptor excision circles; *WG*, week of gestation.

*After the first 2 regular doses at ages 2 and 4 months, third doses of DTPa-HepB-IPV+Hib and PCV13 were administered at age 6 months without prior measurement of specific antibody levels

†After the first 2 regular doses at ages 2 and 4 months, additional doses were administered only if there was no clear increase of the respective specific antibody levels compared with baseline measurements.

‡Age at first abnormal measurement to age at normalization.

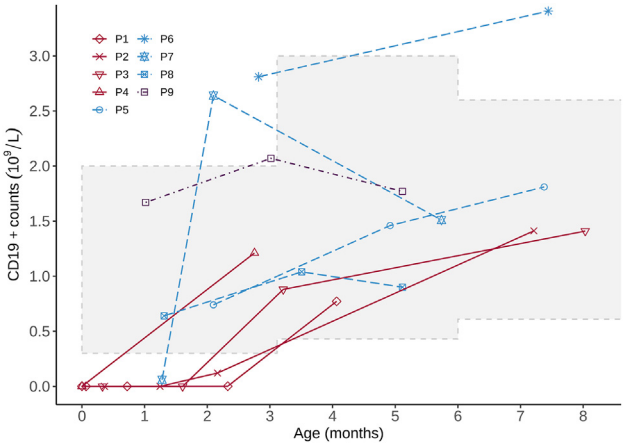

FIGURE 1. Natural development of peripheral blood CD19+ B cells of the children exposed to anti-CD20 therapies *in utero*. Data from infants exposed to rituximab and chemotherapeutic agents are shown in red, rituximab and plasmapheresis/immunoadsorption in blue, and obinutuzumab and venetoclax in purple. The age-appropriate reference range is shaded gray. *CD*, Cluster of differentiation.

In line with earlier reports on fetal RTX exposure,[3] all patients showed a relatively rapid normalization of B-cell counts and remained free from severe infections during periods of transient B-cell deficiency. However, the families' heightened awareness of detected abnormalities likely leading to risk-reducing behaviors and administration of additional vaccine doses might have contributed to their favorable clinical outcomes. Maternal IgG levels were available for only 3 cases, ranging from 2.4 to 12.2 g/L. Two mothers had received IgG infusions as a part of their plasmapheresis protocols. Therefore, the amount of placentally transferred maternal IgG might vary widely among mothers during the pregnancy and after delivery. Thus, caution is warranted in extrapolating these findings to all children exposed to B-cell depleting therapies *in utero*.

Approximately 4 weeks after the initial or second regular vaccine dose, concentrations of specific antibodies against vaccine antigens were either undetectable or indistinguishable from the placentally transferred background level for tetanus toxoid, Hib, and/or pneumococcal polysaccharides in patients tested at that time. The comparison with baseline-specific antibody levels obtained before any vaccine doses were given allowed differentiation between maternally transferred and actively produced IgG, ensuring accurate assessment of the child's immune response. However, vaccine-specific antibody levels similar to normal children were observed in 6 of 7 patients when tested after receiving 3 doses of the respective vaccines. These observations strongly indicate a relevant impact of fetal exposure to B-cell depleting agents on the child's adaptive immune system and underscore the importance of routine immunological monitoring and personalized vaccination schedules in these children, challenging the current recommendation of just delaying live vaccinations until a specific age.[2] Rather than delaying the live vaccines, it seems reasonable to measure baseline vaccine antibody levels before the first dose, administer the inactivated vaccines according to schedule, and check for a significant increase after 2 doses. If needed, additional doses should be administered to ensure an adequate B-cell response. If obtaining repeat samples proves difficult, vaccinating these children with at least 3 doses of the hexavalent and PCV vaccines is as a reasonable alternative.

Transient pneutropenia was observed in one-third of the cohort, a rarely reported finding to date.[8] Although well documented in X-linked agammaglobulinemia,[9] the underlying mechanism remains unclear in both conditions.

As all patients in our cohort were identified through NBS, it is important to acknowledge a potential selection bias toward more severely affected newborns. The total number of exposed infants during this period is unknown.

In summary, this study highlights delayed B-cell maturation, inadequate vaccine responses, and neutropenia in children exposed to anti-CD20 therapies *in utero*, indicating potential vulnerability. These findings emphasize the importance of regular follow-up of such children in a specialized immunology clinic to assess B-cell reconstitution, immunoglobulin production, and vaccine responses. This personalized approach ensures that clinical management, including vaccination schedules, is tailored to the individual needs of these children, thereby optimizing their protection.

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REFERENCES

1. Sammaritano LR, Bermas BL, Chakravarty EE, Chambers C, Clowse MEB, Lockshin MD, et al. 2020 American College of Rheumatology guideline for the management of reproductive health in rheumatic and musculoskeletal diseases. Arthritis Rheumatol 2020;72:529-56.
2. Russell MD, Dey M, Flint J, Davie P, Allen A, Crossley A, et al. British Society for Rheumatology guideline on prescribing drugs in pregnancy and breastfeeding: immunomodulatory anti-rheumatic drugs and corticosteroids. Rheumatology 2023;62:e48-88.
3. Das G, Damotte V, Gelfand JM, Bevan C, Cree BAC, Do L, et al. Rituximab before and during pregnancy. Neurol Neuroimmunol Neuroinflamm 2018;5:e453.

4. Davies A, Kater AP, Sharman JP, Stilgenbauer S, Vitolo U, Klein C, et al. Obinutuzumab in the treatment of B-cell malignancies: a comprehensive review. Future Oncol 2022;18:2943-66.
5. Trück J, Prader S, Natalucci G, Hagmann C, Brotschi B, Kelly J, et al. Swiss newborn screening for severe T and B cell deficiency with a combined TREC/KREC assay—management recommendations. Swiss Med Wkly 2020;150:w20254.
6. Käyhty R, Peltola H, Karanko V, Mäkelä PH. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. JInfect Dis 1983;147:1100.
7. World Health Organization. The immunological basis for immunization series: module 3: tetanus, update 2018 (immunological basis for immunization series; module 3). Geneva: World Health Organization. Accessed June 19, 2024. https://iris.who.int/handle/10665/275340
8. Maggen C, Dierickx D, Cardonick E, Mhallem Gziri M, Cabrera-Garcia A, Shmakov RG, et al. Maternal and neonatal outcomes in 80 patients diagnosed with non-Hodgkin lymphoma during pregnancy: results from the International Network of Cancer, Infertility and Pregnancy. Br J Haematol 2021;193:52-62.
9. Jacobs ZD, Guajardo JR, Anderson KM. XLA-associated neutropenia treatment: a case report and review of the literature. J Pediatr Hematol Oncol 2008;30:631.

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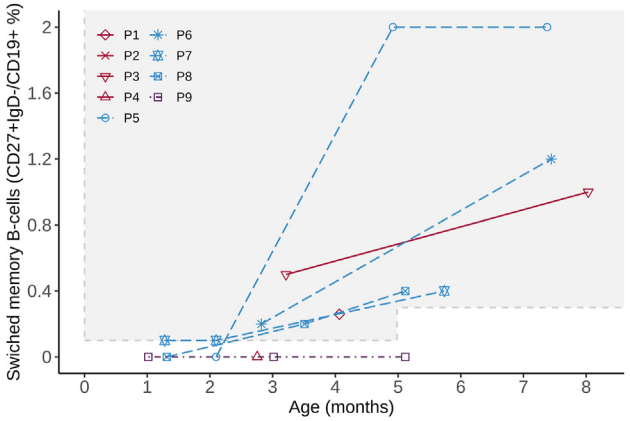

FIGURE E1. Natural course of the fraction of peripheral blood switched memory B cells as a percentage of all CD19+ B cells. B-cell subset data were available on at least 1 occasion for 8 patients. The trajectories varied considerably, with 4 patients showing no switched memory B cells at the first measurement. In 6 of the 7 patients with sufficient data points, the fraction normalized by the age of 5 months or earlier. The age-appropriate reference range is shaded gray.

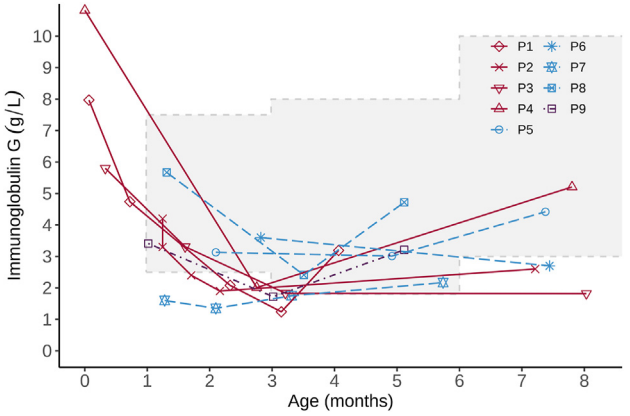

FIGURE E2. Natural course of serum IgG levels. Initial measurement showed a reduced IgG concentration in one child (P7) and levels fell below the reference range in 7 patients during follow-up. Spontaneous recovery to normal IgG levels was observed in 4 of these infants by a median age of 7 months (range: 5-18 months, P3 not shown). Two children had reduced IgG levels at 7 months but did not undergo further follow-up. One child (P1) received immunoglobulin replacement therapy from ages 4 to 5 months. The age-appropriate reference range is shaded gray.