

Immunodeficiency Due to a Unique Protracted Developmental Delay in the B-Cell Lineage

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A unique immune deficiency in a 24-month-old male characterized by a transient but protracted developmental delay in the B-cell lineage is reported. Significant deficiencies in the number of B cells in the blood, the concentrations of immunoglobulins in the serum, and the titers of antibodies to T-dependent and T-independent antigens resolved spontaneously by the age of 39 months in a sequence that duplicated the normal development of the B-cell lineage: blood B cells followed by immunoglobulin M (IgM), IgG, IgA, and specific IgG antibodies to T-independent antigens (pneumococcal polysaccharides). Because of the sequence of recovery, the disorder could have been confused with other defects in humoral immunity, depending on when in the course of disease immunologic studies were conducted. Investigations of X-chromosome polymorphisms suggested that the disorder was not X linked in that the mother appeared to have identical X chromosomes. An autosomal recessive disorder involving a gene that controls B-cell development and maturation seems more likely. In summary, this case appears to be a novel protracted delay in the development of the B-cell lineage, possibly due to an autosomal recessive genetic defect.

The most common types of congenital immunoglobulin G (IgG) deficiencies in human infants are X-linked agammaglobulinemia (XLA) (7, 43) and a transient hypogammaglobulinemia (12, 17, 31, 42, 47) (see Table 1). XLA is due to defects in a member of the Src family of tyrosine kinases, Bruton's tyrosine kinase (BTK) (5, 23, 35, 43, 44, 51, 53, 54). Although some phenotypic variations are found (8, 20, 28, 44, 56), defects usually lead to profound deficiencies in blood B cells, plasma cells, all isotypes of serum immunoglobulins, and specific antibody formation. Consequently, there is a paucity of detectable peripheral lymphoid tissue and an increased frequency of highly virulent, encapsulated respiratory bacterial infections and systemic enterovirus infections.

In contrast to XLA, the diagnostic immunological criteria for transient hypogammaglobulinemia of infancy remain problematical. A brief review of the normal ontogeny of the B-cell lineage (4) will help in the consideration of this problem. (i) B cells first appear in fetal life. The proportions of B cells in the blood and spleen at 22 weeks of fetal life are similar to those in adults, although fetal B cells are not completely mature (24). At birth, the number and function of blood B cells are similar to those of adults. (ii) The fetus can produce pentameric IgM, but that usually does not occur except during intrauterine infections. In normal mature newborn infants, levels of total IgG in serum are similar to those in adults because of placental transfer of that immunoglobulin isotype (18, 30). Because no other immunoglobulins are transmitted to the fetus via the placenta and the fetus is sheltered from foreign immunogens, concentrations of other immunoglobulins in serum are very low at birth. (iii) Soon thereafter, concentrations of IgM in serum rise, whereas the production of other immunoglobulin isotypes is delayed. (iv) The levels of IgG transferred via the

placenta gradually fall and production of that isotype slowly increases during the first months of postnatal life until the nadir of concentrations of IgG in serum is reached at the age of 5 to 6 months (36, 37, 57). (v) IgA production is delayed even further (57). (vi) Furthermore, specific IgG antibodies to T-independent immunogens do not appear until after the age of 2 years (1).

In transient hypogammaglobulinemia of infancy, the numbers of B cells are normal, whereas the synthesis of immunoglobulins is delayed (12, 17, 31, 42, 50). Deficiencies of serum IgG are below 2 standard deviations for the mean for the age (the nadir is usually between 100 to 150 mg/dl) (12, 31, 42, 50) but usually are not as marked as those seen in XLA (43). Concentrations of IgA and IgM in serum are usually not as greatly depressed as those of IgG (12, 31, 42, 50). Specific antibody formation is spared (12, 31, 50). Males and females are equally affected. Peripheral lymphoid tissue is readily detectable, and the clinical consequences are usually restricted to an increased susceptibility to mild to moderate upper- and lower-respiratory tract infections. In that disorder, all serum immunoglobulin concentrations usually normalize by the age of 2.5 to 3.5 years, although persistent IgA deficiencies have been reported (12).

The definition of this problem as a disease has been questioned (12, 31, 57). Wilson and his colleagues (57) argued persuasively that many of these cases represent normal physiological variations in the concentrations of IgG in serum. In this respect, we will describe a transient deficiency in the B-cell lineage that was more extensive than those found in the classical transient hypogammaglobulinemia of infancy. Furthermore, in contrast to previous reports of transient hypogammaglobulinemia of infancy, the sequence of recovery from these immunodeficiencies followed the normal order of development of the B-cell lineage (4). Those immunological abnormalities, as well as the pattern of recovery, suggested that this is a unique immunodeficiency or that certain previous cases of this disorder were categorized as physiological variants of im-

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munoglobulin development because of inadequate longitudinal studies of the B-cell lineage.

CASE REPORT

The patient, a Caucasian nonidentical twin male, was born prematurely with a birth weight of 2.2 kg. His gestational age was clinically estimated to be about 37 weeks. The mother received terbutaline for several days to "delay labor" and dexamethasone for two days at the end of her pregnancy to "help the baby's lung development." No adverse effects of those agents on the newborns were noted.

The patient was breast-fed for the first 4 months of life. Afterward, he developed a symmetrical, eczematous, pruritic dermatitis on the scalp, face, and flexor surfaces of the extremities that was thought to be atopic dermatitis. The dermatitis responded well to topical corticosteroids. At 12 months, recurrent episodes of either otitis media, sinusitis, conjunctivitis, or bronchitis began. Those illnesses were treated with oral antibiotics. In addition, the bronchitis was treated a few times briefly with oral prednisone.

At 21 months of age he developed vomiting, diarrhea, fever, otitis media, and pharyngitis. Soon thereafter, urticaria appeared on the trunk and abdomen. Group A β -hemolytic streptococcus was cultured from the posterior pharynx. Radiograms revealed partial opacification of the maxillary and ethmoid paranasal sinuses. Oral phenoxymethylpenicillin was administered. The fever gradually subsided, but the urticaria spread. A corticosteroid injection was given because of a suspected drug-induced eruption.

Two days later he developed excessive lethargy, fever (40.5°C), rusty brown watery stools, and generalized tonic-clonic seizures. The cerebrospinal fluid (CSF) glucose (~55 mg/dl) and CSF cell count (<5/mm³) were normal, but the CSF protein (261 mg/dl) was elevated. No bacterial pathogens or viruses were cultured from the blood, stool, or CSF. A hemogram revealed neutropenia (105 neutrophils and 376 band forms/mm³); the blood lymphocyte count (3,102/mm³) was normal. The neutropenia resolved 2 days later. Serum aspartate aminotransferase and alanine aminotransferase levels were initially increased (392 and 232 IU/liter, respectively) but were normal 4 days later.

A viral infection involving the brain and liver was suspected. He was initially treated with lorazepam, phenytoin, chloramphenicol, and acyclovir. On the 4th day of hospitalization antibiotics were discontinued, phenytoin was replaced with carbamazepine, and corticosteroids were administered intravenously because of a generalized eruption consistent with drug-induced erythema multiforme. The fever and eruption subsided over the next 4 days. He was then discharged on no medications, except oral carbamazepine for 2 weeks.

One month later (at 24 months of age), we examined him. His weight (11.7 kg) and height (84.5 cm) were normal. The upper eyelids were mildly edematous. The skin was slightly dry and coarse. Lichenifications were present on the face and the antecubital and popliteal fossae. Scalp hair, eyebrows, eyelashes, and nails appeared normal; sweating was detected. Subcutaneous lymph nodes, tonsils, and posterior pharyngeal lymphoid tissue were of normal size. The tip of the spleen was palpable; the liver size was normal. Radiograms revealed a prominent adenoidal shadow and residual evidence of maxillary/ethmoid sinusitis.

MATERIALS AND METHODS

Immunologic investigations. (i) **Quantitation of proteins in serum.** The concentrations of IgG, IgA, IgM, C3, and C4 were determined by nephelometry, and the concentration of IgG subclasses were determined by radial immunodiffusion.

(ii) **Flow cytometry.** Populations and subpopulations of blood lymphocytes were enumerated by two-color flow cytometry (6). Standards and normal controls were run in our laboratory with each specimen. About 96% of total blood lymphocytes were accounted for by these phenotypic analyses. The values for the control subject for that day were within the normal range. Two-color flow cytometry was also performed on blood B cells and B-lymphoblastoid cell lines produced by infecting blood B cells with the Epstein-Barr virus obtained from a marmoset cell line (46) to detect the following surface antigens: CD19, CD20, CD21 (the receptor for complement and the Epstein-Barr virus), CD22 (a marker of mature recirculating B cells) (15), CD23 (the Fcε receptor II) (45), and CD40 (receptor for CD39).

(iii) **In vitro proliferation of blood lymphocytes.** The proliferative response of blood T cells cultured for 3 days with phytohemagglutinin-P (Difco Laboratories, Detroit, Mich.) or for 5 days with *Candida albicans* dialyzed free of glycerol (Hollister-Stier, Division of Miles Laboratory, Elkhart, Ind.) was tested by quantifying the incorporation of [³H]thymidine (ICN Pharmaceuticals, Inc., Costa Mesa, Calif.; specific activity, 2 Ci/mmol; 1 μCi/2 × 10⁵ lymphocytes/well) into those cells.

(iv) **Specific antibody formation.** Antibodies to T-dependent antigens (tetanus toxoid [Wyeth Laboratories, Inc., Marietta, Pa.] and diphtheria toxoids [Connaught Labs, Willowdale, Ontario, Canada]) and T-independent antigens (pneumococcal polysaccharides [Pneumococcal Vaccine Polyvalent, Pnu-Imune 23; Lederle Laboratories Division, Pearl River, N.Y.]) were measured by enzyme-immunoassays (6) before and 14 days after immunizations. IgM antibodies to pneumococcal polysaccharides were determined. IgG and IgA antibodies to all immunogens were quantified.

Family studies. A pedigree chart was constructed from the family history. The occurrence of any increased susceptibility to infection among the family members was sought. In addition, immunologic investigations were conducted on the mother, the nonidentical twin brother, and a maternal cousin.

X-chromosome gene polymorphisms. Polymorphisms of short tandem repeats of the androgen receptor gene (location, Xq12) (48), DXS441 (location, Xq13.3) (40), DXS458 (location, Xq21.1-23) (25, 55), and DXS424 (location, Xq24-25) (25) were used to investigate the inheritance patterns of the X chromosomes in the mother's blood T cells, neutrophils, and B cells and B-lymphoblastoid cells produced by infecting blood B cells with the Epstein-Barr virus (EBV) (46). Blood mononuclear cells and polymorphonuclear leukocytes were obtained from heparinized venous blood by dextran sedimentation and Ficoll-Hypaque centrifugation. T cells were enriched by centrifuging E-rosetted lymphocytes through Ficoll-Hypaque.

DNA from cell preparations was obtained by lysis with sodium dodecyl sulfate, RNase treatment, and protein precipitation (Puregene DNA Isolation Kit; Gentra Systems, Inc., Minneapolis, Minn.) according to the manufacturer's directions. The sense (5'-TCCAGAATCTGTCCAGAGCGTGC-3') and antisense (5'-GCTGTGAAGGTTGCTGTTCCTCAT-3') primers from the androgen receptor gene (12.5 pmol each) were mixed with 250 ng of genomic DNA, 1 U of *Taq* DNA polymerase, 250 μM deoxynucleoside triphosphates (3 μCi of [³²P]dCTP [3,000 Ci/mmol]), and 1.5 mM MgCl₂ in the manufacturer's suggested buffer (Perkin-Elmer Cetus, Norwalk, Conn.) (total volume, 25 μl). DNA was amplified for 25 cycles at 95°C for 1 min, 65°C for 1 min, and 72°C for 1 min. Amplifications were preceded by a primary denaturation step (95°C for 5 min) and followed by a final extension step (75°C for 8 min) after the last cycle. The same procedures were used to investigate the other polymorphisms except that the annealing temperature was 55°C.

The degree of methylation sensitivity to *HpaII* on the paternal and maternal X chromosomes in cells from the mother was determined to investigate the pattern of X-chromosome inactivation (2). This study was restricted to the androgen receptor gene. When the methylation status of the genomic DNA was examined by digestion with *HpaII*, the DNA (250 ng) was digested with *HpaII* (Promega) in a reaction volume of 4 μl (in the manufacturer's recommended buffer) for 2 h at 37°C before amplification. After amplification and the addition of formamide stop buffer, samples were denatured at 95°C for 5 min and electrophoresed on 4% polyacrylamide-8 M urea gels at 38 W for 1.5 h. Resultant bands were visualized by autoradiography with Hyperfilm-MP (Amersham Corporation, Arlington Heights, Ill.).

RESULTS

Initial immunologic findings. The concentrations of lymphocytes (4,712/mm³), neutrophils (4,864/mm³), platelets (370,000/mm³), and other types of leukocytes in blood were normal except for an increased number of eosinophils (2,560/mm³). The concentrations of C3 and C4 in serum (115 and 18 mg/dl, respectively) were normal, but the concentrations of IgG, IgA, and IgM and of IgG subclasses in serum were pro-

TABLE 1. Longitudinal investigations of the patient's blood B cells and serum immunoglobulins

Age	B cells (no./mm ³)	Concn in serum (mg/dl) of:		
		IgM	IgG	IgA
Patient (mo)				
24	120	22	174	<6
25	500	20	104	<6
26	600	19	92	<6
28		35	* ^a	<6
30		60	*	<6
32		54	*	<6
34		58	*	<6
36		63	650	<6
38	800	101	660	10
39		150	920	35
42	1,200	228	1,110	63
50	600	174	992	107
51	600	142	976	95
52	600	156	896	92
53	600			
54	600	131	818	85
Normal children (yr) ^b				
2-3	700-1,300	47-160	407-1,009	14-122
3-4		45-190	423-1,090	22-157

^a *, data not included because specimens were collected after immunoglobulin infusions were begun.

^b Values represent 95 percentile ranges for the ages given.

foundly deficient (Tables 1 and 2). The concentration of IgE in serum was also very low (less than 10 IU/ml).

The numbers of total CD3⁺ T cells (3,920/mm³), CD3⁺ CD4⁺ T cells (3,040/mm³), CD3⁺ CD8⁺ T cells (900/mm³), and CD16⁺ NK cells (500/mm³) were within normal ranges, whereas the number of blood CD19⁺ B cells was greatly reduced (120/mm³; normal range, 700 to 1,300/mm³) (Table 1).

The incorporation of radiolabeled thymidine was normal in blood lymphocytes stimulated with phytohemagglutinin-P (unstimulated, 543 cpm; normal controls, 322 ± 125 cpm; stimulated with phytohemagglutinin-P, 40,050 cpm; normal controls, 49,912 ± 16,839 cpm) or with *C. albicans* (18,460 cpm).

Except for modest, stable titers of IgG antibodies to diphtheria toxoid that were present before and after immunization, the levels of antibodies to other immunogens were negligible before and after immunization (Fig. 1).

Subsequent immunologic findings. Two months following the initial immunologic evaluation (at the age of 26 months), the number of blood B cells rose to just below the normal range (Table 1). Because the concentrations of immunoglobulins including IgG in serum did not improve (Table 1), at the age of 28 months monthly infusions of human IgG were begun at a dose of 400 mg/kg of body weight. Serum IgG concentrations were quantified just before and 24 h after each infusion. Serum IgG levels were maintained between 1,200 mg/dl (peak) and 650 mg/dl (trough) during the infusion periods (data not shown). Measurements of IgG in serum obtained in the first 5 months of treatment suggested that the biological half-life of the infused IgG was about 20 to 21 days. During that period and thereafter the atopic dermatitis improved steadily and no infections occurred except for a brief rotavirus enteritis at 34 months.

Between the age of 28 to 30 months, the concentration of IgM in serum rose to a normal level (Table 1). Because the decreases in serum IgG levels following infusions lessened over time, infusions were withheld after 35 months to determine

whether IgG was produced. Two months later, the serum IgG concentration stabilized at ~650 mg/dl. Furthermore, at 36 months the concentration of each IgG subclass in serum, except for IgG4, was normal (Table 2). This indicated that the patient was synthesizing IgG1, IgG2, and IgG3. Serum IgA was first detected at 38 months. By 39 months, the concentration of IgA in serum was normal (Table 1).

Serum antibody formation was retested at the age of 38 months. Substantial titers of IgG antibodies to T-dependent antigens were detected (Fig. 1). Levels of serum IgM antibodies to pneumococcal polysaccharides rose after immunization. Some IgG antibodies to those polysaccharides were present before immunization, but the titers fell following immunization. One and one-half months later, the level of antibodies of each immunoglobulin isotype directed against pneumococcal polysaccharides rose, although no further immunization was performed (Fig. 1).

Over the next 6 months, the patient was well except for a few brief viral upper-respiratory tract infections. A repeat immunologic survey at the age of 42 months was normal, but an IgG2 deficiency reappeared at the age of 50 months (Table 2). Also at that time, the eosinophilia (1,500/mm³) reappeared. He remained asymptomatic. At the age of 53 months, the IgG2 deficiency resolved (Table 2), and the blood eosinophil count fell to 600/mm³.

Phenotypic investigations of B cells. The expressions of CD19, CD20, CD21, and CD40 on the surface of B cells in blood obtained from the patient at the age of 54 months and from his mother and nonidentical twin brother were similar (data not shown). EBV-transformed B cells obtained from the patient at the age of 28 months and at the age of 54 months were investigated for those same phenotypic markers. The relative frequencies of B-lymphoblastoid cells that displayed CD19, CD20, CD21, CD23, and CD40 and the degree of the expression of those B-cell surface proteins were similar in both cell lines (data not shown).

Family investigations and X chromosome gene polymorphisms. (i) Family history. Neither the mother, father, an older sister, a nonidentical twin brother, nor maternal or paternal relatives had frequent infections in infancy or thereafter except for recurrent upper-respiratory tract infections in a

TABLE 2. Longitudinal investigations of serum IgG subclass concentrations^a

Age (mo)	Serum IgG subclass concn (mg/dl)			
	IgG1	IgG2	IgG3	IgG4
Patient				
24	110	10	<1	<1
36	450	90	22	<1
38	520	80	27	<1
39	570	100	30	3
42	1,090	310	32	<1
50	960	7	33	<1
51	920	10	22	<1
52	800	90	30	<1
53	1,000	90	30	<1
54	1,000	90	30	<1
Normal children ^b				
9-24	286-680	30-327	13-82	1-65
36-48	381-884	70-443	17-90	1-116

^a Data collected between 24 and 36 months were not included because intravenous immunoglobulin infusions were given during that period.

^b Values represent 95 percentile ranges for the ages given.

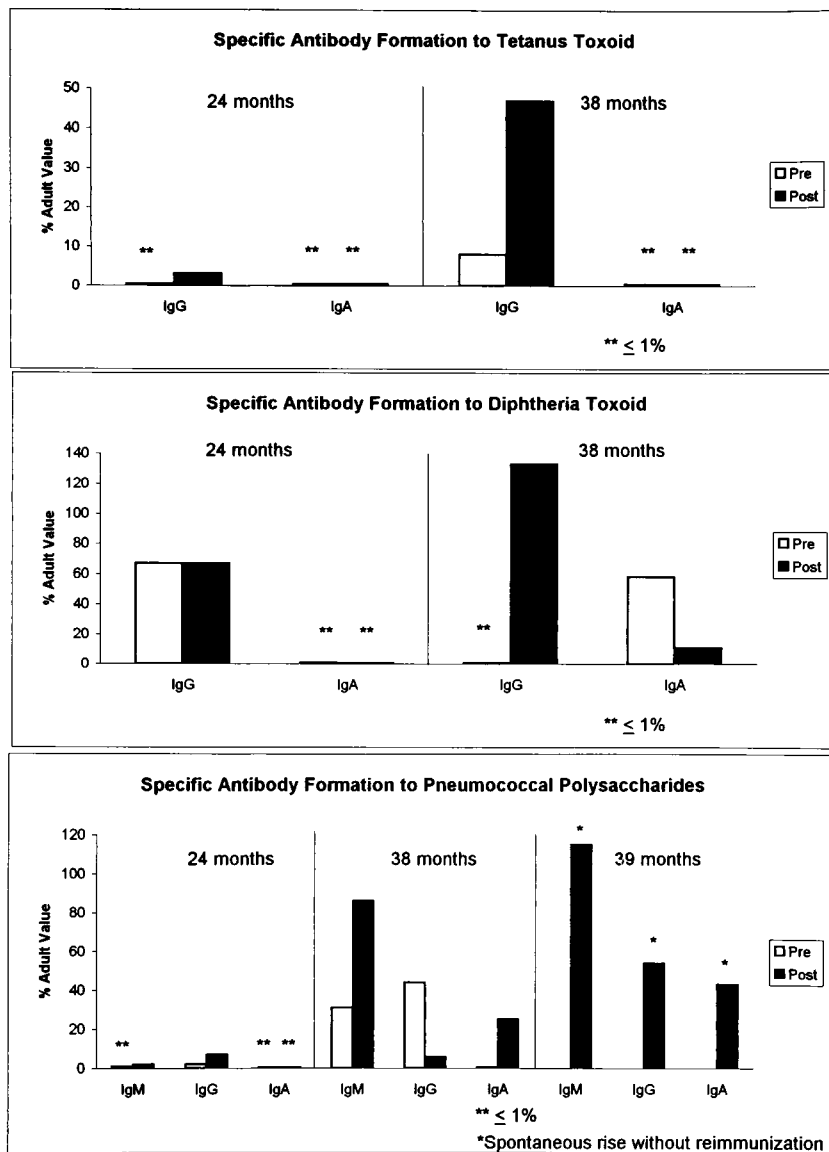


FIG. 1. Concentrations of specific antibodies in serum before (pre) and 2 weeks after (post) immunization. Data are presented as percentages of the activity of a pool of normal human adult sera.

9-year-old boy and a 2-year-old girl of a paternal aunt. In addition, a maternal cousin had Hirschsprung's disease.

The child with Hirschsprung's disease was unavailable for investigation. Immunologic investigations were performed on the mother, the nonidentical twin male, and the younger cousins. The mother and nonidentical twin displayed normal numbers of B cells in blood and normal concentrations of immunoglobulins in serum (data not shown). The concentrations of immunoglobulins in the serum of the cousin were normal (data not shown).

(ii) **X-chromosome gene polymorphisms.** No polymorphisms of each of four tested DNA sequences were found in X chromosomes in the maternal cells (Fig. 2). Because the maternal X chromosomes appeared to be identical, it was not possible to determine the pattern of X-chromosome inactivation in the mother. Therefore, the patterns of androgen receptor gene polymorphisms after treatment with *HpaII* were not shown.

By using certain estimates of the frequencies of homozygous polymorphisms in the general population (25, 40, 48, 55), the

possibility that this pattern was due to chance was calculated by multiplying together the four probabilities of homozygosity (androgen receptor gene, ~0.11; DXS441, ~0.18 to 0.24; DXS458, ~0.34; DXS424, ~0.17). The overall probability was therefore ~0.001. This suggested that the mother's X chromosomes were identical.

The same banding patterns were present in the patient, his nonidentical twin brother, the maternal grandmother, and the maternal grandfather (Fig. 2). However, the maternal grandmother also displayed other polymorphisms that indicated that her X chromosomes were not identical (Fig. 2). Thus, these data suggested that the patient and his nonidentical twin brother had identical X chromosomes.

DISCUSSION

Several well-characterized diseases were considered as possible causes of the immune deficiencies that this patient presented. The first was a genetic defect in BTK that causes XLA

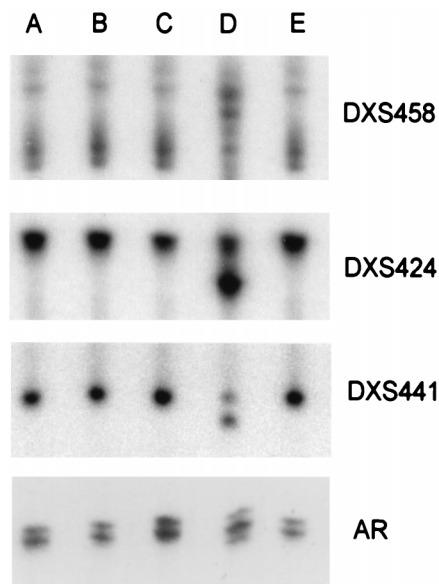


FIG. 2. Patterns of four DNA polymorphisms found on the q region of the X chromosome. X chromosomes in the mother's B cells (lane C) failed to demonstrate polymorphisms. This was consistent with a pair of identical X chromosomes. The patient (lane A), his nonidentical twin brother (lane B), and the maternal grandfather (lane E) displayed the same DNA patterns. The maternal grandmother (lane D) displayed the same banding patterns, as well as bands indicating a nonidentical X chromosome.

(5, 7, 23, 35, 43, 51, 53, 54). As in XLA, the number of blood B cells and the concentrations of IgM, IgG, and IgA in serum were initially greatly reduced. But in contrast to patients with XLA, peripheral lymphoid tissues were readily detected and the defect in specific antibody formation was incomplete. The early age of onset and the deficiency of B cells were not in keeping with common variable immunodeficiency (13). This case also differed from transient deficiencies of certain IgG subclasses (34) or from transient hypogammaglobulinemia found in patients with anhidrotic ectodermal dysplasia (26). A phenytoin-induced immunodeficiency was considered because of the child's brief exposure to that drug, but that was unlikely since the immunodeficiency is restricted to IgA or to certain IgG subclasses (27, 43) and deficiencies in numbers of B cells have not been recognized in that drug-induced disorder.

Because the patient was also briefly exposed to systemic corticosteroid therapy shortly before birth and 1 month before our initial immunological investigations, we also examined the issue whether the alterations in the B-cell lineage and its immunoglobulin products were secondary to that immunosuppressive agent. Corticosteroids are known to directly or indirectly affect the functions of B cells by several different mechanisms (11, 21, 22, 41), but corticosteroids do not commonly affect the numbers of human blood B cells (21). Furthermore, the normal numbers of T-cell subpopulations and the normal response of blood T cells to a mitogen were not consistent with an acquired defect due to iatrogenic immunosuppression (3, 19, 21).

The issue was raised whether the hypogammaglobulinemia developed because of a protein-losing enteropathy secondary to a food allergen. The eosinophilia and eczema were consistent with a food allergy. An allergic gastroenteropathy leading to a massive loss of protein into the gastrointestinal tract seemed unlikely, however, because the biological half-life of intravenously infused human IgG was normal at a time when

the child was deficient in IgG. Further, it would not be anticipated that the recovery of the deficiencies would follow an isotype-specific pattern. Could the problem be due to a loss of B cells into the intestinal tract? In enteropathies characterized by a loss of lymphocytes into the gastrointestinal tract, the lost cells are principally T cells. Consequently, a deficiency of blood T cells develops (49). It would thus be unlikely that the lymphocyte deficiency would be restricted to B cells, as occurred in our patient.

We also considered whether the patient had a severe combined immunodeficiency (SCID) complicated by a mild graft versus host (GVH) reaction due to a T-cell engraftment from the mother or the twin brother. The dermatitis, eosinophilia and transient splenomegaly were consistent with that possibility. When the patient was first evaluated, no HLA typing, gene typing, karyotyping, or skin biopsies were performed to rule out that possibility. However, in contrast to other cases of SCID with a GVH reaction (32), both the proportions of blood T-cell subpopulations and the proliferative responses of blood T cells to a phytomitogen and a specific antigen were normal.

Did this patient have a novel transient deficiency in the B-cell lineage? It was evident from the first immunological evaluation of this patient that the disease was not consistent with previous reports of transient hypogammaglobulinemia of infancy because of the significant quantitative deficiency of blood B cells and because the deficiencies of the serum immunoglobulins exceeded those reported in most cases of transient hypogammaglobulinemia of infancy (12, 17, 31, 42, 50). Most importantly, the pattern of recovery of the B-cell system that recapitulated the normal development of the B-cell lineage and its products (4) has not been reported. For those reasons it does not appear that this disorder is a variant of a "physiologic immunodeficiency," as defined by Wilson and his colleagues (57).

Given the order in which the defect spontaneously resolved, it is likely that the disorder could have been confused with other primary B-cell disorders depending on when in the course of the disease the child was studied. For instance, this disorder could have been mistaken for XLA at 24 months; for common variable immunodeficiency or previously reported types of transient hypogammaglobulinemia of infancy at 26 months; for sporadic, congenital, or phenytoin-induced selective IgA deficiency at 30 months; or for a defect limited to the formation of antibodies to T-independent antigens at 36 months. In that respect, it is possible that deficiencies of blood B cells may have been missed in other cases of transient hypogammaglobulinemia of infancy because phenotypic analyses of blood lymphocytes may not have been performed until the B-cell deficiency spontaneously corrected. In view of the uncertainty that transient hypogammaglobulinemia of infancy exists or is a disease, it will be important to perform longitudinal studies of the B-cell system in future cases of this type.

Although the immunological features of this disorder did not correspond to reports of genetic defects in BTK, we investigated whether the problem was X linked by examining the pattern of X-chromosome inactivation in the mother's B cells, T cells, and neutrophils. The rationale was the previous finding of nonrandom X-chromosome inactivation in leukocytes from female carriers of X-linked immunodeficiencies (9, 10, 38, 39, 46). B cells were compared with leukocytes from other lineages because of the limitation of the immunodeficiency in this patient to the B-cell lineage and the restriction of nonrandom inactivation of X chromosomes to B cells in XLA (9, 10, 38). Because no polymorphisms were found for each of the four DNA sites on the long arm of the X chromosomes that were investigated (Fig. 2), the pattern of X-chromosome inactivation

tion could not be ascertained in the mother's cells. Moreover, since those sites cover a wide area (Xq12 to Xq25), it is likely that the mother has identical X chromosomes, either because of uniparental X-chromosome disomy leading to isodisomy (14) or because of inheritance of the same X chromosome from the mother's father and mother. With respect to the latter, the X chromosome of the maternal grandfather and one of the X chromosomes of the maternal grandmother revealed the same DNA banding patterns for the four markers (Fig. 2). If our conclusion is correct, the patient and his nonidentical twin brother have the same X chromosome. Since the mother and non-identical twin brother were immunologically normal, it appears that the immunodeficiency in this patient was not due to a mutation in an X-chromosome gene.

To further understand the basis of this unusual developmental delay in the B-cell lineage and because of one report of a relative deficiency of blood CD4⁺ T cells in transient hypogammaglobulinemia of infancy (47), blood T-cell populations were enumerated by flow cytometry. The numbers of blood CD3⁺ CD4⁺ and CD3⁺ CD8⁺ T cells were normal. That finding agreed with the report of Dressler et al. (12).

Finally, we considered whether this transient deficiency was due to an autosomal recessive gene defect whose effects are restricted to the B-cell lineage. Indeed, four B-cell defects created by genetic manipulations in experimental mice suggest some possible causes. The murine Pax5/BSAP defect leads to a block in early B-cell differentiation (52), the murine protein kinase C β gene defect leads to a B-cell deficiency (29), and mice lacking genes for either the CXC chemokine PBSF/SDS-1 (33) or its chemokine receptor CXCR4 (58) do not produce B cells. The last possibility is of particular interest, since the chemokine receptor is a cofactor for the entry of the human immunodeficiency virus into CD4⁺ T cells (16). Further investigations of this child for that chemokine receptor and of individuals who are known to be deficient in the chemokine receptor CXCR4 will therefore be of interest.

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