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Research Article

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A Human Non-XLA Immunodeficiency Disease Characterized by Blockage of B Cell Development at an Early ProB Cell Stage

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Abstract

We report a detailed analysis of a B cell defect affecting a patient girl born from first cousin parents, characterized by a severe non–X-linked agammaglobulinemia with a total absence of $CD19^+$ cells in the periphery. In the bone marrow, CD19 expression was also highly impaired, resulting in the absence of both B and preB compartments. By contrast, $CD34^+CD10^+$, $CD34^+\Psi L^+$, and some $CD19^+CD10^+$ mostly $CD34^+$ early proB cells were present, although diminished. Semiquantitative RT-PCR analysis performed on mononuclear bone marrow cells indicated that λ-like, VpreB, Rag-1, Rag-2, and TdT transcripts expressed during proB cell stages were found at normal levels whereas E2A, CD10, Syk, Pax-5, CD19, Igα, Igβ, V_H-Cμ, and $V_κ$-$C_κ$ transcripts characteristic of later stages were severely depressed. This phenotype resembles that of Pax-5 knock-out mice, but since the coding sequence of the patient Pax-5 cDNA was shown to be normal, the defect might rather result from an altered regulation of this gene. All these data indicate that the patient suffers from a new genetic defect that results in an arrest of differentiation within the proB cell compartment, i.e., earlier than X-linked agammaglobulinemia, before the onset of Ig gene rearrangements. (*J. Clin. Invest.* 1996. 98:1519–1526.) Key words: proB cells • surrogate light chain • Ig genes • Btk • Pax-5

Introduction

B cell development from pluripotent stem cells proceeds through proB, preB, and B cell stages that may be followed by a variety of experimental approaches: (*a*) sequential acquisition and/or loss of a number of cell surface markers (1, 2), such as, in humans, CD34, CD10, CD19, CD20; (*b*) occurrence of successive Ig gene rearrangements that follow an essentially regulated kinetics, H→ κ→ λ (3); (*c*) follow-up of fully rearranged gene expression and also detection of germ-line transcripts that precede most rearrangement events (4); and (*d*) identification of mutations occurring in genes responsible for a block in B cell differentiation (5).

Among the earliest cell surface markers of the B lineage, the surrogate light chains (ΨL) are of special interest. They are encoded by the λ-like gene in humans (6–8) or the λ5 in the mouse (9), and the VpreB gene (10, 11). At the proB cell stage, ΨL are expressed at the cell surface in the absence of μ chains (12–14). Recently, using "proB specific" anti-VpreB mAbs, we have identified proB cell subpopulations that are $CD34^+\Psi L^+$ and do or do not express CD10 and/or CD19 markers in normal human bone marrow (see reference 22). Normal preB cells have been identified within the $CD19^+$ population (15), either as having ΨL associated with μ (6, 14–17), or as a $CD34^-CD19^+$ fraction that contains intracytoplasmic μ chains (18). In addition, Igα and Igβ, which constitute the signaling elements of the B cell receptor, and are encoded by the mb1 and B29 genes, respectively (19), were also detected at the cell surface of preB (20, 21), but not proB cells (22).

X-linked agammaglobulinemia (XLA),[1] also known as Bruton's disease, is a rare immunoglobulin deficiency characterized by severe decrease or absence of mature B cells and serum immunoglobulins (23). Mutations in the Bruton's tyrosine kinase (Btk) gene have been shown to be responsible for a blockage at the preB stage (24, 25), resulting in the accumulation of proB ($CD34^+CD19^+$) and presence of preB ($CD34^-CD19^+$) cells in the bone marrow (26). The preB cells express normal amounts of λ-like and VpreB transcripts and a V-D-J-Cμ repertoire characteristic of normal preB cells (26). Conley and Sweinberg have reported in two girls a recessive disorder that is phenotypically identical to XLA but which is of possible autosomal origin (27). Recently in a similar case, defect in the Btk gene was also ruled out (28). In this patient, presence of $CD10^+$ cells but absence of $CD19^+$ cells and a 10-fold decrease of mature V-D-J-Cμ transcripts suggested a blockage at an earlier stage of B cell development.

In this paper, we describe another young female patient with a severe agammaglobulinemia and demonstrate, by a detailed analysis of B cell subpopulations and B cell specific transcripts, a blockage at an early proB cell stage of the B cell differentiation pathway before the onset of Ig gene rearrangement. This case is reminiscent of the $Pax5^{-/-}$ phenotype in the mouse (29).

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1. *Abbreviations used in this paper:* Btk, Bruton's tyrosine kinase; XLA, X-linked agammaglobulinemia.

Methods

Case report. The patient is a girl born to healthy first cousin parents. From the age of 4 mo, she suffered from recurrent infections, consisting of otitis media, candidiasis, arthritis caused by *Hemophilius influenzae*, and *Herpes simplex* stomatitis associated with failure to thrive. She was referred to Hôpital des Enfants-Malades at the age of 11 mo for suspicion of an immunodeficiency condition. Lymphocyte counts were within normal range or increased (4,000–13,000/μl) while a neutropenia was detected (500/μl). In peripheral blood no serum immunoglobulins were detectable (IgG < 0.07 grams/liter, IgA < 0.08 grams/liter, IgM < 0.07 grams/liter). Lymphocyte phenotyping showed the absence of $sIgM^+CD19^+$ mature B cells, while T lymphocyte subsets were present (Table I). The CD4 to CD8 ratio that was inverted in the first analysis (presumably due to an infectious episode) was normal as shown when rechecked 6 mo later. Mitogen- and antigen-induced proliferation was also found to be normal (Table I). Cytogenetic analysis of lymphocytes did not detect any anomaly. In particular, a normal 46XX karyotype was observed both in blood and bone marrow of the patient (data not shown), excluding the possibility of a Turner syndrome or an X-autosome translocation. Furthermore, absence of detectable X-allelic polymorphism in this patient prevented X inactivation pattern analysis. After onset of intravenous immunoglobulin substitution, clinical status improved, growth normalized, and neutropenia resolved.

The parents gave informed consent for this study. The bone marrow sample was obtained when the patient was 18 mo old and under general anesthesia required for central line insertion. Age-matched control cells were an aliquot obtained from a healthy donor in the course of a bone marrow transplantation.

X inactivation pattern analysis. X inactivation pattern analysis was performed using a PCR-based detection of the X-linked androgen receptor gene as previously described (30), on DNA isolated from $CD19^-$ and $CD19^+$ sorted cells from the patient's mother using a FACStar® cell sorter (Becton Dickinson, San Diego, CA).

Antibodies. Cell surface phenotyping was performed by direct immunofluorescence using FITC-mAb anti-CD10, anti-CD13, anti-CD19, anti-CD20, anti-CD22, anti-CD34, anti-CD38, or PE-mAb anti-α/β TcR (BMA031, IgG1) or anti-γ/δ TcR (IMMU510, IgG1) all purchased from Immunotech SA (Marseille, France). FITC-anti-CD3 (Leu 4, IgG1), anti-CD2 (Leu 5b, IgG2a), anti-CD4 (Leu 3a, IgG1), and PE-anti-CD8 (Leu 2a, IgG1) were from Becton Dickinson. FITC-sIgM was from Nordic Immunology (Tilburg, The Netherlands) and FITC-anti-CD24 was from Tebu Caltag Laboratories (San Francisco, CA). Indirect immunofluorescence using 9C2 (14) and 3C7 (22) anti-VpreB mAbs, and PE-labeled $F(ab')_2$ goat anti–mouse IgM (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) as revealing antibodies.

Cell surface staining. Cells (2×10^5) were incubated with mAb for 30 min on ice. After three washes with PBS 0.2% BSA, 0.1% NaN_3, cells were incubated for 30 min with 100 μl of revealing antibodies. Irrelevant IgM mAb (IgM negative control; Immunotech SA) was included as a control. Immunofluorescence was analyzed using a FACScan® analyzer (Becton Dickinson).

Proliferation assays. PBMC were isolated from freshly drawn heparinized blood by means of Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient centrifugation. Proliferation assays were performed as described elsewhere (31). In brief, PBMC were stimulated either with 50 ng/ml of anti-CD3 (OKT3, IgG2a; Ortho Pharmaceutical, Raritan, NJ) in a 4-d culture or by 1:250 final dilution of tetanus toxoid (Pasteur-Merieux, Lyon, France) and 50 μg/ml of Candidin (Pasteur Diagnostic, Marne la coquette, France) in a 6-d culture. Data are the mean incorporation of $[^3H]$thymidine for the last 18 h of culture.

PCR. cDNA from bone marrow cells was first prepared from total RNA of 4×10^6 cells, using 150 ng of oligo dN6 as primer and the reverse transcriptase (BRL, Cergy-Pontoise, France), according to the manufacturer's instructions. PCR was performed in a Cetus apparatus using 1/20 of cDNA with 50 pmol of each oligonucleotide as primer (Table II). To ensure that PCR products were not derived from DNA, primers were defined from discrete exons for each transcript, except Rag-1 and Rag-2. In these cases, RNA preparations were treated with DNase (Boehringer Mannheim Corp., Indianapolis, IN) and a PCR control without RT was included. Quantification was performed by reference to amplification of 25, 2.5, and 0.25 ng actin mRNA. The first cycle was run as follows: denaturation at 94°C for 3 min, annealing at temperatures indicated in Table II for 1 min, and synthesis at 72°C for 2 min. The next 28 cycles were run similarly, except that denaturation lasted only 1 min. For the last cycle, synthesis time was 7 min. One-fourth of the amplified products was run in agarose gel made 1 or 2% in 1× TBE.

Cloning and sequence analysis of the Pax-5 cDNA. PCR product of Pax-5 cDNA (1,208 bp) derived from patient bone marrow mononuclear cells was purified using QIA quick spin PCR purification kit (Qiagen, Coger, France) and cloned in competent *Escherichia coli* by using the TA cloning™ system (Invitrogen, Leek, The Netherlands). Double-strand DNA sequence of the PCR product was performed using the dideoxy chain termination protocol with ^{35}SdATP, a modified T7 DNA polymerase, a Sequenase kit (United States Biochemical Corp., Cleveland, OH), using −40 and SP6 oligonucleotides and the following internal specific primers derived from the Pax-5 cDNA published sequence (32): antisense 5′ATCCTGTTGATGGAACTGACGC3′ position 468–488; sense 5′CTCTGTGGTGGTGAAGATGTCT3′ position 794–815; antisense 5′TGTTTGAGAGGCAGCACTACTC3′ position 816–837.

Table I. Characterization of Patient's Peripheral Blood Lymphocytes

	Patient (18 mo)		Controls (age-matched)
Lymphocyte counts (per μl)	$4–13 \times 10^3$		$4–6 \times 10^3$
Lymphocyte phenotype (%)			
CD19	< 1	(< 1)*	5–25
Surface μ	0	ND	5–25
CD2	98	ND	75–95
CD3	92	(94)	70–90
CD4	36	(59)	35–65
CD8	55	(33)	15–35
TcRαβ/CD3	99	(96)	90–99
TcRγδ/CD3	1	(3)	1–10
Lymphocyte proliferation (cpm × 10^3)			
None (day 3)	0.2		0.7±0.3
Anti-CD3 mAb	30		36±23
None (day 6)	0.5		0.5±0.2
Tetanus toxoid	43		31±21
Candida Ag	37		34±21

*Numbers in parentheses correspond to a second sampling performed 6 mo later.

Results

Random X inactivation pattern of the patient's mother. Using a PCR-based detection of the X-linked androgen receptor gene (30), a similar random X inactivation pattern was observed in the patient's mother for both the $CD19^+$ and $CD19^-$ sorted PBMC, indicating that she was not an XLA carrier (data not shown).

Table II. Oligonucleotide Primers Used for cDNA-PCR Amplification

		Primers used (5′ to 3′)	Annealing temperature	Product size (bp)
Actin	sense	TACCACTGGCATCGTGATGGACT3′	65°C	507
	antisense	TCCTTCTGCATCCTGTCGGCAAT3′		
Rag-1	sense	CCAGCTGTTTGCTTGGCCATCCGT	65°C	353
	antisense	TTGGGATCTCATGCCTTCCAAGAT		
Rag-2	sense	ATGTCCCTGCAGATGGTAACA	55°C	246
	antisense	GCCTTTGTATGAGCAAGTAGC		
TdT	sense	TATGAGAGCAGCTTCTGTATTG	58°C	375
	antisense	TCTTTAACCAGCACACTGACG		
λ-like	sense	*ATGCATGCGGCCGCG*GCATGTGTTTGGCAGC	60°C	194
	antisense	*ATCCGCGGCCGCATCGAT*AGGTCACCGTCAAGATT		
VpreB	sense	CATGCTGTTTGTCTACTGCACAG	60°C	396
	antisense	TGCAGTGGGTTCCATTTCTTCC		
Iμ/CH1μ	sense	AGATTCTGTTCCGAATCACCGATGC	68°C	340
	antisense	AAGGAAGTCCTGTGCGAGGCAGC		
VHcons.	sense	GACACGGCCGTGTATTACTG	60°C	150–200
Cμ	antisense	*ATCCGCGGCCGCGG*AATTCTCACAGGAGACGA		
Vκ cons.	sense	ATGACCCAGTCTCCATCCTCCCTG	65°C	390–410 (Vκ/Cκ)
Iκ	sense	*GTTGAGCTTCAGCAGCTGACCCA*		207 (Iκ/Cκ)
Cκ	antisense	*ATGCGGCCG*CGGGAAGATGAAGACAGATG		
Igα	sense	*TAGTCGAC*ATGCCTGGGGGTCCAGGAGTCCTC	68°C	697
(mb1)	antisense	*TAGGATCC*TCACGGCTTCTCCAGCTGGACATC		
Igβ	sense	*CGGTCGAC*ATGGCCAGGCTGGCGTTGTCTCCT	68°C	716
(B29)	antisense	*ATGGATCC*TCACTCCTGGCCTGGGTGCTCACC		
CD10	sense	CTGTGACAATGATCGCACTCTATG	65°C	584
	antisense	GATTCCAGTGCATTCATAGTAATCTC		
CD19	sense	CGAGTTCTATGAGAACGACTC	60°C	596
	antisense	ACTGGAAGTGTCACTGGCATG		
IL7Rα	sense	CAGAATGACAATTCTAGGTACAACT	62°C	1431
	antisense	AATCATCTTTGTCGCTCACGGT		
E2A	sense	GCTGACCACTCGGAGGAG	62°C	297
	antisense	CGCTCTCGCACCTGCTGC		
Id	sense	CTGCTATACAACATGAACGACTGC	62°C	285
	antisense	GCTGTCATTTGACATTAACTCAGAAGG		
Syk	sense	GCATGGCTGACAGCGCCAAC	62°C	1810
	antisense	GTTAGTTCACCACGTCATAGTAG		
Btk	sense	*GGATCC*GGTGAACTCCAGAAAGAAGAAG	58°C	2095
	antisense	*GCGGCCGCCCGGG*CTCCAGGGCTCCTAAGCTTGG		
Pax-5	sense	CTGTCCATTCCATCAAGTCCTG	63°C	1218
	antisense	TCAGTGACGGTCATAGGCAGTG		

Linker sequences are italicized.

Phenotypic analysis of patient bone marrow cells reveals a major defect in B cell differentiation. The main characteristic of the patient's PBMC was the absence of $CD19^+$ and surface μ^+ cells (Table I). For a more precise focus of the B cell impairment, bone marrow mononuclear cells were screened for a number of surface markers and compared with age-matched control, as indicated in Table III. CD34 expression was within normal values, whereas that of CD19, CD20, CD22, CD24, and surface μ^+ which clearly assign commitment to the B lineage were severely depressed. Expression of CD10, although markedly diminished points to a defect which should be located at early B cell precursor stages. As shown earlier, 3C7 mAb iden-

Table III. Phenotypic Characterization of Patient's Bone Marrow Mononuclear Cells

	Patient (18 mo)	Control (age-matched)
CD34	13	15
CD38	63	76
ψL (3C7)	2	4
ψL (9C2)	3	5
CD10	7	42
CD19	2.5	47
CD20	1	27
CD22	2	11
CD24	2.5	42
Surface μ	0	22
CD33	15	11
CD13	13	5
CD3	41	38

Cell populations were gated for the lymphoid light scatter. All values are percentages.

tifies proB cells ($\Psi L^+\mu^-$) whereas 9C2 mAb also encompasses identification of preB stages (22). Identification of surrogate light chain (ΨL) by both reagents therefore indicates that the B differentiation pathways proceeds at least to the proB stage.

Two-color FACS® analysis performed on lymphoid cell gated populations visualized the absence of immature and/or mature B cells in patient's bone marrow (absence of μ and CD20, Fig. 1 *B*). The $CD34^-CD19^+$ preB cell population was also absent (Fig. 1 *B*), and the $CD34^+CD19^+$ proB cell population decreased (1.6 vs. 8.6%). The few $CD19^+$ cells were positive for CD10 (2.5%, Fig. 1 *A*) and were mostly $CD34^+$, strongly suggesting that differentiation did not proceed beyond proB cells. Presence of proB cells within the $CD34^+CD10^+$ population was also supported by the identification of ΨL chain at the surface of 1.7% of $CD34^+$ cells. The $CD34^+CD10^+$ was however smaller than that of the control (3.5 vs. 11%), indicating that the early proB compartment was already affected.

Identification of transcriptional defects in patient bone marrow cells. To approach the molecular defect, characterization of transcripts present in patient's cells was performed by RT-PCR using the primers described in Table II. Transcripts of total bone marrow cells from patient and age matched control were compared by reference to actin calibration (Fig. 2 *A*). We chose to analyze transcripts directly linked to Ig gene expression or rear-

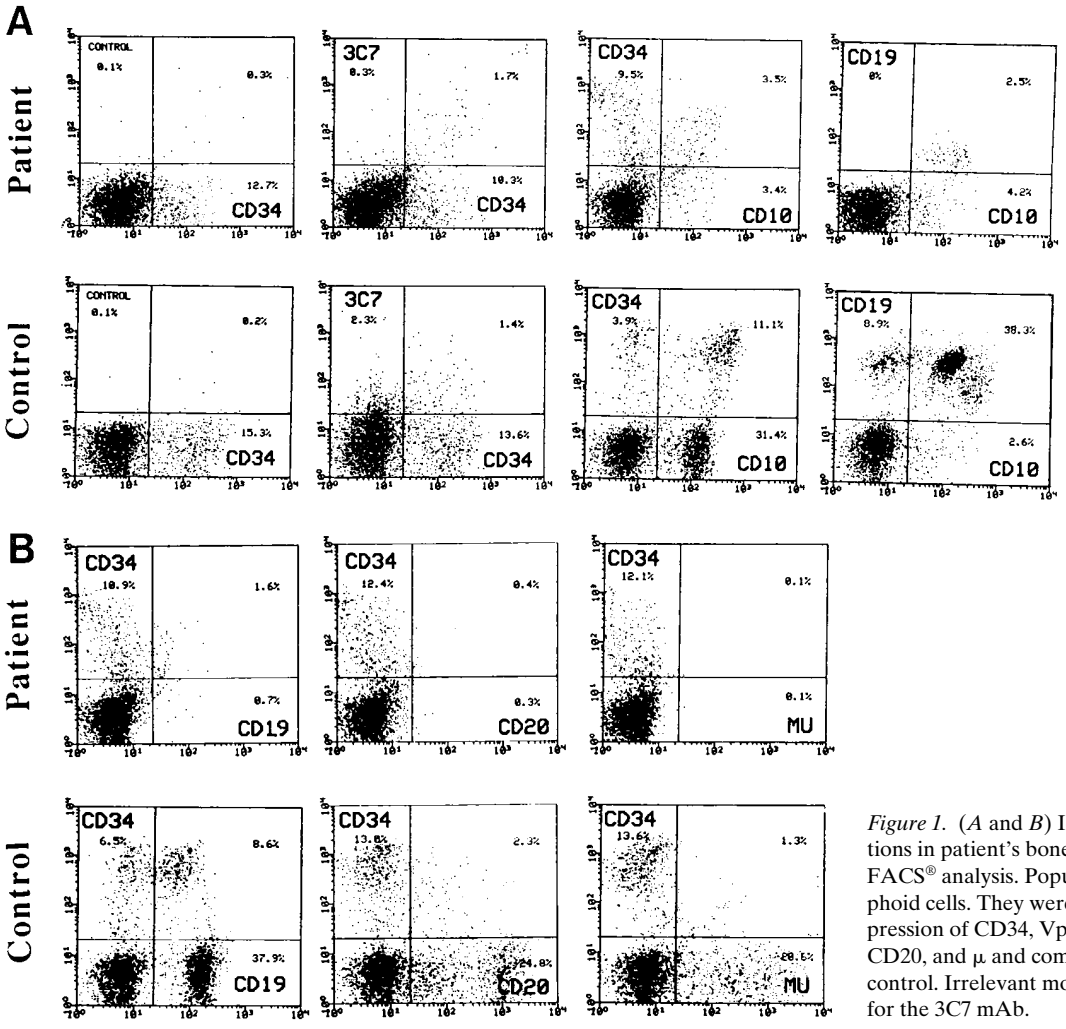

Figure 1. (*A* and *B*) Identification of cell populations in patient's bone marrow by two-color FACS® analysis. Populations were gated on lymphoid cells. They were analyzed for surface expression of CD34, VpreB (3C7), CD10, CD19, CD20, and μ and compared with age-matched control. Irrelevant mouse IgM served as control for the 3C7 mAb.

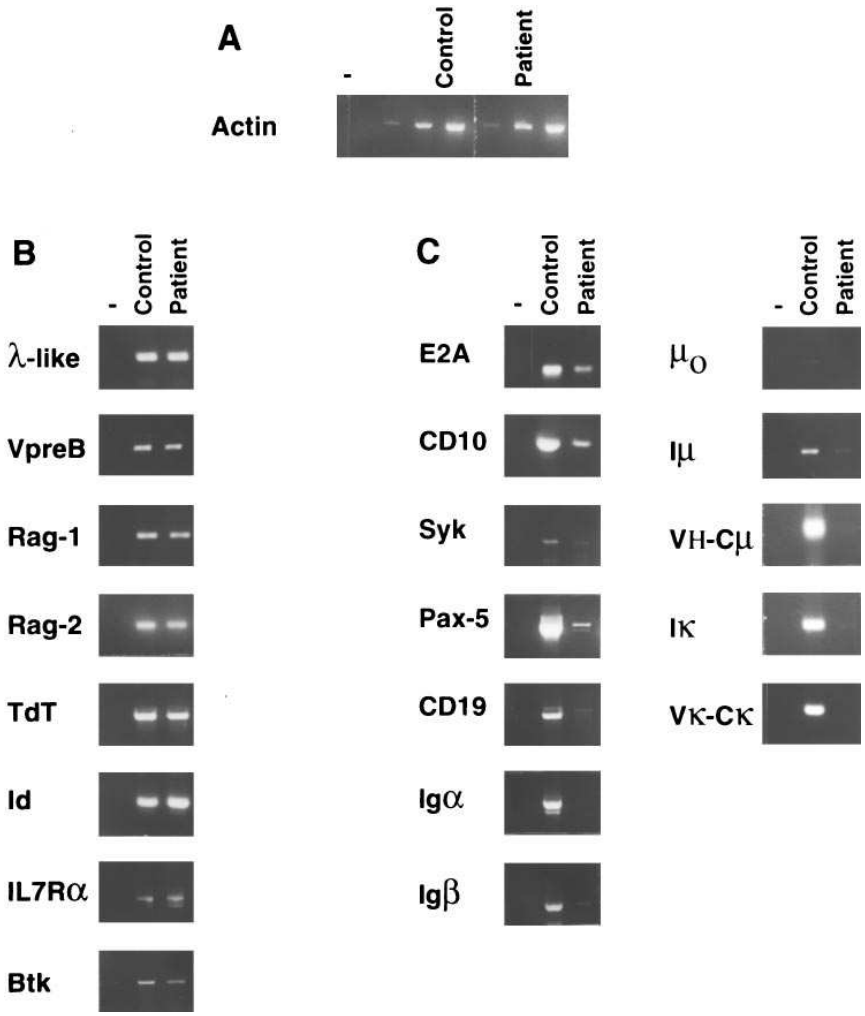

Figure 2. Transcriptional activity of the patient's bone marrow cells compared with an age-matched control. (*A*) Quantitative RT-PCR controls were performed on three dilutions (25, 2.5, and 0.25 ng) of cDNA with actin primers. (*B* and *C*) PCR were performed on 250 ng of cDNA, using primers and conditions depicted in Table I. A control without cDNA was included in the left column of each series (−).

rangements but also transcripts derived from genes which have been shown of importance in B cell differentiation at the proB stage, as evidenced from gene targeting or transgene overexpression experiments in the mouse, namely E2A (33), Pax-5 (29), Id (34), and IL7Rα (35). The expression of Btk (36) and Syk (37), involved at a slightly later stage of differentiation, was also performed.

Among the classical markers directly linked to Ig gene expression, λ-like, VpreB, Rag-1, Rag-2, and TdT transcripts were clearly present in the same amount between the 2 samples (Fig. 2 *B*). Pertaining to this group of nonaffected transcripts were also found Id, IL7Rα, and Btk (Fig. 2 *B*). By contrast, expression of transcripts corresponding to the heavy (V-D-J-Cμ) and light (Vκ-Cκ) Ig rearranged gene loci was highly impaired or absent, respectively (Fig. 2 *C*). Iμ (38) and Iκ (39) sterile transcripts that initiate 5′ of the Eμ and Eκ intronic enhancers, respectively, and which are normally expressed before the corresponding Ig gene rearrangements, were only faintly visible, as was the μ_0 sterile transcript that initiates 5′ of the J_H locus (40), but it should be emphasized that in the control it was already rather weak. Finally, E2A, CD10, Syk, Pax-5, CD19, Igα, and Igβ expression was also affected in the patient, although at variable levels (Fig. 2 *C*). A more precise evaluation of the affected transcripts could not be given due to the limits of the quantification technique.

Taken together these results are in agreement with the above FACS® analysis, pointing to a blockage of differentiation at the proB stage in this patient, before the heavy chain locus rearrangements are completed.

Sequence determination of the Pax-5 cDNA. Because Pax-5 might be a potential candidate accounting for the observed defect (see below), PCR product encompassing the entire coding region (1,218 bp) was cloned and sequenced, using two external and three internal primers as indicated in Methods. Three independent clones failed to reveal any difference with the Pax-5 coding region previously reported (32).

Discussion

In this paper, we report the case of a young girl suffering a complete agammaglobulinemia with total absence of peripheral B cells, and normal T and myeloid compartments, thus resembling the phenotypic pattern of XLA. However, this syndrome may be eliminated for several reasons: (*a*) the patient is a girl born to first cousin healthy parents; (*b*) a normal 46XX karyotype was observed both in the blood and bone marrow of the patient, excluding the possibility of a Turner syndrome or an X-autosome translocation; and (*c*) the patient's mother was not an XLA carrier and therefore this case cannot result from

a maternal carrier associated to a spontaneous mutation from the father. Therefore, the origin of the disease may result rather from an homozygous defect of an autosomal gene. Although similar non-XLA primary immune deficiencies have been reported (27, 28, 41), the precise stage of differentiation that was affected by the genetic defect was not characterized.

Systematic analysis of most classical markers of the B cell lineage in the patient's bone marrow indicates that this deficiency is the consequence of a block of differentiation at an early stage of B cell development first evidenced from the absence of surface μ and the quasi-absence of CD19, CD20, CD22, and CD24, leading to the lack of immature B cells. In humans, preB cells may be characterized by either the presence at their surface of the μ-ΨL complex (16, 17) or by the presence of intracytoplasmic μ chains in a $CD34^{-}CD19^{+}$ population (18). Patient's bone marrow cells were negative for surface μ, V-D-J-Cμ transcripts, and $CD34^{-}CD19^{+}$ cells, pointing to the absence of preB compartment. Identification of $CD34^{+}CD10^{+}$ cells and of a small population that was $CD19^{+}CD10^{+}$ and essentially $CD34^{+}$ is consistent with the presence of a proB cell population, a conclusion which is strengthened by the fact that these cells expressed ΨL epitopes in the absence of μ chains at their surface. However, this proB cell population is diminished, indicating a blockage within this compartment, i.e., earlier than the X-linked Btk defect characterized by the presence of both $CD34^{+}CD19^{+}$ proB cells and $CD34^{-}CD19^{+}$ preB cells (26, 42).

In the mouse, gene targeting has shed light on the sequential events that drive the early steps of B cell differentiation. Most of these genes encode transcription factors which control regulatory points that are critical in early stages of lymphopoiesis. They can be ordered as indicated in Fig. 3, altogether with the kinetics of expression of the major transcripts that are involved in the differentiation of the B lineage. When searching for a gene that could account for the reported defect, one obvious track is to identify impairments of transcription. However, one should realize that several difficulties must be faced. First, extrapolation of results derived from gene targeting in the mouse to human situation may require some caution. For instance, clear differences have been identified between phenotypes of human XLA and Btk gene targeting (23, 26, 42–44). Second, it may be difficult to decide whether the very low level of any one transcript is directly linked to the corresponding gene defect or whether it is diminished because of the lack of subsequent stages of differentiation. As appreciated from semiquantitative RT-PCR, two levels of transcript expression pattern could be identified (Fig. 2). In the first one, composed of λ-like, VpreB, Rag-1, Rag-2, TdT, Id, IL7Rα, and Btk transcription level was identical to that of normal age-matched control indicating that the steps leading to the early proB were normal. Btk deserves a special comment in that it is also found expressed in the myeloid lineage (36), which thus might contribute to the level of transcription observed in this patient. Absence or very low efficiency of Ig gene rearrangements was

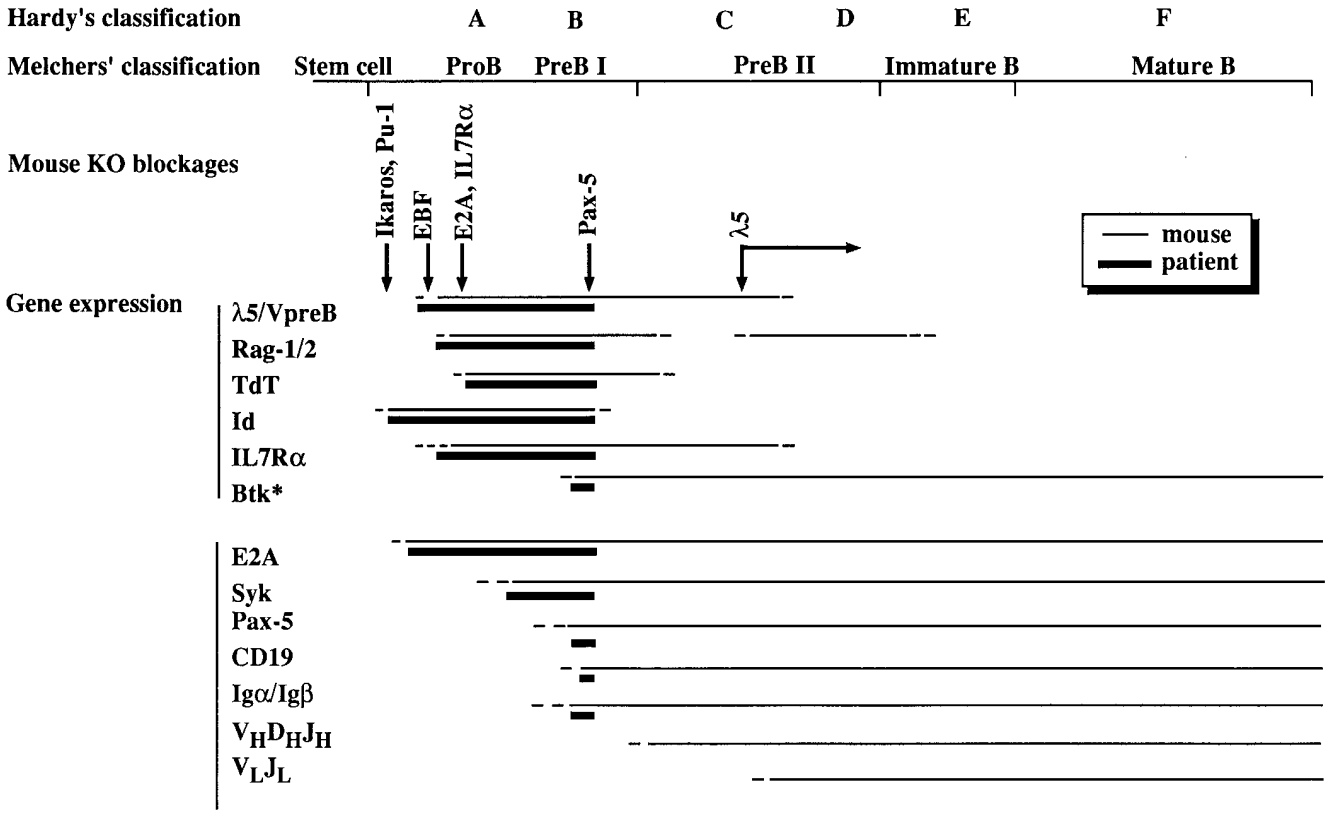

Figure 3. General scheme of B cell differentiation in the mouse, based on Hardy's (51) and Melchers' (52) classifications. The extent of mouse transcripts expression is indicated by horizontal thin lines (51, 53) and blockage point resulting from gene targeting is shown by vertical arrows (5, 29, 33, 35, 43, 44, 54). The right-angle arrows indicates that blockage upon λ5 targeting is leaky. The thick bars indicate the hypothetical extent of the patient's transcripts in the B lineage, expressed by reference to the mouse diagram. *It should be noted that most of the patient's Btk transcripts must originate from the myeloid lineage.

not due to an alteration of Rag-1 nor Rag-2, since the T compartment was normal. Nevertheless, a mutation that would affect the control of Rag transcription solely in the B lineage could be invoked, as this was reported to be the case for the $E2A^{-/-}$ phenotype (33). In the second group, which included E2A, CD10, Syk, Pax-5, CD19, Igα, Igβ, μ_0, Iμ, V_H-C_μ, I_κ, and V_κ-C_κ, gene expression was severely impaired. As expression of these genes extends beyond the preB stage, the lower level of transcripts is most simply explained by the absence of more mature cells. The precise step of differentiation which is directly affected by the defect must be very close to the onset of transcription of these genes. In this regard, several genes must be critically analyzed. The presence of λ-like, VpreB, Rag-1 and Rag-2 transcripts in the patient rules out E2A defect, which was shown to severely impair transcription of these genes in the mouse (33). The phenotype that results from gene targeting of Igβ is close to that of the patient in that blockage arises at the proB stage. However, its implication appears unlikely in our case, since in $Ig\beta^{-/-}$ mice V_H-D-J_H occurs, although with a low efficiency, and the level of Igα transcription remains normal (45). Gene targeting of CD19 did not affect B cell differentiation until the immature B lymphocyte stage (46), making it unlikely a direct defect of this gene in the patient. Among factors that control CD19 expression, Pax-5 transcription factor has been shown to be clearly involved (47). Binding sites for Pax-5 have been also identified in the promoters of other B specific genes such as λ-like/λ5, VpreB, Blk, and at multiple sites within the IgH locus (48). The very low level of CD19 transcription, the strong impairment in Ig gene rearrangements, and the presence of normal levels of λ-like and VpreB transcripts were reminiscent of the phenotype reported in $Pax\text{-}5^{-/-}$ mice (29, 49) and therefore made the Pax-5 gene the most likely candidate to date (Fig. 3). However, sequencing of the coding portion of the cDNA did not differ from the previously reported sequence, thus leaving the definitive answer with a question mark. Using a molecular genetic approach of primary immune deficiencies that may be similar to our case, Vorechovsky et al. concluded that involvement of the coding portion of the Pax-5 gene was unlikely (41). However, implication of Pax-5 cannot be definitively ruled out, either directly through a mutation in the regulatory boxes of this gene, or by a defect of a gene encoding a factor regulating the Pax-5 gene. It should be recalled that Pax-5 also drives important steps of development in the posterior midbrain and anterior cerebellum in the mouse (29). The patient had no evidence of such a syndrome, but this might be explained by the redundancy in the Pax gene family, Pax-2 being expressed in the central nervous system (50).

Therefore, this new non-XLA primary immunodeficiency provides a potential model to attempt elucidation of very early stages of human B cell differentiation.

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