Predominance of Fetal Type DJ_{H} Joining in Young Children with B Precursor Lymphoblastic Leukemia as Evidence for an In Utero Transforming Event

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Summary

The presence of N sequences in the complementarity determining region 3 (CDR3) of the rearranged immunoglobulin H chain is developmentally regulated: N regions are generally present in the DJ_H joinings of adult B cells but are often absent in fetal B cells. Analysis of the CDR3 in 61 B precursor acute lymphoblastic leukemias indicated that 87.5% of the leukemias obtained from children ≤ 3 yr old lacked N regions at the DJ_H junction. In contrast, in children >3 yr old, only 11.1% of the leukemias lacked N regions at this junction, a frequency similar to what we have observed in B cells from children and adults. These findings suggest that the majority of leukemias presenting within the first 3 yr of age arise from an in utero transforming event.

Hypervariability within the CDR3 of the human Ig H chain is initially generated at the time of VDJ joining by the recombination of multiple V_H , D, and J_H gene segments (1-3). This VDJ recombination process is dependent upon two recombinase enzymes, RAG1 and RAG2, since mice deficient in either gene fail to produce lymphocytes with rearrangements in their Ig or TCR loci (4-8). Exonucleolytic activity produces joinings in which germline nucleotides are lost from the ends of the joined segments (1-4). Variability is increased when nontemplate-derived nucleotides (N regions) are added between joined gene segments through the action of another enzyme, terminal deoxynucleotidyl transferase (Tdt)¹ (1-4). In both IgH and TCR rearrangements, palindromic (P) mono- or dinucleotides may be found adjacent to a recombined gene gene segment when the segment is present in its entirety. These germline-encoded nucleotides arise from a flip-over mechanism of the 5' end of one strand of the joining segment (9).

The developmental regulation of N region addition has been demonstrated in both mice and humans (10–13). DJ_H joinings that lack N regions are found more frequently at the fetal stage of development. No more than 5% of the DJ_H junction sequences of B lymphocytes present in murine fetal liver contained an N region, whereas in newborn mouse spleen and liver, 5–23% of the DJ_H junctions had N regions The functional significance of the absence of N regions in the early stage of development is not clear. Gu et al. (12) speculate that the absence of N regions implies predominant expression of germline-encoded specificities. Thus, idiotypic interactions in a germline-encoded network might play a functional role in the development of the antibody repertoire early in ontogeny. In newborns, Feeney (10) found a higher percentage of N regions in productive vs. nonproductive rearrangements and speculated that this increase suggests a preferential activation of B cells whose IgH sequences contain N regions by antigens or cellular interactions. Alternatively, IgH sequences with N regions might have an advan-

^{(10-12).} In contrast, a significantly higher percentage of DJ_{H} junctions with N regions (64-73%) were found in B cells from adult (4-8 wk) murine spleen (10-12). A similar trend was found in human B cells; evaluation of $>500 DJ_{H}$ joining DNA sequences obtained from human fetal, neonatal, and adult lymphoid tissue revealed N regions at the DI_H junction at frequencies of 68%, 86%, and 91-100%, respectively (13, 14). Together, these observations suggest that CDR3 sequences lacking N regions at the DJ_H junction are representative of a DJ_H recombination event that occurred during the time of fetal development when TdT activity may have been absent. Indeed, in the murine system, TdT levels rise slowly in the developing thymus, which correlates with the absence of N regions in fetal TCR- γ/δ rearrangements (15, 16). Similarly, in the murine B lymphoid system, TdT was not detected in fetal liver but was demonstrated in adult bone marrow B lineage cells (16).

¹ Abbreviation used in this paper: Tdt, terminal deoxynucleotidyl transferase.

tage in the transition from pre-B cell to B cell, perhaps through enhanced binding to surrogate L chains.

B lineage acute lymphoblastic leukemia (ALL) of childhood results from the transformation of B precursor cells and their clonal expansion (17–19). We reasoned that if lack of N regions at the DJ_H junction was a marker for fetal-derived B cells, then leukemias arising from an in utero transforming event should show a bias for DJ_H joinings that lack N regions. Furthermore, the age distribution of leukemias lacking N regions at their DJ_{H} joinings might provide insight into the length of time required to develop clinical disease from the time of the transforming event.

Materials and Methods

Source of Cells. Bone marrow samples with >70% lymphoblast replacement were obtained at diagnosis from 63 patients (6 mo

PATIENT CORS CODE	RGE (NONTHS)		Q	N	د		REF
P46-111		(184)	9679FF867567997		CTRCTRCTRCTRCTRCRT6GRC6TT6668CRRA66RCCRC	(6)	26
C1-102	5	(81)	STATACTSSA		ACTITISACTACT SOBSCCABOBRACCT	(1)	27
C1-110		(84)	TORTARA		CTACTACTACSSABCSTCT8BBSCCARBBBACCAC	(6)	27
C208-116	7	(H4)	TGTABCAGETEGEEC	TCC	ACTACTACTACTACG5TASTC15586CCAR656ACCAC	(6)	27
208-129		(21/10)	TATSATIALSTITEGBARSTATEGTTATAGG	(1)	TRETRETRETRETRETESTETSSECCRASESSECRE	(6)	27
C208-136		(LR9)		-	AACTESTTCSACCCCTESEBCCACCCAACCCT	(5)	•
C76-128	14	(XP4)	GRATINGENTITIGBAGTGATTATTA		LacTACTACTACTACGGTRTGGACGTCTGGGGGCCARGGGACCAC	(6)	
C439-111	18	(XP4)	AGTGGTTRICCCT		ATTACTACTACTACCACGG TATGGACGTCTG GBGCCARBOGACCAC	(8)	
C135-124	18.5	(LNZ)	BT 5 B 9 NG T NC T		CTRCTACTACTACGBTATGGACSTCTSGSBCCARBURCCAC	(6)	28
265-117	22	(LM)	STRSTRCCRSCTUCT		ACTACTASASACCASASASCCCT	(4)	-
C85-113	24	(XP1) (LB1)	GRINTITARCIUGIJNINI Artagtartatatarta	510	GACTACTAGGGCCAGGGARCCCT	(1)	
C96-119	63.3	(XP1)	ATTTRACTABTA	• • •	LLCCTRCTRCTRCTRC66TRT66RC6TCT6666CCRASSERCCR6	(6)	
C433-88	26	(XP11)	CGGGERE A (HI) SGGTA		TIGACTACTOGOGCCAOSSANCCCT	(1)	27
C433-107		(HP1)	ARGEBTT		I THE THE THE THE THE BACK TO THE BABCCHARGE CAL	(6)	27
C100-131	27.5	(81)	TATABCABCASCISG		Lattac66TRT06AC6TCT6558CCRR88RCCRC	(6)	27
CI78-104	4r.5	(XP1)			LEBTTCBACCCCTBUBGCCRUBBAACCCT	(5)	27
C731-94	30	(K1)	BRITAC		TACTTTGACTACT5666CCABT66ARCCCT	(1)	
C77D-101	32.5	(#4)	TGATABTTETE (DIA1) CCTGCCC			(1)	
C770-107		(HQ)	TAACTEBBBA TBAD (DIAI) TTADGAGCTITGA		ATECTITIESTATE SEBACCASANCEL	a	28
C111-105	33.5	(HQ) (WB11)			GACTACTESESCCARSBARCCCT	(4)	27
C68-101	16.5	(LR1)	A GENTAL STATECA	00000	BRETRETBEBESCEREBBRACECT	(1)	
C68-105		(XP4)	RTTTTTBBASTSSTATCTATATACC	CGC	GACTACTESEBCCASSGAACCCCT	(1)	
C68-122		(K1)	STBOATATABTSBTCTCGA (HI) RGCRGCRBCTGGTAC				27
C26-105	38	(81)	GTRCCABETBCT	1001506	TTGBETACTSBEECCABBERACCT	(4)	
C200-98	39	(#4} /#81)	565TAYABCASCIC5IC	AACCECCTGGA	TACTACGS TATEGACSTCTGSSSCCAASSACCAC	(6)	
C200~119	41.5	(10)	TITIENCIGNING	A	TCT0 BOSCERNOSBACAAT	(3)	
C527-112	44	(L4)	TAGTAGTACCABCTGCTATG	6	TGGTTCGACCCCTGGGGCCABGGAACCCT	(5)	
C\$27-121		(L1)	GGTGTATBCTATAC	TGGB	THETTIGHEINCIGESSSCCHOODHHELEEI		
C69-104	44.5	(M1)	TACMBTRACTAC	656(T)	1 TEACTACTA BEACCASSARECCT	à.	
C69-105		(104)	NINCONIIIINOCUNIADIIN Tattatastantarenti fot (VPS) protobergente	ATCCT	TACTACTACSSTATOGACOTCTIOSSCCAASBAACCAC	(6)	27
C132-137	46	(LA2)	GENTATTATABIBETABCIGC CIGA (K4) AGROCICANIGGT	A150	ACTACTACTACOS TATSSACSTCTBUSECCAASBACCAC	(6)	27
C72-968	15	(LR4)	GTAGTAGTACCASCTGCT	TAGBBECTA		(5)	
C344-99	47	(HQ)	ACCT6666A	TCCGGTTGG	SALIALISSELLABORANCELL SALIALISSELLABORANCELL	ä	
0174-113	47.5	(XPIR)	TATTACTATESTICSSGAST	CAC 8521551	ACTACTEBBBCCABBBARCECT	(4)	
C310-100	53	(184)	In JAGEAGE		ACTACTACTAC6GTAT6GAC8TCT6688CCAA688ACCAC	(5)	
C92-123	63	(801)	ACGRINTITACTSSTATTA	GGCCCCTGGG	CTACTACOG TATGGACGTCTGBGBCCAABBBACCAC	(6)	28
C\$15-116	51.5	(XP4)	GTRTTRCGRTTTTTGGRBTGGTTRT	CGARTS	A LOCITITICATATCI SOBOCLADONCANT	(ii)	
C113-105	66.5	(N1)	NGCT66	010010	CTRETRETACTALEGTATGGACGTETGB68CCAA686ACCAC	(6)	
C113-140		(21/8)		R	TGACTACTEBBBBCCASSBAACCCT	(1)	26
C136-114	70.5	(LR4)	SATATTSTAGTAGTAGTAGCTGCT		ACTESTICEACCCTESSECCAEBBAACCCCT	(5)	28
C552-104	70.5	(#1)	ACAGTARCT ACAGAAATGAAGTA (21/9) ATGATA	M	TSOUSCENSERRECCET	8	
C422-111	71	(LM3)	TATTOTOGTESTESTERCTECTAT GACCT (HQ) ACTEGAGER	TTCIG	TITGACTACTABABACCASTA	- ä	26
C432-90	73.5	(DIA2)	GGAGGC Eccippes fittert	00000011	ACTACTITIGACTACTBGGGGCCAGSGAACCCT	(1)	26
C721-123	74	(21/9)	our iterol in 1801	CAGAGE	ACTITGACTACT&BBBBCCABBBAACCCT	(1)	26
C148-106	90	(#1)	ANCTESAAC	CETTR	GGRCTRCTRC5GTRT6GRC6TCT8888CCRR868ACCRC	(6)	
C130-114	85.5	(11)	GGGTATAGCAGTGGCTGG	GGTG	116C [[]] 6H] H [C] 6 888 CCM 899 HC HH] 1768 CT9C TE 688 PC 868 PC PC F	(1)	26
C182-109	87.5	(81)	ATABCASCASCASE		TGGBSCCRSSBRCCCT	- ä	24
ALL1-155	102.5	(21/10)	TITEGEGRAFINT GECCEGESTEL (UIR2) LERCELEN GEGELLE GEGEL (ALUMAN) Avantarianeteristentet (Constantarianeteristentetista) (21/4) retatariastastastastastastastastastastastastasta		CTBBBCCRBBBACCCT	(0)	24
F52-121	112	(179)	Ganarizatashekarcagelgetat		CTRETACTACATGGACGTET6568CCAR688ACCAC	(6)	28
C52-132		(LR3)	NGENTATIGEGEGEGEGECTECT	GRGCARC(GT)	ACTACTITGACTACTOGGGCCADBBARCECT	(1)	28
C60-60	128.5	(D1R2)	CCGGCAGCCCG	GGACT	TATAGACATC TAGGACCAGAGAAAAACCCAC	(6)	
C24-104	130	(XP'1)	GETICOBE	COCCOOMI	TRETTTERCTACTEGESCERESERRECCT	(4)	28
C238-133	146.5	(181)		CGITAT	TACTACTACCAGCAGCATCTAGGGCCAAGGGACCAC	(6)	
C280-120	148.5	(LR4)	TTGTBBBABBACCASCISC	111111	TINCTACTACTACTACTACTACATGGACGTCTGGGGCAAAGGGACCAC	(6)	
C290-112R	168	(LM3)	CATATT&T>>&ATT	ATR	QUACLAC ACTITODOTOCIONACIONACIONACIONACIONACIONACIONACION	(0)	21
C211-123	169	(21/9)	GINTINCIA TGATAGATAGATAC	CAGAGG	TTACTACTACTACTACATGGACGTCTGGGGGCCAAGGGGACCAC	(6)	26
C211-135		(197)	SATITIGANGTAGILATI ICDIA (21/9) ADIGULAALI Bernatatevaltevaltekatarikertet (31/9) adigulaali	66666	CTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCAC	(6)	27
C155-137	175	(11)	GGCTREBATTA BGTC (21/9) ATTACTATGATAGTAGTAGTAGTAGA		CLACTRCTRCTRC66TRT66RC6TCT66666CCRR666ACCAC	(6)	
C771-132	178	(LR4)	ATTGTAGTAGTACTACTACTAT TT (L4) TAGTCGARGCGABCTSCTAT	TGTAGE	CTACTGBGGCCMGGGAACCCT	(1)	
C771-158		(LR4)	GRTATIBTAGTAGTAGCAGCTGCTAT CGAGGTCTTGTGGACCCCA (21/9) ATTACTATGACAGTAGTGGTTATT	THATCCCA	CTACTACGGTATSGARCHTCTSGGGCCARRAGACTAC	(6)	
C372-115	183.5	(XP4) (LP4)	GIGGITATTAIN II LUTRIJ BAGGATAG Actorect	000	CTESTTCGACCCCTEGEECCAGEGAACCCT	(5)	28
C277-119	191	(K1)	REGEBECTACE (DIR2) GEGEGETCCERGE	TREG	CIEGTICGACCCCIEGEGCCASEGAACCCI	(5)	
C252-113	191.5	(LR4)	GATRITBINGTAGTACCAGCIGCIAT	ABC	GACTACTEGEGCCAGEGANCCCT	(1)	
2252-123		(81)	GGGTATGS STITATGGGT (21/9) TATGATAGTAGT	CCCTCCCCGA	ACTRET&GGGECHGGGRACEET	(1)	
0130-122	204	(LR2)	GETGETARCTCC	000	ATAACTEGTTCGACCCCTGGEGCCASEGAACCCT	(5)	26
0260-106	210.5	(X₽'1) (#1)	551165656911M Actort	66000666	66 TA TEGACETC TEGESCCAAGEBACCAC	(6)	
r 301-A3	212.3	1417					

Figure 1. DNA sequence of the DJ_{H} region from 61 cases of B lineage lymphoblastic leukemias. The first column indicates the sequence code (case number and the length of the CDR3, including the V_{H} and J_{H} primers). Each sequence is subdivided into D (and when applicable multiple D with intervening N regions), N, and J_{H} (5' end) regions. Germline D and J_{H} segments with maximum homology to the segments used in the CDR3 sequences are shown to the left and right of these segments, respectively. P nucleotides are enclosed by parentheses. 5' ends of J_{H} segments sharing homology with the 3' end of the D segment are indicated by lower-case letters. References are shown where the indicated sequences have been previously published.

to 17 yr) with ALL. Morphologically normal bone marrow samples from three children (21–26 mo) with solid tumors obtained on routine staging were used as a source of normal marrow B cells. Consent was given by the patients or their parents and by the Committee for Protection of Human Subjects at the Children's Hospital of Philadelphia.

Diagnosis of ALL in each patient was based on standard morphologic and histochemical parameters of the leukemic cells. The B cell precursor lineage of these leukemias was confirmed by immunophenotyping in which all the leukemias expressed the B lineage antigens CD19 or CD10 and were negative for T cell antigens (20).

DNA Amplification, Cloning, and Sequencing of CDR3. Marrow cells were fractionated on a Ficoll-Hypaque gradient (d = 1.077) and genomic DNA was isolated from mononuclear cells by established procedures (21) or by rapid cell lysis using nonionic detergents (22).

PCR was carried out as described (23) using an annealing step at 55°C for 1 min, an elongation step at 72°C for 30 s, and a denaturation step at 92°C for 1 min (14, 24). The V_{μ} and J_{μ} nucleotide consensus primers used in the PCR and the J_{μ} consensus ($J_{\mu}C$) probes used for screening have been described (14, 24). These consensus primers do not preferentially amplify CDR3 sequences containing specific J_H segments (14). Restriction endonuclease digestion of the PCR-amplified DNA, electrophoresis in a 4% Nusieve agarose gel (FMC Bioproducts, Rockland, ME), purification, and cloning of CDR3-containing bands were performed as described (14, 24). Positive clones were picked up randomly (10-20/sample), and a double-stranded DNA template was prepared and screened by T lane sequencing. In each leukemia, from one to three different T lane sequences were identified that were present in multiple copies and were fully sequenced using the dideoxy method (25). In each normal marrow all the T lanes contained different sequences and \sim 20 sequences were obtained from each marrow.

DNA Sequence Analysis. DNA sequencing data were analyzed for utilization of D and J segments using the sequence analysis software pack (Release 5; Genetic Computer Corp., Madison, WI) at the University of Wisconsin and a Micro Vax II computer (Digital Equipment Corp., Marlboro, MA), according to described criteria (14).

Results and Discussion

80 DNA sequences of VDJ joinings from 61 B lineage ALLs were analyzed for the presence of N regions at the DJ_H junction (Fig. 1). Overall, 31/80 (38.8%) DJ_H junctions lacked N regions, a frequency higher than that reported for human adult tissues and similar to that found in human fetal tissue (13). When the percentage of leukemias lacking N regions was analyzed as a function of age at diagnosis, a striking pattern emerged: in patients ≤ 3 yr old, 87.5% (14/16) of the leukemias were comprised entirely of CDR3 sequences lacking N regions; whereas in children >3 yr old, only 11.1% (5/45) of the leukemias met this criterion.

The percentage of leukemias without N regions in children ≤ 3 yr old was much higher than expected even when compared with data reported for normal human fetal liver or neonatal cord blood (13), whereas the frequency observed in children >3 yr old was close to that observed in adults (13, 14). To exclude the possibility that the paucity of N regions in these young patients could be due to an inherent abnormality in TdT activity, we examined the DJ_H joinings in lymphocytes obtained from the end of therapy marrows of three of these patients when residual leukemia was not detectable using PCR analysis (Fig. 2). N regions were present

PRTIENT CDR3 CODE		D	N	J	
C100-91	(LR3)	GGAGGTGAT	с	TTGACAACTGGGGCCAGGGAACCCT ((4)
C100-918	(LR2)	GGTAGC GA (HQ) TGGGGA	-	CTGGGGCCAGGGAACCCT (5)
C100-94	(K4)	GCTATGG		ttTGACTACTGGGGCCAGGGACCCT (4)
C100-102	(21/10)	GGGGAGCT (DF16) ACTACGGGGT	(T)	ACTACTTTGACTACTGGGGCCAGGGAATCCT (47
C100-103A	(HQ)	CTARCTGGGGA	RGATTT	TITGACTACTGGGGCCAGGGAACCCT ((4)
C100-106	(21/9)	TAGTGGTTATACCAC (DIRI) CCACCAC		TGACTACTGGGGCCAGGGACCCT ((4)
C100-109	(LR4)	GTACCAGCTGCTATAT	CGATCACGGGG	GGTATGGACGTCTGGGGCCAAGGGACCAC (6)
C100-109A	(N1)	STATAGCAGTGGCTGGTAC (N1) CAGCCGGT		CITIGACTACTGGGGCCRGGGAACCCT ((4)
C100-1098	(K1)	RETGECTRCGA	CGGGCC	RATTGGTTCGACCCCTGGGGCCAGGGAACCCT ((5)
C100-115	(11)	GGATAGCRGTGGCTGGT (DIRI) CCCACCC	C	CTACGGTATGGACGTCTGGGGCCAAGGGACCAC ((6)
C100-1158	(XP4)	GAGTGG GAGC (XP4) TACTACGATGTT	ATCTC	ATTACTITGACAACTGGGGCCAGGGAACCCT ((4)
C100-132	(XP'1)	RGGETTCG (LR3) TGTTGATTG GET (K1) TGGATATAGTGGCTACGAT	CC	ACAACTEETTCEACCCCTEEEECCAEEEAACCCT ((5)
C100-136	(112)	TACGGGTTA GGACTT (LR5) TACTACTCT TCGGGACCC (DIR2) GGGCGGGG		CTACTATTATTREGGTATGGACGTETGGGGGCEAAGGGACEAC (.6)
C178-97	(21/9)	ATTACTATGATA		TACGGTATGGACGTCTGGGGGCCAAGGGACCAC ((6)
C178-103	(LR+)	GTTGGTACTA (DIR1) GGGGACGGGGT		TACGGTATGGACGTCTGGGGCCAAGGGACCAC ((6)
C178-117	(XP1)	GTTCGGGGGGTT	CAC	AATACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCAC ((6)
C178-121	(LR4)	TCTAGGAGTACCAGCTGCTATG	AGA	ACTACTACTACTACGGTATGGACGCTCGGGGCCAAGGGACCAC ((6)
C178-122	(N1)	AGCAGCAGCTG	CCR	ACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCAC ((6)
C178-1228	(XP'1)	GTATTACTATGGTTCGGGGGGTTATT	TCC	CTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCAC ((6)
C178-135	(21/9)	TACTATGATAGTAGTAGTGGTTATTACTAC (21/10) ATCATTAC	C	AATACTACTACTACTACGGTATGGGGCCAAGGGACCAC ((6)
C178-136	(DIR1)	SGTCCTCCRGG CACG (21/9) ATTRCTATGATAGTAGTGGT	TCGTC	TTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCAC ((6)
C178-136R	(LR4)	TAGTACGGCAGCTGCTA CA (N2) AACCGGGA	C	TACTACTACTACTACGGTATGGACGTCTGGGGGCCARGGGACCAC ((6)
CI11-103	(21/9)	AGTAAGTGGT	c	CTACITCGGTAIGGACGICIGGGGCCAAGGGACCAC ((6)
C111-106	(XP4)	GETTITEGAGIGETTATIACE	GG	GACTACTGGGGCCAGGGACCCT ((4)
C111-1068	(XP'1)	TTACTATGGTTCGGGGAG	CCGTGCGTCA	TTTGACTACTGGGGCCAGGGACCCT ((4)
C111-112	(K4)	GTGGATACAGCTATGG		ARTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCAC	(6)
C111-124	(21/10)	TTATGATTACGTTTGGGGGGGTATCGTTA	C(T)	ACAACTGGTTCGACCCCTGGGGCCAGGGAACCCT ((5)
C111-139	(21/10)	TGGGGGRGAGA ACCTTTGCRAAAGATTA (DIR2) TCGGGATTGGGCTT A (NI) ATAGCAGCAGCTGG		1 TGACTACTGGGGCCAGGGAACCCT ((4)
C111-145	(LR2)	GGACATTGTAGTGGTGGTAGCTGCTAC	AAGATAGGGTT	TTTCTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCAC ((6)

Figure 2. DNA sequences of the DJ_{μ} regions derived from normal B cells obtained from the end of therapy remission marrow of three patients with B lineage lymphoblastic leukemia exhibiting fetal type DJ_{μ} joinings. See Fig. 1 for further explanation.

in the majority of the joinings, indicating the presence of TdT activity. An alternative explanation for the lack of N regions is that bone marrow B cells (which give rise to ALL) might normally have a higher frequency of DJ_H junctions that lack N regions as compared with the other lymphoid tissues. N region frequency was calculated from the joining sequences obtained from normal bone marrow of three young children (21–26 mo old). N regions were present in 50/53 (94.3%) DJ_H junctions (data not shown), a frequency comparable with the 86% and 90–100% reported for newborns and adults, respectively (13, 14). A high frequency of multiple D regions was noted in the DJ_H junctions obtained from the normal B cells of the young leukemic patients, suggesting the possibility that abnormalities in the VDJ recombination process are present in these children.

To determine whether the DJ_{H} junctions without N regions in the 14 leukemias in children ≤3 yr old utilized specific D or J_H segments, we examined the frequency distribution of such segments. The D gene family is composed of \sim 30 segments including multiple and duplicated loci, and thus patterns of utilization are difficult to analyze (1). XP was found to be the most commonly used D family in the leukemias, consistent with the report by Sanz (13) for fetal tissue. However, the leukemias showed a lower frequency of HQ52 utilization (9.1%) than that reported by Sanz (13) (14%) and Schroeder and Wang (29) (50%) for fetal tissue. $J_{\rm H}4$ and $J_{\rm H}6$ were the two predominant gene segments used in the leukemias comprising 80% of the sequences, whereas $J_{\rm H}1$ and $J_{\rm H}2$ usage was absent. In fetal tissue, Sanz (13) found J_{H4} usage to be the most common followed by J_{H3} and J_{H6} , whereas Schroeder and Wang (29) found J_H3 usage to be the most common followed by J_H4 and J_H5 . These differences in I_H and HQ52 usage found in fetal liver tissue may reflect individual variation as samples were obtained from only one or two fetuses in each study (13, 29).

Consequently, developmental stage-specific trends in the usage of D and J_{μ} gene segments in fetal B cells have been consistently demonstrated only for the D segment HQ52, and this usage varies widely (13, 29). While HQ52 usage appears to be a marker of fetal-derived B cells, its absence does not imply a more developmentally mature stage. Thus, the absence of N regions in the CDR 3 sequences appears to be a more useful marker of fetal origin. The vast majority (87.5%) of leukemias occurring in children ≤3 yr old lacked N regions at the DJ_H junction, suggesting that they arose from a transformation event in utero when the pool of fetal lymphoid cells is largest. Furthermore, the sharp decrease in the frequency of DJ_{H} junctions lacking N regions after 3 yr of age suggests that some leukemias initiated during fetal life require a maximum of 3 yr to become clinically evident. The absence of N regions observed in a small fraction of leukemic and normal B lymphocytes present later in life may represent a DJ_H joining event that occurred during fetal development in a long-lived B cell or may simply indicate that Tdt was inactive at the time of DJ_H joining in a developmentally mature lymphoid cell.

Greaves (30) has proposed a two-step mutation model in B lineage ALL in which the first event occurs in utero in association with an ontogenic drive to expand B cell precursors in liver and marrow. The second event produces clinically overt leukemia and is associated with the proliferative stress elicited in infants by the rapid onset of immune responses to exogenous antigens. Support for this latter event stems from the observation that the steep rise in the incidence rate of B precursor ALL follows shortly after serum antibody levels sharply rise toward adult levels during the first 2 yr of life (31). Our observation would be compatible with Greave's hypothesis but would limit the in utero transforming event to primarily those leukemias that occur during the first 3 yr of age.

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