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Research Article

We have explored the relationship among immunoglobulin gene rearrangement, cytoplasmic immunoglobulin production, and cell surface antigen expression within 37 cases of acute lymphocytic leukemia. All 12 cases of the T cell type had germ-line kappa and lambda genes and 11 of 12 had germ-line heavy chain genes. In contrast, all 25 cases of the "non-T, non-B" classification, which lacked both definitive T cell markers and surface immunoglobulin, had rearranged immunoglobulin genes, indicating that they represent precursor cells already committed to the B cell lineage at the gene level. 14 had rearranged heavy chain genes, yet retained germ-line light chain genes, whereas 11 cases had both heavy and light chain gene reorganizations. All patterns of immunoglobulin gene rearrangement predicted by a model that proceeds from heavy chain gene recombination to light chain genes were observed. Despite the uniform presence of rearranged immunoglobulin genes, only five cases produced cytoplasmic mu-chain, one exceptional case produced gamma-chain, and another produced only lambda-chain. The cases of B cell precursor type that do not produce immunoglobulin may represent cells that frequently possess ineffectively rearranged immunoglobulin genes. Included in this group may be a set of cells trapped within the B cell precursor series because their ineffective rearrangements have eliminated certain gene subsegments necessary for the assemblage of an effective heavy chain gene. All seven cases of [...]

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Immunoglobulin Gene Rearrangement and Cell Surface Antigen Expression in Acute Lymphocytic Leukemias of T Cell and B Cell Precursor Origins

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ABSTRACT We have explored the relationship among immunoglobulin gene rearrangement, cytoplasmic immunoglobulin production, and cell surface antigen expression within 37 cases of acute lymphocytic leukemia. All 12 cases of the T cell type had germ-line κ and λ genes and 11 of 12 had germ-line heavy chain genes. In contrast, all 25 cases of the "non-T, non-B" classification, which lacked both definitive T cell markers and surface immunoglobulin, had rearranged immunoglobulin genes, indicating that they represent precursor cells already committed to the B cell lineage at the gene level. 14 had rearranged heavy chain genes, yet retained germ-line light chain genes, whereas 11 cases had both heavy and light chain gene reorganizations. All patterns of immunoglobulin gene rearrangement predicted by a model that proceeds from heavy chain gene recombination to light chain genes and from κ to λ within the light chain genes were observed. Despite the uniform presence of rearranged immunoglobulin genes, only five cases produced cytoplasmic μ -chain, one exceptional case produced γ -chain, and another produced only λ -chain. The cases of B cell precursor type that do not produce immunoglobulin may represent cells that frequently possess ineffectively rearranged immunoglobulin genes. Included in this group may be a set of cells trapped within the B cell precursor series because their inef-

fective rearrangements have eliminated certain gene subsegments necessary for the assemblage of an effective heavy chain gene. All seven cases of the non-T, non-B subgroup that bore HLA-DR but lacked CALLA (the common acute lymphocytic leukemia-associated antigen) represented the earliest recognizable stage of B cell precursors with rearranged heavy chain genes but germ-line light chain genes. Correlations here suggest that cells entering B cell development express HLA-DR and rearrange heavy chain genes before the expression of a B cell-associated antigen recognized by the antibody BA-1, the antigen CALLA, and any subsequent light chain gene rearrangements.

INTRODUCTION

The study of acute lymphocytic leukemia (ALL)¹ has provided important insights into early human lymphoid differentiation (1-5). Yet, considerable controversy has surrounded the cellular origin of such leukemias as only ~20% bear cell surface markers restricted to T cells and only extremely rare cases possess the cell surface immunoglobulin (SIg) of a mature B cell (6). Because of this, the majority of cases have frequently been termed "non-T, non-B" ALL and they often, but not uniformly, bear an additional marker, the common ALL-associated antigen (CALLA) (7). Initial studies of eight such cases of common, non-T,

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¹ *Abbreviations used in this paper:* ALL, acute lymphocytic leukemia; C, constant (segment); CALLA, common ALL-associated antigen; D, diversity (segment); FCS, fetal calf serum; H, heavy (chain); J, joining (segment); kb, kilobase; L, light (chain); SIg, cell surface immunoglobulin; SRBC, sheep erythrocyte; V, variable (segment).

non-B ALL illustrated the usefulness of the immunoglobulin (Ig) gene arrangement patterns in classifying these controversial malignancies (8). Such analysis takes advantage of the fact that before its expression an Ig gene must first undergo a rearrangement at the DNA level that assembles the various subsegments of this discontinuous gene. A heavy (H) chain gene must successfully recombine separate variable (V_H), diversity (D_H), and joining (J_H) gene subsegments before cytoplasmic μ -chain can be produced (Fig. 1) (9–11). Likewise, the light (L) chain genes must correspondingly recombine V_κ and J_κ or V_λ and J_λ subsegments before their effective expression (12–15).

Recently, a number of monoclonal antibodies have been developed that recognize antigens that are primarily expressed on either T cells or B cells (4, 16–18). In the present study we compare the patterns of Ig gene recombination and cytoplasmic Ig production with the cell surface phenotype of 28 unselected fresh presentations of ALL in addition to nine well-characterized cell lines arising spontaneously from such ALL cases.

We demonstrate that all 25 cases of ALL without definite T cell surface markers were apparently committed to B cell development at the Ig gene level. Furthermore, all patterns of Ig gene rearrangements predicted by a model that proceeds from H chain gene recombination to the L chain genes and from κ to λ within the L chain genes were observed. Although B cell precursor ALL uniformly displayed rearranged H chain genes and often L chain genes, they usually failed to produce detectable cytoplasmic Ig. These observations may indicate that many of these leukemias represent monoclonal expansions of B cell precursors at stages in which they have frequently made ineffective, aberrant rearrangements of their Ig genes.

Overall, there was a very good correlation between the cell surface markers associated with B cells (especially HLA-DR and p30 as detected by BA-1) and the presence of rearranged Ig genes, as well as the presence of T cell-associated antigens (especially that detected by 3A-1) and the retention of germ-line Ig genes. Within the B cell precursor series there were seven cases of non-T, non-B ALL bearing HLA-DR antigen but lacking CALLA. This subset represents the earliest identifiable stage of B cell precursors, as the cells had H chain genes that were rearranged but L chain genes that remained germ line. These observations suggest a coordinate sequence of cell surface antigen expression and Ig gene recombination during early B cell differentiation.

METHODS

Initial characterization of ALL. Leukemic cells were obtained by Ficoll/Hypaque gradient centrifugation of either heparinized peripheral blood, the leukapheresis-ob-

tained lymphocyte fraction, or bone marrow from patients with ALL. The leukemic lymphocytes constituted 90% or more of the total cells in each preparation. 28 cases of newly diagnosed ALL before therapy, plus nine cell lines that were established spontaneously from similar such cases (Molt-4, CCRF CEM, CCRF HSB-2, RPMI 8402, HPB-Null, NALM-16, NALL-1, NALM-6, and REH) (19) were examined. All cases were assessed for their capacity to form rosettes with sheep erythrocytes (SRBC) and all values of 15% or greater were considered positive for this T cell marker. Similarly, all cells were examined for the presence of SIg by direct immunofluorescence with F(ab')₂ fluorescein-conjugated reagents of rabbit anti-human polyclonal Ig and/or similar reagents to the specific classes of γ , α , μ , δ , κ , and λ (20). None of the 37 cases examined had detectable SIg.

Monoclonal antibody studies. When adequate numbers of cells were available, they were assessed for the presence of a variety of cell surface antigens. Cells were washed in Hanks' buffered saline solution without pH indicators, with 3% fetal calf serum, and with 0.1% sodium azide. 1 million cells per test were reacted at 4°C for 30 min with a predetermined concentration of a monoclonal antibody, washed twice, and then reacted at 4°C for 30 min with a fluorescein-labeled second antibody that recognized the H chain of the initial mouse monoclonal antibody. After three washes, the cells were analyzed by flow microfluorometry with a fluorescence-activated cell sorter (FACS II, B-D FACS Systems, Becton, Dickinson & Co., Sunnyvale, CA) (21). Appropriate controls of cells without antibody were run to assess autofluorescence, and cells treated with normal mouse serum and the fluorescein-labeled second antibody were both used as a control for background staining. A fluorescence profile of each antibody used was produced and the appropriate background control subtracted. This provided an estimate of the percentage of cells within the total population bearing that antigen. If >50% of the cells bore the antigen, the monoclonal antibody reactivity was scored as positive (+), whereas <10% was scored as negative (-), and those with values between 10 and 50% were specifically listed in Tables I and II.

Monoclonal antibodies used here include the J-5 monoclonal that recognizes CALLA (22), and the DA-2 antibody that detects a nonpolymorphic HLA-DR determinant (23). The BA-1 monoclonal antibody identifies an antigen, p30,² which is expressed on cells at multiple stages of B cell development (17). The BA-2 monoclonal antibody identifies a 24,000-D, B cell-associated antigen (18). The 3A-1 (16), OKT3, OKT4, OKT6, and OKT8 monoclonal antibodies (4) recognize T cell-associated cell surface antigens. The OKT9 monoclonal, which recognizes the transferrin receptor (24), and the OKT10 antibody, which recognizes another non-lineage-dependent antigen, were also used (4).

Cytoplasmic Ig studies. The presence of cytoplasmic μ , γ , κ , λ , and in some instances α and ϵ , was determined whenever possible. Approximately 40×10^6 of the fresh leukemic cells from a given case were washed free of residual plasma Ig by centrifugation through six 10-cm³ fetal calf serum (FCS) gradients. These cells were then incubated in the presence of 0.25% trypsin for 30 min at 37°C with rocking to digest any trapped Ig between aggregated cells. FCS was then added as a source of alpha₁ antitrypsin to inactivate the trypsin. Residual Ig fragments were then removed by three additional washes. Cells were recounted and resuspended in 1% Triton at 10×10^6 /ml and extracted at 37°C for 30 min in a shaker bath. Cellular debris was removed by centrifugation of these extracts at 40,000 rpm for 30 min and su-

² LeBien, T. W. Unpublished observations.

pernatants were saved. The cell lines were examined by the same procedure except that the initial FCS washes and trypsinization proved unnecessary as such cells were grown in media free of any human serum. The Ig in these Triton extract supernatants were measured by using separate, extended curve, double antibody radioimmunoassays (RIA) sensitive within the picogram per milliliter range (25). Values were expressed as picograms of Ig per 10⁶ cells. Negative results for μ -chain represent values at or below <200 pg/10⁶ cells, for γ -chain values < 300 pg/10⁶ cells, for κ -chain values < 320 pg/10⁶ cells, and for λ -chain values < 200 pg/10⁶ cells. These values represent the cut-off limits of sensitivity for each of the assays. Control experiments in which known quantities of an Ig class were added before extraction indicated that this Triton procedure neither destroyed nor spuriously elevated the values for the amount of Ig detected by the RIA. All RIA assays were performed in duplicate and all positive values represent the average of at least two separate extraction determinations.

Determination of Ig gene configurations. High-molecular weight DNA was extracted from the leukemic cells of each of the cases of ALL as well as from the cell lines. These genomic DNAs were digested to completion with BamHI or EcoRI restriction endonuclease, size fractionated over agarose gels by electrophoresis, and transferred to nitrocellulose or diazobenzoyloxymethyl paper (26, 27). Such paper-bound DNA fragments were then hybridized to nick-translated [³²P]DNA probes of the germ-line Ig genes at ~200 cpm/pg sp act (28). After washes at the appropriate stringency, the Ig gene configurations were visualized on autoradiograms (as seen in Fig. 2 B). The human Ig gene probes used are shown in Fig. 2 A and were capable of detecting germ-line and rearranged genes. The J_H probe, which consisted of a 2.4-kilobase (kb) germ-line Sau3a fragment, could recognize rearrangements in either BamHI- or EcoRI-digested DNA (10). When non-B cell sources of tissue were examined from > 50 individuals, this J_H gene segment was routinely found

on a 17-kb BamHI fragment without evidence for polymorphism within the population. The J_H segment was also routinely seen on a 16-kb EcoRI fragment without polymorphic variations. A 1.3-kb germ-line EcoRI probe containing the constant (C) _{μ} region also recognized H chain gene rearrangements within BamHI digests and consistently identified a 17-kb size BamHI fragment in germ-line DNA (10). A D_H segment probe was comprised of a 1.8-kb germ-line BamHI fragment, and it detected recombinations of this particular D_H gene family within BamHI-digested DNA (11). κ gene rearrangements were detectable within the BamHI digests of DNA by a 2.5-kb germ-line EcoRI C _{κ} probe (14). This probe detected a 12.0-kb BamHI fragment for germ-line κ genes in all individuals tested. The combined C _{λ} probe used to detect λ gene rearrangements in EcoRI digests consisted of both a 0.8-kb germ-line BamHI-HindIII fragment containing the C _{λ 1} gene and a 1.2-kb germ-line BamHI-EcoRI fragment containing the C _{λ 2} gene (15). The cluster of C _{λ} gene segments involved in rearrangements within λ B cells was present upon 8-, 14-, and 16-kb sized EcoRI fragments in their most common polymorphic form (15). Other polymorphic patterns of the λ genes were also clearly identifiable in which several other differently sized fragments could substitute for the 8-kb band. In addition, a weakly hybridizing λ pseudogene was frequently seen on a 5-kb sized EcoRI fragment (29). Whenever a leukemia DNA was examined, a non-B cell source of DNA was simultaneously run as a control to clearly identify the germ-line position of the Ig genes.

RESULTS

Phenotypic markers of ALL: T cell cases. 8 of the 28 cases of freshly diagnosed ALL bore distinguishing T cell markers (Table I). Seven formed >30% rosettes with SRBC and the remaining case (No. 9), which

TABLE I
T Cell All

Case identification	Phenotype markers										Ig gene patterns§		
	ER*	Monoclonal antibodies†								H chain	κ	λ	
		J5 CALLA	DA2 HLA-DR	BA-1 p30	BA-2 p24	3A-1	T3	T4	T6				T8
1	+	(15)	(32)	(49)	-	+	-	-	-	(35)	C μ ,J _H ,D _H :Germ	Germ	Germ
2	+	-	-	-	-	+	-	+	+	-	C μ ,J _H ,D _H :Germ	Germ	Germ
3	+	-	-	-	-	+	+	-	-	-	C μ :Germ	Germ	Germ
4	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	C μ ,J _H ,D _H :Germ	Germ	Germ
5	+	-	-	-	-	NA	NA	NA	NA	NA	C μ :Germ	Germ	Germ
6	+	-	-	-	-	+	-	-	(27)	-	C μ ,J _H ,D _H :Germ	Germ	Germ
7	+	NA	-	NA	NA	+	-	-	+	(10)	C μ :Germ	Germ	Germ
8 (Molt 4)	+	-	-	-	+	+	-	-	+	+	C μ ,J _H ,D _H :Germ	Germ	Germ
9	(14)	+	-	-	-	+	+	+	+	(38)	C μ :Germ	Germ	Germ
10 (CCRF-CEM)	-	(42)	-	-	-	+	-	+	+	+	C μ ,J _H ,D _H :Germ	Germ	Germ
11 (CCRF-HSB-2)	-	-	-	-	-	+	-	-	-	-	C μ + J _H :1 Rearr 1 D _H :Germ	Germ	Germ
12 (RPMI 8402)	-	+	-	-	+	+	-	-	-	-	C μ ,J _H ,D _H :Germ	Germ	Germ

Germ, germ line; Rearr, rearranged; NA, not available.

* Sheep erythrocyte rosette formation.

† Fluorescence-activated cell sorter data in which a (-) is <10% of the cells showing reactivity, a (+) is >50% reactivity, and specific values are given for the 10-50% range.

§ H and L chain gene patterns with C μ , J_H, D_H, C κ , and C λ probes.

formed 14% rosettes, had numerous T cell-associated antigens as detected by the monoclonal antibodies of 3A-1, OKT3, OKT6, OKT4, and OKT8 (Table I). In addition, four well-studied cell lines that arose spontaneously from T cell type ALL (CCRF CEM, CCRF HSB-2, RPMI 8402, and MOLT-4) were examined and reacted with the most useful distinguishing antibody,

3A-1. Thus, within this group of 12 T cell ALL, 8 formed definitive numbers of rosettes, whereas 4 cases did not form rosettes and may represent earlier precursor T cells or T cells with defective SRBC receptors. None of these 12 cases displayed any detectable cytoplasmic Ig (data not shown in Table I). Of note, 4 of the 12 cases bore detectable amounts of CALLA,

TABLE II
Non-T, Non-B All

Case identification	Phenotypic markers										Ig gene patterns§		
	Monoclonal antibodies*					Cytoplasmic Ig†				Heavy chain			
	J5 CALLA	DA2 HLA-DR	BA-1 p30	BA-2 p24	3A-1	μ	γ	κ	λ		κ	λ	
1	-	(37)	(32)	(30)	-	-	-	-	-	C _μ :1 Rearr 1 Del	Germ	Germ	
2	+	+	+	+	-	715	-	-	-	J _H :2 Rearr C _μ + J _H :2 Rearr D _H :Del	Germ	Germ	
3	-	+	-	+	-	-	-	-	-	C _μ :1 Rearr 1 Germ	Germ	Germ	
4	+	+	+	+	-	-	-	-	-	C _μ + J _H :1 Rearr 1 Del	Germ	Germ	
5	+	+	+	+	-	570	-	-	-	C _μ + J _H :1 Rearr 1 Del	Germ	Germ	
6 (HPB-Null)	(10)	+	+	+	-	2420	-	-	-	C _μ :1 Rearr 1 Del	Germ	Germ	
7 (NALM-16)	+	+	+	+	-	-	-	-	-	C _μ + J _H BamHI:Germ J _H EcoR ₁ :1 Rearr 1 Germ D _H :Germ	Germ	Germ	
8	-	+	+	-	NA	NA	NA	NA	NA	C _μ :2 Rearr	Germ	NA	
9	-	+	+	+	NA	NA	NA	NA	NA	C _μ :1 Rearr 1 Del	Germ	Germ	
10	+	+	+	+	NA	NA	NA	NA	NA	C _μ :2 Rearr	Germ	Germ	
11	-	+	-	+	NA	NA	NA	NA	NA	C _μ BamHI:Germ J _H EcoR ₁ :1 Rearr 1 Germ D _H :Rearr	Germ	Germ	
12	+	+	+	+	NA	NA	NA	NA	NA	C _μ :2 Rearr	Germ	Germ	
13	-	+	-	+	NA	NA	NA	NA	NA	C _μ :1 Rearr 1 Germ	Germ	Germ	
14	-	+	+	+	NA	NA	NA	NA	NA	C _μ :2 Rearr	Germ	NA	
15	+	+	-	-	NA	-	-	-	-	C _μ :1 Rearr 1 Germ	1 Rearr	Germ	
16	+	+	+	(33)	-	-	-	-	-	C _μ :2 Rearr	1 Rearr	Germ	
17	+	+	+	(13)	-	-	-	-	-	C _μ :2 Rearr	1 Rearr	Germ	
18 (NALL-1)	+	+	+	NA	-	-	-	-	-	C _μ + J _H :2 Rearr D _H :Rearr	2 Del	Germ	
19	+	+	+	+	-	-	-	-	-	C _μ :1 Rearr 1 Germ	2 Del	Germ	
20	+	+	+	+	-	-	-	-	-	C _μ :2 Rearr	2 Del	Germ	
21	(47)	+	+	(46)	-	-	-	-	-	C _μ :1 Rearr 1 Germ	2 Del	Germ	
22	+	+	NA	+	-	1540	-	-	-	C _μ :2 Rearr	2 Del	1 Rearr	
23 (NALM-6)	+	+	+	+	-	4400	-	-	-	C _μ :2 Rearr	2 Del	1 Rearr	
24	+	+	+	+	-	-	592	-	-	C _μ :2 Rearr	2 Del	1 Rearr	
25 (REH)	+	+	+	+	-	-	-	-	545	C _μ + J _H :2 Rearr D _H :Del	2 Del	2 Rearr	

Germ, germ line; Rearr, rearranged; Del, deleted; NA, not available.

* Fluorescence-activated cell sorter data in which a (-) is <10% of the cells showing reactivity, a (+) is >50% reactivity, and specific values are given for the 10-50% range.

† Values represent picograms of cytoplasmic Ig per 10⁶ cells as determined by a sensitive RIA on Triton extracts of the cells' cytoplasmic contents.

§ H and L chain gene patterns with C_μ, J_H, D_H, C_κ, and C_λ probes.

and one of these cells was positive for HLA-DR and reacted with the BA-1 antibody. Two cells also reacted with the BA-2 antibody (Table I).

Phenotypic markers of ALL: Non-T, Non-B cases. The remaining 20 fresh cases of ALL and five cell lines were felt to represent the non-T, non-B form of ALL as they all lacked SIg and distinguishing T cell markers such as rosette formation and reaction with the 3A-1, as well as OKT3, OKT4, OKT8, and OKT6 monoclonal antibodies. Of these, 18 bore CALLA, whereas all displayed HLA-DR molecules on their surface (Table II). Of note was the reactivity with BA-1 in 20 of 24 cases tested and of BA-2 in 22 of 24. The vast majority displayed the transferrin receptor as detected by the OKT9 monoclonal and reacted with the OKT10 antibody (data not shown in Table II). 5 of the 18 cases that could be examined demonstrated detectable amounts of cytoplasmic μ -chain, testifying to their obvious B cell commitment. Only one case (No. 24) displayed another H chain class, in this instance, cytoplasmic γ -chain without either L chain protein. In addition, one case (No. 25, REH) had only cytoplasmic L chain, in this instance lambda in type, without detectable H chains (Table II).

Ig gene configuration: H chain genes. A probe comprised of the human C_{μ} region was used to examine the H chain gene configurations of all 37 cases of ALL,

while J_H and D_H region probes were used on selected cases. We observed that all 25 of the cases classified initially as non-T, non-B had H chain gene recombinations (Table II). In contrast, fully 11 of 12 T cell type ALL retained germ-line H chain genes when examined with these probes (Table I). Any cell that ultimately becomes a B cell must undergo a rearrangement of its H chain genes in order to assemble V_H , D_H , and J_H subsegments when forming a fully intact variable region gene (Fig. 1). The data here indicate that attempts to assemble H chain genes rarely occur in human T cells, although it has routinely already taken place within these non-T, non-B ALL. This is strong evidence that such non-T, non-B cells are really precursor cells already committed to B cell differentiation at the Ig gene level.

The patterns of recombination of H chain gene alleles seen in these 25 non-T, non-B cases included: one rearranged with one germ-line (seven cases), two rearranged (13 cases), and one rearranged with one deleted (five cases). When this latter set was examined in detail the deletions along the one allele sometimes included the J_H as well as the C_{μ} region (cases 4 and 5). However, one case (No. 1) had both J_H segments present in a rearranged state yet had deleted one C_{μ} gene consistent with a H chain class switch to a more 3'-located C_H gene region on that allele (Fig. 2 B). This cell did not

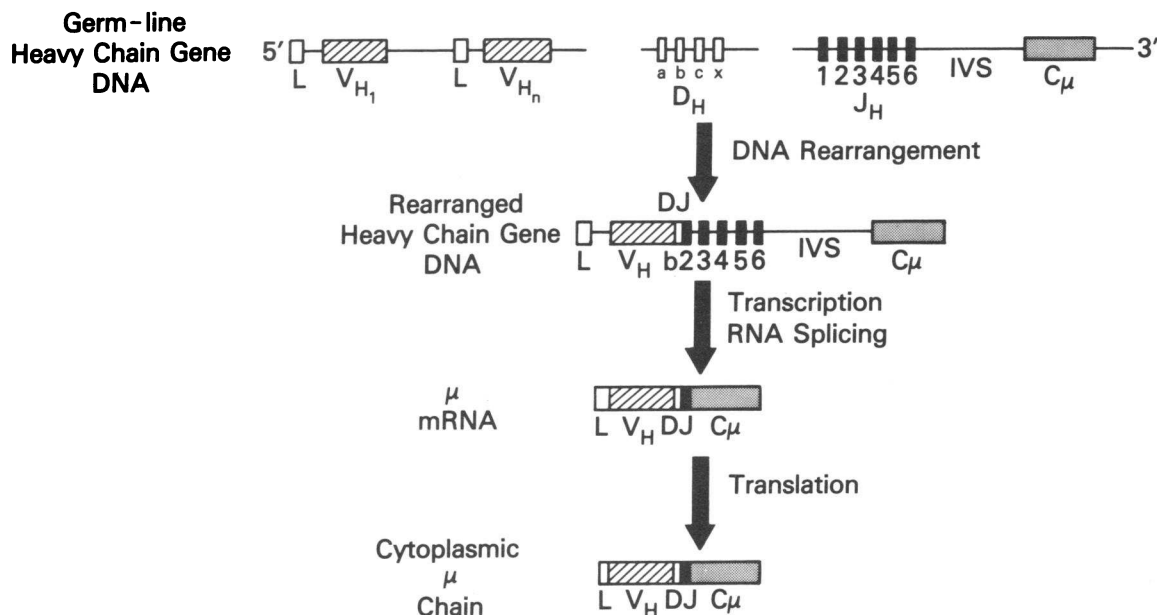


FIGURE 1 A schematic representation of the germ-line organization and subsequent assembly of the human Ig H chain gene. In addition to multiple (V_H) regions each with its own leader (L) sequence, there are six functional J_H segments, families of D_H segments, and but one constant C_{μ} region per allele. A single V_H , D_H , and J_H region must be correctly recombined at the DNA level to form an active gene. The remaining intervening sequences (IVS) are then removed at the RNA level by splicing and the μ -mRNA is then translated into the cytoplasmic form of μ -chain protein.

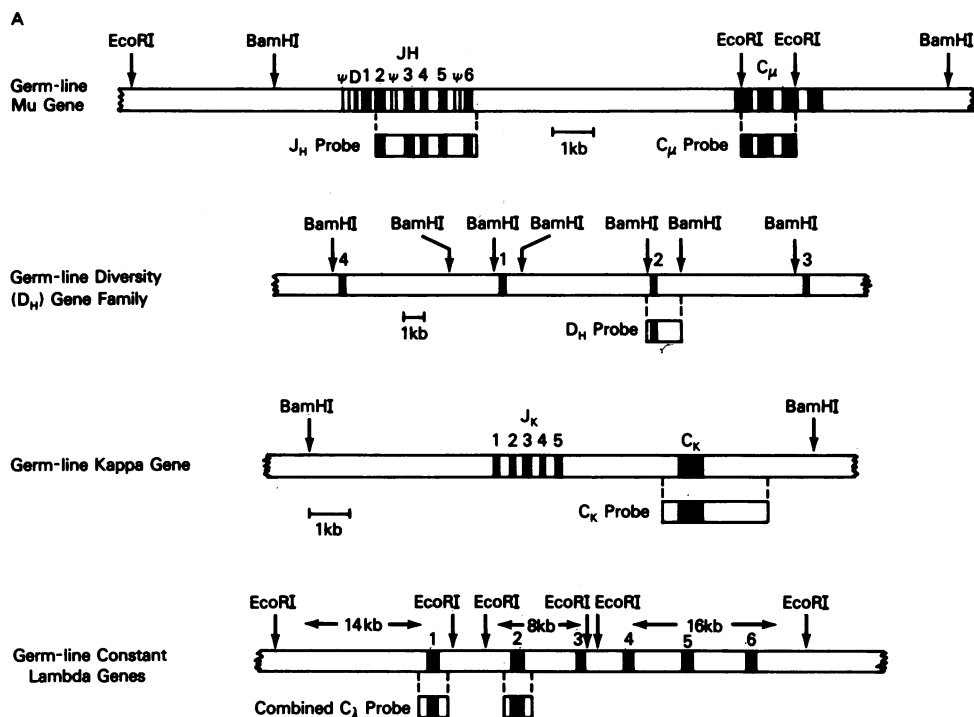


FIGURE 2 A. The human Ig gene probes used in this study to detect germ-line and rearranged genes. The J_{H1} probe consisted of a 2.4-kb germ-line *Sau3a* fragment that could recognize rearrangements in either *Bam*HI- or *Eco*RI-digested DNA. A 1.3-kb germ-line *Eco*RI fragment containing the C_{μ} region recognized H chain gene rearrangements within *Bam*HI digests. A (D_{H1}) segment probe was comprised of a 1.8-kb germ-line *Bam*HI fragment that detected recombinations of this particular D_{H1} gene family within *Bam*HI-digested DNA. κ gene rearrangements were detected in the *Bam*HI digests of DNA with a 2.5-kb germ-line *Eco*RI C_{κ} probe. The combined C_{λ} probe used to detect λ gene rearrangements in *Eco*RI digests consisted of a 0.8-kb germ-line *Bam*HI-*Hind*III fragment containing the $C_{\lambda 1}$ gene and a 1.2-kb germ-line *Bam*HI-*Eco*RI fragment containing the $C_{\lambda 2}$ gene. B. Representative Ig gene arrangement patterns in four non-T, non-B, B cell precursor ALLs. Case 1 leukemic cells (lane L) has rearranged (arrows) H chain genes but germ-line (dash marks) L chain genes when compared with the non-B cell source of control tissue (C). The presence of two rearranged J_H segments with the apparent loss of one C_{μ} segment suggests a H chain class switch event on one allele. Case 7's H chain gene rearrangement was only discernible by using the *Eco*RI restriction sites but not the *Bam*HI sites. A germ-line D_H gene family and germ-line L chain genes were evident. Case 15 had rearranged μ and κ genes but retained germ-line λ genes, whereas, the leukemic lymphocytes (L) of case 25 with two rearranged λ genes had deleted both C_{κ} genes. Case 25, with two rearranged H chain genes, had also deleted both sets of the D_H gene family examined.

produce any detectable cytoplasmic γ -chains (Table II), nor any α -, or ϵ -chains (data not shown). In addition, no cases were observed in which both C_{μ} regions had been deleted by switching, however, this is the most common finding in human mature B cell lines and leukemias that have switched to γ -, α -, or ϵ -chain production (unpublished observations). Thus, although H chain class switching might occasionally occur within B cell precursors, it is not a common event.

Of considerable interest, case 24 (Table II) did reproducibly demonstrate small amounts of cytoplasmic γ -chain. This has been noted to occur infrequently in human ALL by Vogler et al. (30) and has also been seen within a subclone of an Abelson pre-B cell line

in the mouse (31). Provocatively, however, both C_{μ} genes were retained in a rearranged position in case 24 even though it produced γ -chain and not μ -chain. Thus, this case has switched production to a more 3'-located constant region (C_{γ}) but did not appear to undergo the usual deletional loss of C_{μ} genes seen in the classic H chain class switch. Of note, the Abelson pre-B cell subclone (81A-2), which produces γ -chain, also has both C_{μ} genes present (31). This raises the possibility that alternative mechanisms may exist that allow expression of the more distal constant regions. These might include a reorganization of the H chain gene order that would move C_{γ} in a more 5' direction but not delete C_{μ} , or perhaps the generation of an ex-

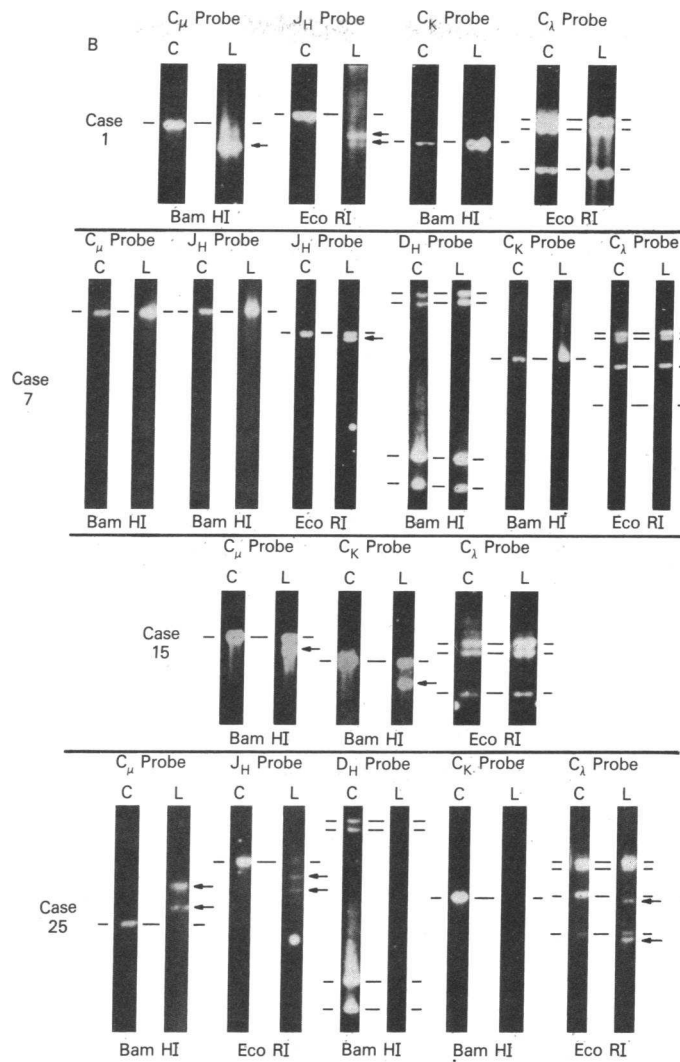


FIGURE 2 (Continued)

tremely large nuclear RNA transcript that might subsequently be processed to juxtapose variable and C_γ information. However, further studies of the leukemic cells of case 24 indicated that only a subpopulation of cells (~5%) within this leukemia actually had demonstrable cytoplasmic γ -chain. This was demonstrated by a direct test for cytoplasmic Ig on cytocentrifuge preparations using a sensitive Avidin-Biotin complex assay (data not shown in Table II).³ Thus, another possibility is that only a subpopulation of the leukemic cells have undergone a classic, deletional H chain class switch and that this was undetectable when the genomic DNA of the entire leukemic population was examined.

³ Braziel, R. M., and S. J. Korsmeyer. Unpublished observations.

There were two cases that initially appeared to have germ-line H chain genes when examined with either the J_H or the C_μ probe against a BamHI digest (cases 7 and 11) (Fig. 2 B and Table II). However, when the EcoRI digests were examined with the J_H probe, rearrangements surrounding the J_H segments were indeed present in both cases. This indicates that the particular genomic reorganization in these H chain genes introduced a new 5'-located BamHI site indistinguishable from the germ-line configuration of these genes. This emphasizes the need to use a second restriction endonuclease when analyzing such cases.

Examination of five B cell precursor ALLs with a D_H segment probe revealed examples in which the tandemly linked members of this D_H gene family were either germ line, used in recombinations, or totally deleted by recombinations (Table II, Fig. 2 B): Analyses of both mouse and human aberrant H chain gene

rearrangements has revealed that many of these involve recombinations of just a D_H and J_H segment failing to introduce a V_H gene region (10, 32). The various patterns seen here for this D_H gene family suggest that although this family is used in some recombinations, other additional D_H gene families exist and may be recombined in some of these cells. In contrast, none of the T cell ALLs examined had obvious rearrangements of their D_H gene segments, including the CCRF HSB-2 cell line (case 12, Table I), which did display a J_H region rearrangement.

Thus, all of the non-T, non-B cells demonstrated H chain gene rearrangements, indicating that attempts at assemblage of D_H/J_H intermediates or $V_H/D_H/J_H$ recombinants has always occurred by this stage of B cell maturation. Despite this, the majority of these B cell precursors failed to make detectable amounts of cytoplasmic H chain (Table II). This may in part reflect the relative difficulty in correctly assembling three different discontinuous gene subsegments (V_H , D_H , and J_H) so as to produce a valid gene with an intact open reading frame.

Ig gene configuration: L chain genes. Within the 25 cases of non-T, non-B ALL, a considerable amount of L chain gene rearrangement and even deletion occurred with approximately half (11 of 25) revealing κ and/or λ reorganizations (Table II). In contrast to the 14 cases with H chain gene rearrangements that had germ-line L chain genes, all 11 cases with rearranged L chain genes had already rearranged their H chain genes. Thus, it appears that attempted H chain, $V_H/D_H/J_H$, gene assemblage precedes L chain gene rearrangements. In contrast, all of the T cell ALLs uniformly retained germ-line κ and λ genes (Table I).

The 11 cells with L chain gene recombinations were no more likely to have undergone recombinational events on both H chain gene chromosomes than were the cells with germ-line κ and λ genes. Of these 11 cells displaying L chain gene reorganization, only three were shown to have detectable cytoplasmic H chain (Table II). This constellation of findings raises the possibility that an effectively recombined H chain gene producing detectable amounts of cytoplasmic μ may not be a prerequisite for L chain gene rearrangements.

All four of the non-T, non-B cells with rearranged λ genes (cases 22–25) had no remaining germ-line κ genes, having deleted them, similar to observations made on mature λ -producing B cells (33). The three cases (cases 15–17) with κ genes present in a rearranged state retained their λ genes in the germline configuration, similar to the findings in mature κ -producing B cells (33). An additional four cases (cases 18–21) had deleted their κ genes but had not yet rearranged their λ genes. Studies of short- and long-term λ B cell lines suggest that these deletions of the κ gene complex may

be the frequent fate of aberrantly rearranged κ genes (34). Representative cells with a κ rearrangement (case 15) and λ gene rearrangements (case 25) are presented in Fig. 2 B.

Of interest was the infrequent production of detectable L chains despite the recombination of these genes in 11 cases, some of which had undergone multiple reorganizations. One cell line (REH, case 25, Table II) without detectable cytoplasmic H chain (no μ -, γ -, α -, or ϵ -chain were detectable) reproducibly showed cytoplasmic λ -chain and even small amounts of secreted λ -chain. Evidence from this leukemic cell suggests that productive λ gene rearrangements can exist in the face of nonproductive H chain genes and would imply that there is no strict requirement for H chain to make L chain. The small amount of L chain produced does raise the possibility that some type of coordinate regulation of H and L chain synthesis might exist.

Correlation of surface antigen expression and Ig gene arrangement. Some rather striking correlations were noted when the cell surface phenotype and the Ig gene patterns of the 37 ALL cases were compared. The ALL cases bearing distinguishing T cell antigens usually retained all of their Ig genes (both H and L chain genes) in the germ-line configuration (Table I). The single T cell case displaying a H chain gene rearrangement (case 12, CCRF HSB-2), although it displayed no B cell-associated antigens, demonstrated reactivity with only 3A-1 out of all the T cell markers examined. The rare recombination observed in this T cell is likely to be an aberrant event incapable of complete Ig chain production. This would indicate that the J_κ - C_κ , J_λ - C_λ , and J_H - C_μ segments, and at least one D_H gene family are not contributing genetic information to any antigen-specific receptor that these T cells might possess. Earlier cells within the T cell lineage, those lacking the SRBC receptor, are the subsets most difficult to assign a cellular origin. The 3A-1 antibody of Haynes et al. (16) appears to be quite valuable in this assignment as it recognized T cells with germ-line Ig genes but did not recognize any of the cases we would classify as B cell precursors (Tables I and II).

Similarly, the antibodies of BA-1 and BA-2, although not solely restricted to the cells we would classify as B cell precursors, proved to be helpful when used together with T cell markers. Both BA-1 (20 of 24) and BA-2 (22 of 24) reacted with the vast majority of the B cell precursor leukemias showing Ig gene rearrangements (Table II). In contrast, they were each reactive with only one and two, respectively, of the 12 T cell leukemias. CALLA was present on some early T cell subsets (four of 12 cases here, Table I) and was not present upon the HLA-DR⁺ CALLA⁻ subset of non-T ALL. We feel that the HLA-DR⁺, CALLA⁻ group may represent the earliest stages of B cell development be-

cause all seven cases had H chain gene rearrangements without L chain gene recombinations. Three of these CALLA⁻ cases also lacked BA-1 reactivity, yet had rearranged H chain genes, emphasizing the use of the Ig gene studies in defining the origin of HLA-DR⁺ leukemias lacking other B cell lineage markers.

DISCUSSION

Categorization of the cellular origin of ALL by Ig gene rearrangement and surface antigen expression. The coordinate examination of cell surface antigens by monoclonal antibodies and the analysis of Ig gene rearrangements proved to be of value in defining the cellular origin and state of maturation of ALL cells. Comparisons presented here indicate that in the presence of discriminatory T cell markers, cells in general have germ-line H and L chain genes, whereas cases lacking such definitive markers (non-T) uniformly have Ig gene recombinations and often display B cell-associated antigens as well. It is clear however, that no single cell surface antigen examined in this study strictly predicts the configuration of Ig genes in all cases. Even the p30/BA-1, p24/BA-2, and HLA-DR/DA-2 molecules that are so frequently detected on the B cell precursor ALLs with Ig gene rearrangements are also present on a neuroblastoma cell line (AG 3320) with germ-line Ig genes (unpublished data). As noted, even occasional T cells (CCRF HSB-2) can have a rearrangement of H chain genes that appears not to result in complete H chain production. Yet, use of several monoclonal antibodies (especially 3A-1, DA-2, BA-1, and BA-2) indicates that good correlations do exist between cell surface markers and the configuration of Ig genes. Thus, use of such a combination of monoclonal antibodies helps confirm the assignment of cellular origin in a given case of leukemia.

The HLA-DR⁺ CALLA⁻ subset of non-T ALL may represent the earliest identifiable B cell precursors. All seven cases of non-T, non-B ALL studied that bore HLA-DR but lacked CALLA and T cell-associated antigens had rearranged H chain genes yet retained germ-line L chain genes. That the CALLA⁻ cases have less in terms of L chain gene rearrangements when compared with CALLA⁺ cases, suggests that they may be the malignant counterparts of the earliest recognizable stage of B cell precursors. Of interest, none of the cases lacking T cell-associated antigens had totally germ-line J_H gene regions. One would predict that a truly uncommitted B cell precursor would have all of its Ig genes in the germ-line configuration. Yet all cases of B cell precursor ALL examined to date have represented more mature stages of genetic commitment displaying rearranged Ig genes. The finding of Ig H chain gene recombinations within the subset of HLA-DR⁺ CALLA⁻ non-T ALL further illustrates the use-

fulness of Ig gene studies in providing insights into the origin and stages of differentiation of leukemias and lymphomas of controversial phenotypic classification.

A coordinate sequence of B cell surface antigen expression and Ig gene recombinations. The existence of 14 leukemic cells with only H chain gene rearrangements and the fact that none of the cells with L chain gene rearrangements retained totally germ-line H chain genes is further evidence for a H chain before L chain order to Ig gene rearrangements (Fig. 3 A). Furthermore, of the 11 cases with L chain gene recombinations, seven displayed κ gene reorganizations (rearrangements or deletions) while retaining germ-line λ genes. In contrast, the four cases with λ L chain gene recombinations had no remaining germ-line κ genes. These patterns of gene arrangement support the hypothesis of a sequential order to Ig gene rearrangements within these B cell precursors in which H chain genes rearrange before L chains and κ L chain genes rearrange before λ L chain genes (Fig. 3) (8, 33, 34).

The presence of HLA-DR, but not CALLA in many of the cases that had rearranged H chain genes but retained germ-line L chain genes offers evidence that expression of HLA-DR may well precede CALLA during B lymphoid differentiation. In addition, of the B cell precursor ALLs examined, three of the four that lacked BA-1 antibody reactivity fell within the HLA-DR⁺ CALLA⁻ subset. These findings suggest a coordinate sequence of Ig gene rearrangement and B cell surface antigen expression (Fig. 4). By this proposal the earliest B cell precursors that initiate Ig gene rearrangements about the J_H gene segments would bear surface HLA-DR, but later acquire the p30 surface antigen detected by BA-1 and the antigen CALLA (Fig. 4). All 11 cases that had moved on to their L chain genes bore cell surface CALLA as well as HLA-DR and nine of 10 reacted with BA-1 (Table II).

L chain gene configurations observed are predicted by a hierarchical model of L chain gene rearrangements. A model in which κ gene rearrangements including those which are ineffective and thus fail to make detectable L chain, precede those of the λ genes was first suggested by an examination of transformed B cells by Hieter et al. (33). Supporting evidence for this apparent sequence was also found in normal mature B cells and in non-T, non-B leukemic cells (8, 34). Such a postulate would predicate a series of intermediate patterns of L chain gene configurations within B cell precursors that are indeed observed in this study (Fig. 3 B). These patterns included cells with H chain gene rearrangements that have but a single κ gene recombined (cases 15-17) followed by cells that recombined and at times deleted both copies of their κ genes (cases 18-21). After attempted use of both sets of κ genes, cells could proceed to the remaining germ-

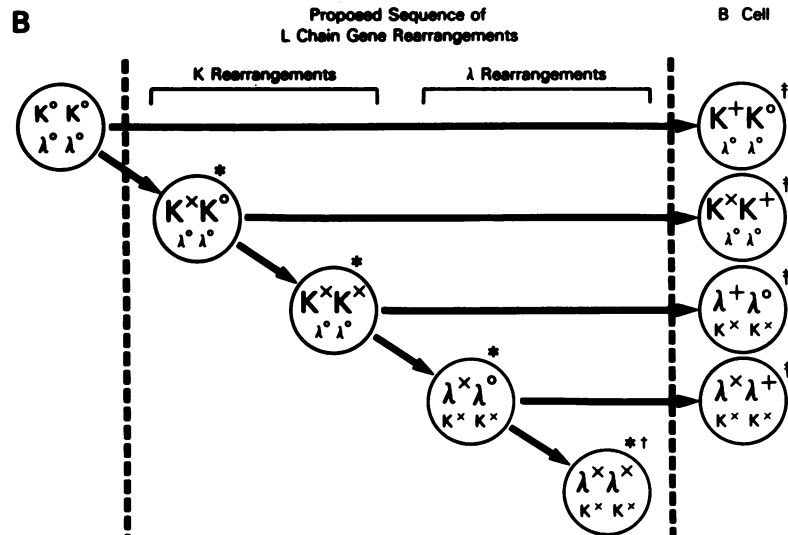
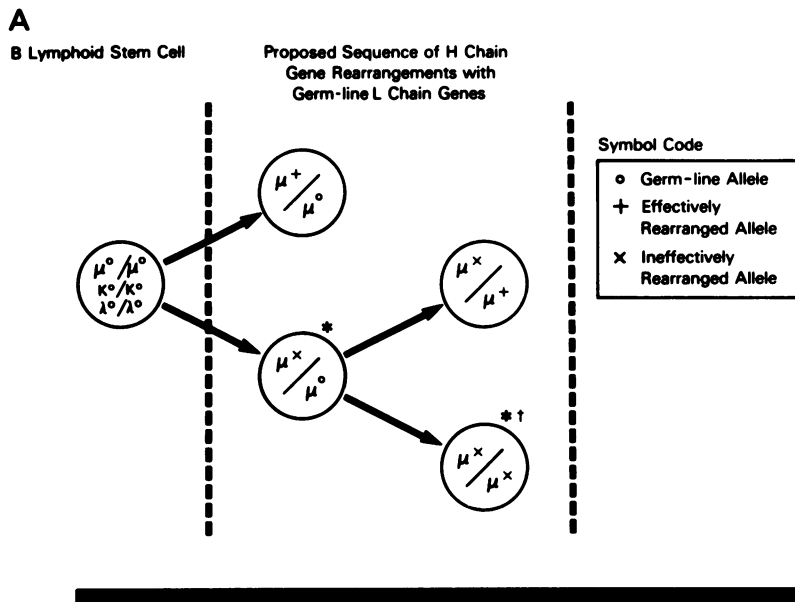


FIGURE 3 B cell precursor series. **A.** Proposed sequence of H chain gene rearrangements within B cell precursors. A B lymphoid stem cell uncommitted at the Ig gene level would have its H chains (μ) and L chains (κ and λ) in the germ-line form. Gene rearrangement would begin with the H chain genes and if effective, this cell (μ^+) would be capable of μ -chain production and further maturation. Many rearrangements are ineffective and would generate cells lacking μ -chain production regardless of whether their L chains could subsequently rearrange. Included here is a hypothetical set of cells trapped within the B cell precursor series (\dagger) because they have no remaining germ-line J_H or D_H segments with which to assemble an effective gene. **B.** Proposed sequence of L chain gene rearrangements within B cell precursors with rearranged H chain genes. In general, L chain rearrangement would begin with the κ genes and if ineffective (\times), λ rearrangements could follow. Cells with effectively rearranged κ or λ genes could be mature B cells (\ddagger) if prior H chain rearrangements were effective, whereas, the cells with only ineffectively rearranged L chain genes ($*$) would lack L chain production and would be B cell precursors. A theoretical population of cells might exist which had exhausted all L chain recombinational opportunities (\dagger).

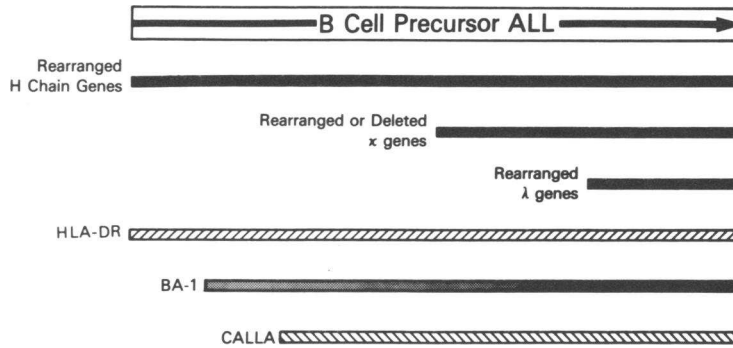


FIGURE 4 A coordinate sequence of Ig gene rearrangements and B cell surface antigen expression. Comparisons here suggest that the presence of rearranged H chain genes and HLA-DR molecules precede the appearance of BA-1 reactivity and CALLA. Cases with recombined L chain genes always had rearranged H chain genes and usually displayed all three of the surface antigens.

line λ L chain genes. Correspondingly, we found three cases (Nos. 22–24) with single λ rearrangements with no remaining germ-line κ genes and one case (No. 25) that had rearrangements of both λ gene sets (Figs. 2 B and 3 B).

Lack of Ig production in the presence of rearranged Ig genes. Despite the presence of H chain gene rearrangements in all 25 of the B cell precursor ALLs, only 6 of 18 examined had detectable cytoplasmic H chain protein. In addition, the paucity of L chain production in these cells (1 of 11) is especially surprising considering how frequently the L chain genes have been recombined. As noted above, L chain gene rearrangements were frequently present in the absence of detectable cytoplasmic μ . If the capacity of L chain genes to rearrange effectively occurs irrespective of whether the preceding H chain gene rearrangement was productive, it is somewhat surprising that more cases of ALL do not produce free L chains. The cell line, REH, which has no detectable μ but produces small amounts of λ L chain suggests that there is no absolute prevention of L chain synthesis in the absence of measurable H chain production (case 25, Table II), however, it is as yet uncertain whether normal bone marrow B cell precursors may display this phenomenon seen within this leukemic cell line.

The lack of Ig production by the vast majority of these leukemic B cell precursors could be accounted for at a number of levels. The Ig gene assembly process that moves and combines different V_H , D_H , J_H , V_L , and J_L subsegments, although extraordinarily flexible and efficient at generating antibody diversity, is also remarkably prone to error. A number of aberrantly rearranged genes are created by this process that would not result in detectable protein production. Within a population of B cell precursors, a high pro-

portion of such mistaken rearrangements might be expected (Fig. 3). Certain aberrant events, occurring on both chromosomes bearing H chain genes, could potentially render a cell incapable of producing an Ig molecule and thus prevent it from maturing into a B cell. Predicted examples of such cells trapped within the B cell precursor series would be cells that had aberrantly rearranged D_H segments to the 3'-most situated J_H region (J_6) on each H chain gene-containing chromosome. Such cells would have no remaining germ-line J_H segments for any additional attempts at forming a valid gene. Alternatively, another example of cells that might be unable to differentiate into Ig-bearing B cells would be those with aberrant $V_H/D_H/J_H$ recombinations of both H chain alleles. Such cells might well have deleted all of their D_H gene families and thus, would be incapable of assembling a complete variable H chain gene (Figs. 1 and 3 A).

In addition to the blockade at the DNA level it is quite probable that transcriptional and/or translational regulatory events can affect the expression of rearranged Ig genes within some leukemic cells at this stage of B cell differentiation. We have seen a case with rearranged μ and κ genes lacking *de novo* Ig produce both cytoplasmic and ultimately surface IgM $_{\kappa}$ after induction with the phorbol ester, TPA (12-O-tetra-decanoylphorbol-13-acetate) (35). In addition, Nadler et al. (36) have observed cytoplasmic μ -chain after induction with TPA in some non-T ALL cases. The Ig genes in such a cell are almost certainly effectively rearranged but are not being expressed in their *de novo* state due to a regulatory event.

Cases of classic non-T, non-B ALL appear to be monoclonal expansions of B cell precursors representing serial stages of Ig gene rearrangement and surface antigen expression. The uniform presence of Ig gene

rearrangements, yet the frequent lack of Ig production in such B cell precursors may arise from a variety of mechanisms. These mechanisms include regulatory events that prevent the further maturation of some B cell precursor leukemias as well as ineffective, aberrant Ig gene rearrangements that may be frequently present at this stage of development. Thus, these leukemias are serving as a model system to identify the genetic events during early B cell stages of human lymphoid differentiation, and in addition are providing insights into the failure of maturation of certain cells within the B cell precursor series.

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