LYMPHOID BLAST CRISES OF CHRONIC MYELOGENOUS LEUKEMIA REPRESENT STAGES IN THE DEVELOPMENT OF B-CELL PRECURSORS

AJAY BAKHSHI, M.B., B.S., JUN MINOWADA, M.D., ANDREW ARNOLD, M.D., JEFFREY COSSMAN, M.D., JANE P. JENSEN, M.S., JACQUELINE WHANG-PENG, M.D., THOMAS A. WALDMANN, M.D., AND STANLEY J. KORSMEYER, M.D.

Abstract The origin and stage of differentiation of the blast-crisis cells in chronic myelogenous leukemia have remained uncertain. Because immunoglobulin heavy-chain and light-chain genes must undergo a DNA rearrangement during B-cell development but rarely do so in human non–B-cell lineages, we examined these genes in 18 episodes of chronic myelogenous leukemia. In eight of nine episodes of lymphoid blast crisis, heavy-chain genes were rearranged, and in three, rearrangements in light-chain genes were also present. In contrast, cells from chronic myeloid, myeloid blast, and erythroid-like phases retained germ-like immunoglobulin genes. The observed phenotypic markers and gene configurations revealed that most lymphoid blast crises represent stages of development of B-cell precursors. In two separate episodes of lymphoid crisis, cells from a single patient possessed identical heavy-chain but different light-chain-gene configurations. Thus, the precursor cells that monoclonally expand to produce a lymphoid crisis are capable of immunoglobulin-gene rearrangements and represent discrete steps in early B-cell maturation. (N Engl J Med 1983; 309:826-31.)

CYTOGENETIC and isoenzyme studies have established that chronic myelogenous leukemia is a clonal proliferative disorder arising from a progenitor cell with a rather pluripotential capacity.[1-6] Studies of glucose-6-phosphate dehydrogenase isoenzymes in female heterozygotes with chronic myelogenous leukemia have revealed that involved granulocytes, erythrocytes, platelets, monocytes, and some lymphocytes displayed only a single glucose-6-phosphate dehydrogenase allele.[3-6] This finding indicated that in this disease, cells in multiple hematopoietic lineages could be of clonal origin. Because of this multipotential capacity of the clonally derived cell in chronic myelogenous leukemia, the exact cellular origin and indeed the state of differentiation of the cells comprising the blast-crisis phases of the disorder have remained uncertain. Occasionally, lymphoblasts from the acute phase have been classifiable as pre-B cells because of the presence of cytoplasmic immunoglobulin.[7-9] However, most cases of lymphoid blast crisis lack both detectable cytoplasmic and surface immunoglobulin as well as definitive T-cell antigens that would permit their clear assignment to either B-cell or T-cell lineage.'

Both heavy-chain and light-chain immunoglobulin genes must undergo a successful DNA rearrangement during B-cell development before a complete immunoglobulin molecule can be produced.[10,11] These rearrangements begin with an attempt to juxtapose the separated variable (V_H), diversity (D_H), and joining (J_H) gene subsegments of the heavy-chain gene.[12] Subsequently, germ-line light-chain genes must be similarly rearranged to recombine V_κ and J_κ or V_λ and J_λ subsegments before a light chain can be produced.[10,1] Because these rearrangements are mandatory in B cells but only rarely occur within other human hematopoietic lineages,[13] we examined the status of immunoglobulin genes in chronic myelogenous leukemia.

In eight of nine episodes of lymphoid blast crisis. rearrangements of heavy-chain genes were observed and in three progression to light-chain-gene rearrangements had occurred as well. In contrast, cells from chronic myeloid and acute blastic myeloid phases had germ-line immunoglobulin genes. Thus, the lymphoid blast crises had initiated immunoglobulin-gene rearrangements and appear to represent monoclonal expansions of cells at B-cell-precursor stages ofdevelopment. Serial studies of a single patient indicated that the separate cellular expansions during different crises can originate from distinct B-cell precursors at discrete genetic stages of maturation.

METHODS

Cases Examined

The leukemic cells were obtained from the peripheral blood or bone marrow during 14 episodes of chronic myelogenous leukemia and four representative spontaneous cell lines (RPMB, NALM-I, PTF, and K562) established during blast crisis of the disease were examined.[8,14,15] Eight patients were studied during nine episodes of lymphoid blast crisis (Cases 1 through 8 in Table 1). Two patients were examined during a chronic granulocytic phase (Cases 2c and 9), and six during a myeloid biast crisis (Cases 10 through 15) (n· listed in Table 1). The K562 erythroid-like cell line was examined the 16th case." Case 2 was studied on three occasions: during tw·· separate lymphoid blast crises (Cases 2a and 2b in Table 1) and during an intervening chronic granulocytic phase (Case 2c in Fig. 1B). Cells from 14 patients possessed a t(9;22) translocation, Philadelphia chromosome, whereas the RPMB and PTF cell lines (Cases 6 and 8) had no t(9;22) translocation, representing the form of chronic myelogenous leukemia lacking the Philadelphia chromosome."

None of the cells studied during the 18 episodes examined had cell-surface immunoglobulin, and none formed rosettes with sheep red cells. The presence of cytoplasmic immunoglobulin of classes μ, γ, κ, and λ was assessed when possible either by cytoplasmic i·· munofluorescence or by measurements of the actual immunoglol lin present in cytoplasmic extracts of leukemic cells with a sensitive double-antibody radioimmunoassay.[13] Terminal deoxynucleotidyltransferase (TdT)[16] was present in all cases of lymphoid blast crisis tested but was absent during the chronic and acute myeloid phases.

From the Metabolism Branch, Laboratory of Pathology, and Division of Cancer Treatment. National Cancer Institute. National Institutes of Health. Bethesda. Md.. and Hines Veterans Administration Medical Center. Hines, Ill. Address reprint quests to Dr. Bakhshi at Bldg. 10. Rm. 4N104. National Institutes of Health. Bethcsda, MD 20205.

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Table 1. Studies of Nine Episodes of Lymphoid Blast Crisis in Eight Patients with Chronic Myelogenous Leukemia.

Case No	Phenotypic Markers											Immunoglobulin-Gene Patterns †		
	Surface Antigens *							Cytoplasmic Immunoglobulin						
	TdT	J5 CALLA	DA-2 HLA-DR	BA-1 p30	BA-2 p24	TAg	MAg	μ	γ	κ	λ	*heavy chain*		I
1	+	+	+	+	+	–	–	–	–	–	–	Cμ: 2 Rearr J_H: 2 Rem	Germ	Germ
2a	+	+	–	–	+	–	–	NT	NT	NT	NT	Cμ: 2 Rearr J_H: 2 Rearr	Germ ‡	1 Rearr
2b	+	+	–	–	+	–	–	NT	NT	NT	NT	Cμ: 1 Rearr J_H: 2 Rem	Germ	Germ
	+	+	+	+	+	–	–	–	–	–	–	Cμ: 1 Rem J_H: NT	Germ	NT
	+	+	+	+	36	–	–	–	–	–	–	Cμ: 2 Rem J_H: 2 Rem	Del	Germ
5	+	17	20	17	+	–	30	–	–	–	–	Cμ: Germ J_H: Germ	Germ	Germ
6 RPMB	NT	–	+	NT	NT	–	–	+	–	–	–	Cμ: 1 Rem. 1 Del J_H: 2 Rearr	1 Rem	Germ
7 NALM-1	+	+	+	19	+	–	NT	+	–	–	–	Cμ: 2 Rem J_H: 2 Rem	Germ	Germ
8 PTF	+	+	+	+	–	–		–	–	–	–	Cμ: Del J_H: 2 Rem	Germ	Germ

†Plus signs indicate that <10 per cent of the cells reacted with the monoclonal antibody J5, which recognizes the common acute-lymphoblastic-leukemia antigen (CALLA), DA-2, BA-1, or in a fluorescence-activated cell sorter, or possessed T-cell associated antigens (TAg) or myeloid-associated antigens (MAg). Plus signs indicate >50 per cent reactivity, and values between 10 and 50 per cent are specifically listed. Tdt denotes deoxynucleotidyl transferase, and NT not tested.

‡Heavy-chain and light-chain gene patterns were determined with the Cμ, J_H, Cκ and Cλ probes. Rearr denotes rearranged, Germ, germ-line; and Del, deleted.

§Germ-line κ genes may have been contributed by normal cells.

Immunoglobulin-Gene Analysis

High-molecular-weight DNA prepared from leukemic cells or cell lines was digested to completion with *Bam*HI or *Eco*RI restriction endonuclease, size-fractionated by agarose-gel electrophoresis, and transferred to nitrocellulose paper.[17] These nitrocellulose blots were hybridized with nick-translated ^{32}P DNA probes of the human immunoglobulin genes that were capable of detecting germ-line and rearranged alleles (Fig. 1A).[18,19] Such blots were washed at the appropriate stringencies and were visualized on autoradiograms. A population of normal, polyclonal B cells contains many differently sized immunoglobulin-gene rearrangements and when collectively analyzed reveals no detectable individual rearrangement. In contrast a B-cell-lineage leukemia is a monoclonal expansion in which each cell has an identical immunoglobulin-gene recombination and displays an identifiable rearranged immunoglobulin-gene fragment. Such rearrangements are a sensitive marker capable of detecting even minority populations (5 per cent) of clonal B cells when admixed with non-B cells or polyclonal B cells.

Surface-Antigen Characterization

When available, leukemic cells were reacted with monoclonal antibodies or directly fluoresceinated antiserum and were analyzed by flow microfluorometry as previously described.[11] When more than 50 per cent of the cells of a case reacted with an antibody it was scored as positive, less than 10 per cent was scored as negative, and values between 10 and 50 per cent are specifically listed for the lymphoid crises in Table 1. The antibodies used include the J5 monoclonal antibody (kindly provided by Dr. Jerome Ritz, Harvard University), which recognizes the common acute-lymphoblastic-leukemia antigen,[20] the DA-2 monoclonal antibody (a kind gift of Dr. Peter Parham and Jack Strominger, Harvard University), which identifies a nonpolymorphic HLA-DR determinant,[21] the BA-1 [monoclonal antibody (courtesy of Dr. Tucker LeBien, University of Minnesota), which detects a p30 antigen that is expressed on cells at multiple stages of B-cell development,[22] and the BA-2 monoclonal antibody (provided by Dr. Tucker LeBien, University of Minnesota), which identifies a 24,000-dalton leukemia and B-cell-associated antigen[23] (Table 1). The presence of T-cell-associated antigens was assessed with either the OKT3, OKT4, OKT6, and OKT8 monoclonal antibodies (Ortho Pharmaceuticals, Raritan, N.J.)[24] or an absorbed heteroantiserum specific for human T-cell-surface antigens.[25] Results for the lymphoid cases are listed under TAg in Table 1. The existence of myeloid antigens was determined with the OKM1[26] (Ortho Pharmaceuticals) and MCS-2 monoclonal antibodies[27] and another absorbed heteroanriserum recognizing myeloid antigens.[28] Results are listed under MAg in Table 1.

Results

Immunoglobulin-Gene Rearrangements

Cells from seven of the eight patients examined during a lymphoid blast crisis possessed rearranged heavy-chain immunoglobulin genes, and three of these also had rearranged light-chain genes (Table 1 and Fig. 1B). These rearrangements result from attempts at recombinational joining of the separated immunoglobulin-gene subsegments V_H, D_H, and J_H, which comprise the final heavy-chain variable region, or V_L and J_L, which comprise the final light-chain variable region. These DNA reorganizations within B cells occur in a sequential order in which rearrangements of heavy-chain genes precede those of the light-chain genes.[19,29] In contrast, human leukemic T cells representing various stages of maturation have not shown light-chain rearrangements and have usually (18 of 20

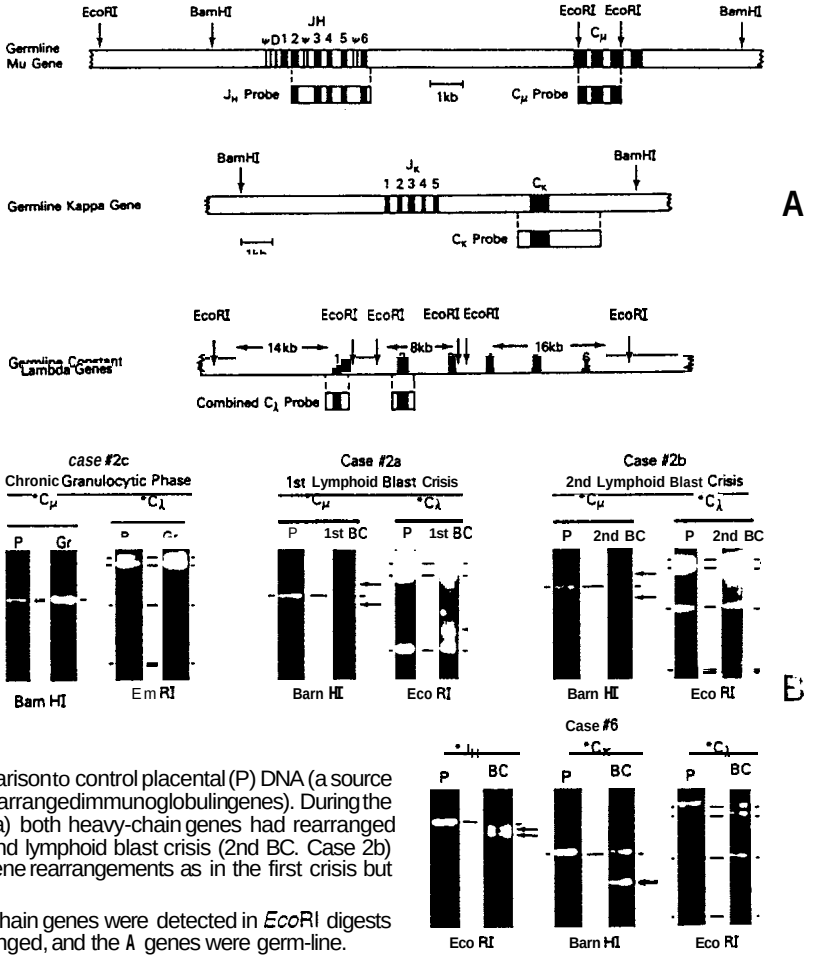

Figure 1. Immunoglobulin-Gene Rearrangements in Chronic Myelogenous Leukemia.

Part A shows the human immunoglobulin-gene probes used in this study to detect germ-line and rearranged genes. The 1.3-kb germ-line *Eco*RI probe containing the C_μ region hybridized to a 17-kb *Bam*HI fragment in germ-line DNA and was capable of detecting rearrangements. Such rearrangements alter the size of this fragment by introducing a new 5' *Bam*HI site when a V_H and D_H segment combine with a J_H region. The 2.4-kb Sau3a J_H probe recognized the same 17-kb *Bam*HI germ-line fragment and a 16-kb germ-line *Eco*RI fragment. The 2.5-kb *Eco*RI C_κ probe recognized a 12.0-kb germ-line *Bam*HI fragment. We have not observed any polymorphism with the above three probes in over 50 subjects examined. The combined C_λ probe used consisted of a 0.8-kb *Bam*HI–*Eco*RI fragment containing the $C_{\lambda 1}$ gene and a 1.2-kb *Bam*HI–*Eco*RI fragment containing the $C_{\lambda 2}$ gene. This probe could discern the several polymorphic patterns of C_λ genes in human beings.[**] Panel B shows the results of examinations of Case 2 on three occasions (asterisks indicate radiolabeled probe). DNA genomic blots during the chronic granulocytic phase (Gr, Case 2c) revealed germline (dash marks) $C\mu$ and C_λ genes in comparison to control placental (P) DNA (a source of non-B-cell DNA that has germ-line, non-rearranged immunoglobulin genes). During the first lymphoid blast crisis (1st BC, Case 2a) both heavy-chain genes had rearranged (arrows), as had one C_λ allele. In the second lymphoid blast crisis (2nd BC. Case 2b) the cells displayed identical heavy-chain-gene rearrangements as in the first crisis but then had germ-line CA genes.

In Case 6, rearrangements of both heavy-chain genes were detected in *Eco*RI digests with the J_H probe. One C_κ allele had rearranged, and the A genes were germ-line.

examined) had germ-line heaw-chain genes as well.[13] Similarly, in both cases of chronic myelogenous leukemia examined during a chronic granulocytic phase, all six cases of myeloid blast crisis, and the early erythroid-like K562 cell line, germ-line heavy-chain and light-chain immunoglobulin genes were retained (data not shown). These contrasting findings offer strong evidence that the vast majority of the episodes of lymphoid blast crisis in chronic myelogenous leukemia represent monoclonal expansions of cells that are genetically committed to the B-cell lineage. The only potential exception (Case 5 in Table 1), with germ-line immunoglobulin genes, also had detectable myeloid surface antigens and had B-cell–associated antigens on only a minority of the cells examined. Therefore, the cells from this patient appeared to represent a mixed population, perhaps accounting for the lack of demonstrably rearranged immunoglobulin genes.

The demonstration of rearranged immunoglobulin genes is of particular importance in establishing the B-cell origin of lymphoid crisis in chronic myelogenous leukemia, since each of the other markers that were tested (p30/BA-1, p24/BA-2, HLA-DR, the common acute-lymphoblastic-leukemia antigen, and TdT) can be found on cells of lineages other than B cells.[13,16,30] In fact, HLA-DR and p24/BA-2 were detected on some of the cells from myeloid phases of chronic myelogenous leukemia, even though these same cells also displayed myeloid antigens. Of note. the common acute-lymphoblastic-leukemia and HLA-DR antigens were present during all lymphoid crises. whereas the p30/BA-1 and p24/BA-2 B-cell-associated antigens were more variable in their presence (Table 1). Thus, when such markers are present the. are consistent with a B-cell lineage, but their presence alone is insufficient for assigning a B-cell origin to a cell.

Of the seven cases with rearranged heavy-chain genes, five (Cases 1, 2b, 3, 7, and **8)** retained germ-line light-chain genes, indicating that they represented an early B-cell-precursor stage of development. Two cases had progressed to κ-gene recombination (both alleles were deleted in Case 4 and a rearrangement had occurred in Case 6; Table 1 and Fig. 1B). These cells retained their A genes in the germ-line form; this has been observed in κ-producing B-cells[19] and is consistent with an ordered usage of light chains in

which κ generally precedes λ. Case 2a had moved on to A-gene rearrangement (Table 1 and Fig. 1B). We have previously noted the loss of germ-line κ genes in λ-producing B-cells.[19] The status of germ-line κ genes in Case 2a, however, could not be accurately assessed because of the admixture of some normal cells (approximately 25 per cent of the total), which contributed their own germ-line κ genes. The precise gene-rearrangement patterns demonstrated during the chronic granulocytic phase and two lymphoid crises of Case 2 and during the lymphoid crisis of Case 6 are shown in Figure 1B. Although seven of eight cases of lymphoid blast crises had immunoglobulin-gene rearrangements, only two had detectable cytoplasmic immunoglobulin (Table 1). This may reflect the error-prone nature of immunoglobulin rearrangements, in which many rearrangements are nonproductive, and is similar to our findings in the B-cell-precursor form of acute lymphoblastic leukemia, in which heavy-chain genes were always rearranged but were productive of μ chain in only 25 per cent of cases.[13]

Serial Examinations in a Single Patient

In one patient (Case 2) leukemic cells were examined during an initial lymphoid blast crisis (Case 2aj), an intervening chronic granulocytic phase (Case 2c), and a clearly separate later lymphoid blast crisis (Case 2b) (Table 1 and Fig. 1B). The myeloid cells from the intervening chronic phase had the expected germ-line configuration of heavy-chain, κ, and λ immunoglobulin genes and displayed a 46,XY t(9;22) karyotype. In both episodes of lymphoid blast crisis, however, the cells possessed a new unique cytogenetic marker with a 45,XY-7 karyotype but retained the t(9;22) Philadelphia chromosomal translocation. Since blast crisis of chronic myelogenous leukemia is thought to represent the evolution of an altered clone of cells, the crisis cells not infrequently display additional chromosomal changes.[31] In this particular case a single chromosome 7 had been lost in the lymphoid crises. In addition, the cells from the first and second lymphoid blast crises shared identical rearrangements of both alleles of their heavy-chain genes (Cases 2a and 2b in Figure 1B). The individual cells that enter the B-cell pathway rearrange their immunoglobulin genes in a unique fashion to create a certain antibody specificity.[10-12] Yet, there were identically sized rearrangements of both heavy-chain gene alleles in the two separate clinical blast crises. This finding argues strongly that both these episodes can ultimately be traced to a common lymphoid progenitor cell that had undergone heavy-chain but not light-chain gene rearrangements (Fig. 2). The two lymphoid crises did have a distinguishing immunoglobulin-gene marker, however. The first lymphoid crisis possessed a light-chain rearrangement that was λ in type (Case 2a), whereas the second lymphoid crisis had totally germ-line light-chain genes (Case 2b, Fig. 1B). This indicates that the immediate precursor cells that gave rise to the separate lymphoid blast crises in this patient were distinctly different. In the first instance, the precursor cell had advanced to λ-light-chain-gene rearrangement, whereas in the second, the source of the blast-crisis expansion was a less mature cell that had retained germ-line λ genes (Fig. 2).

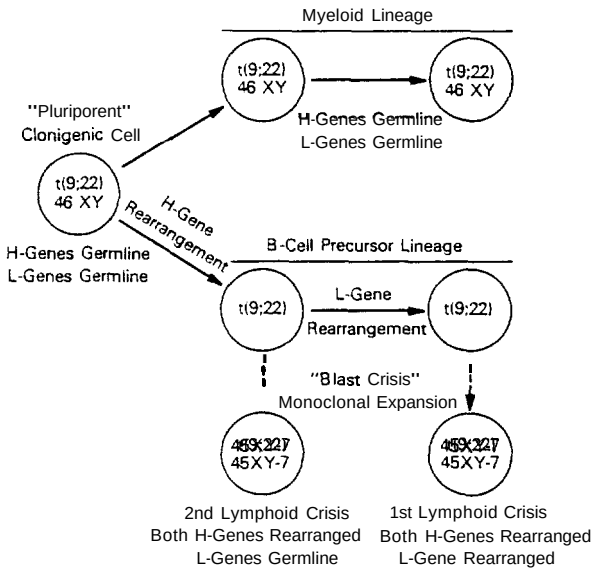

Figure 2. A Differentiation Scheme in Chronic Myelogenous Leukemia Suggested by Serial Analysis of Case 2.

The pluripotent stem cell bearing the Philadelphia chromosome t(9;22) may pursue a chronic myelogenous or acute myeloblastic course, in which it would retain germ-line immunoglobulin genes. When the affected cells pursue B-cell differentiation they are capable of sequential rearrangements of their heavy-chain (H) and then their light-chain (L) genes. Separate episodes of lymphoid blast crisis are monoclonal expansions of affected lymphoid precursor cells, which represent discrete genetic steps of B-cell maturation.

Discussion

The patterns of immunoglobulin-gene rearrangement observed in this study indicate that the cells that comprise most episodes of lymphoid blast crisis in chronic myelogenous leukemia are genetically committed B-cell precursors. Consistent with these findings, Ford et al. have also observed the presence of heavy-chain-gene rearrangements in lymphoid but not myeloid phases of this disease.[32] The determination of immunoglobulin-gene rearrangements in other human cancers has allowed cases of "non-T,non-B" acute lymphocytic leukemia,[13] hairy-cell leukemia,[33] and certain lymphomas[34] to be assigned to the B-cell lineage. The genetic information we now report is particularly useful in classifying the lymphoid blast crises of chronic myelogenous leukemia, because of their morphologic variation and because many of the phenotypic markers expressed, such as TdT, Ia antigen, and the common acute-lymphoblastic-leukemia antigen, are known to be present on multiple different cellular lineages.[13,16,30] The cells comprising these crises are similar in genotype to the B-cell-precursor type

in acute lymphocytic leukemia.[13] The fact that they represent similar stages of differentiation may account for the known responsiveness of some lymphoid crises in chronic myelogenous leukemia to chemotherapeutic regimens that are similar to those successfully used in acute lymphocytic leukemia.[1] Our findings show no evidence for involvement of the T-cell lineage within the lymphoid blast crises examined here. However, a rare T-cell-type lymphoid blast crisis has been reported.[35]

We have demonstrated that the clonally abnormal cell of chronic myelogenous leukemia can advance along the B-cell lineage and is capable of undergoing the normal genetic process of DNA rearrangement of the immunoglobulin loci (Fig. 2). Even though this may include both heavy-chain and light-chain-gene rearrangements, the cells in these lymphoid blast crises lack surface immunoglobulin and thus appear to be a population of B-cell precursors that have not advanced to more mature stages of development. The mechanism that arrests these malignant cells at a certain point of B-cell differentiation is unclear. Considerable attention has focused on the normal cellular oncogenes as elements that may effect the uncoupling of growth and differentiation capacities. Klein has suggested that chromosomal translocations may alter the expression of a cellular transforming gene by placing it into another locus.[36] For example cAbl (the Abelson murine leukemia virus cellular oncogene analogue), which is normally on chromosome 9, has been translocated to chromosome 22 in some cases of chronic myelogenous leukemia.[37] The A immunoglobulin genes are located at the very band, 22q11, involved in the Philadelphia translocation (Kirsch I: unpublished data). This area is also the breakpoint of some variant translocations found in Burkitt's lymphoma, suggesting that it is a vulnerable site for interchromosomal recombinations.[38] However, the lack of rearrangement of the A-gene locus in all the myeloid episodes of chronic myelogenous leukemia and in all but one of the lymphoid episodes suggests that this translocation does not occur directly within this portion of the A immunoglobulin-gene locus.

Our serial examination of one patient demonstrated that the monoclonal expansions recognized clinically as separate lymphoid blast crises arose from precursor cells at slightly different stages of genetic maturation. Thus, even though all affected lymphoid cells in chronic myelogenous leukemia may ultimately be the progeny of a common lymphoid progenitor cell, blast-crisis expansions appear to represent unique subclones at different stages of differentiation. Consistent with this, Martin et al.[39] have shown that both κ-producing and A-producing B cells bearing a Philadelphia chromosomal marker can be observed after the transformation of lymphocytes in chronic myelogenous leukemia with the Epstein–Barr virus. This indicates that clonally affected B-cell precursors in chronic myelogenous leukemia can be induced to differentiate to mature B-cell stages of development. The immunoglobulin-gene analysis reported in the present study has shown that the episodes of lymphoid blast crisis in chronic myelogenous leukemia involve clonal proliferations of cells at an earlier, B-cell-precursor stage of differentiation.

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PRENATAL DIAGNOSIS OF SICKLE-CELL ANEMIA IN THE FIRST TRIMESTER OF PREGNANCY

MICHEL GOOSSENS, M.D., YVES DUMEZ, M.D., LILIANA KAPLAN, M.D., MIEKE LUPKER, B.S., CWUDE CHABRET, B.S., ROGER HENRION, M.D., AND JEAN ROSA, M.D.

Abstract To investigate the usefulness of chorionic biopsy for prenatal diagnosis of sickle-cell anemia by restriction-endonuclease analysis of fetal DNA, we studied 30 pregnancies before elective abortion. When the reproducibility of the technique for obtaining adequate DNA samples was established, we successfully applied the test to five pregnancies at risk for sickle-cell anemia. In two cases, sickle-cell disease of the fetus led to a decision to terminate the pregnancy. In three other cases, a normal or AS genotype was demonstrated. One normal infant has been born, and one other pregnancy is continuing normally. In one case in which fetal death was observed three weeks after sampling, placental abnormalities found on histologic examination were compatible with a chromosomal aberration.

Our study shows that chorionic biopsy is feasible for the prenatal diagnosis of sickle-cell disease before the 10th gestational week. If subsequent experience demonstrates this technique to be safe enough for mother and fetus, the ability to test in early pregnancy may make prenatal diagnosis acceptable to more couples at risk for serious genetic disorders. (N Engl J Med 1983; 309:831-3.)

SINCE restriction-enzyme techniques for prenatal diagnosis of sickle-cell anemia by amniocentesis at about 17 weeks of gestation were developed by Chang and Kan[1] and by Orkin et al.,[2] emphasis has focused on techniques to permit diagnosis earlier in pregnancy, particularly with methods using trophoblast biopsy.[3] One report of three instances of prenatal diagnosis using DNA from trophoblast tissue obtained by suction has recently appeared.[4] We have been developing techniques for the diagnosis of fetal sickle-cell disease in France. We report here our results in five fetuses at risk for sickle-cell anemia, for which we used the restriction enzyme *Cvn*I to analyze trophoblast DNA obtained at six to eight weeks of pregnancy. The biopsy can be performed during outpatient consultation without anesthesia, and sufficient quantities of DNA are routinely obtained to permit direct analysis with the sensitive assay recently described.[1,2]

From the Service de Biochimie and INSERM, U.91, Centre Hospitalier Universitaire Henri Mondor. 94010 Creteil, France, and the Clinique Universitaire Port-Royal and INSERM. U.166, Maternité de Port-Royal, 75014 Paris. France. Address reprint requests to Dr. Goossens, at the Service de Biochimie, INSERM U 91. Hôpital Henri Mondor. 94010 Creteil, France.

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METHODS

Trophoblast Biopsy

As a preliminary chorionic biopsy specimens were obtained (after informed consent) from 30 women at 7 to 10 weeks of gestation, before elective abortion. The biopsies were performed using a Dyonics biopsy forceps (1.7 mm in diameter), introduced transcervically. High-resolution real-time ultrasound scanning was used to estimate gestational age and to guide insertion of the forceps to an area adjacent to the implantation site (Fig. 1). Biopsy specimens were taken from the placental villi. The small fragments of tissue obtained were examined under a dissecting microscope, and chorionic villi were separated from maternal decidua as previously described.[5]

Once the reproducibility of the technique was established, the possibility of using it was carefully discussed with couples at risk for having a child with sickle-cell anemia. We emphasized that the risk to the fetus was not known. Five women requested the early prenatal test, which was performed at six to eight weeks of gestation. Two of them already had a child affected by sickle-cell anemia, two had no previous children, and one, who had sickle-cell anemia herself, had already terminated a pregnancy because the fetus had the dis-