T Lymphocytes Lack Rearrangement of the bcr Gene in Philadelphia Chromosome-Positive Chronic Myelocytic Leukemia

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To study the possible involvement of T lymphocytes in Philadelphia chromosome (Ph)-positive chronic myelocytic leukemia (CML) we analyzed the arrangement of the bcr gene in T cell and non-T cell samples of 12 CML patients. Although all the patients showed bcr rearrangements in non-T cell fractions, T cell populations lacked respective gene recombinations. Moreover, by Southern blot analyses using T cell receptor \(\beta\) chain sequences our data indicate polyclonality of T cell samples from 11 of 12 cases; in one patient a clonal T cell population could be identified. These results suggest that T lineages of most Ph-positive CML patients are not derived from pluripotent stem cells involved in leukemogenesis and thus confirm previous investigations based on cytogenetic or glucose-6-phosphate dehydrogenase analyses. The demonstration of polyclonal T cell populations may reflect persistence of stem cells committed to differentiate only into T cells.

CHRONIC MYELOCYTIC LEUKEMIA (CML) originates in a pluripotent hematopoietic stem cell that gives rise to granulocytes, eosinophils, basophils, erythrocytes, megakaryocytes, monocyte/macrophage cells, and B lymphocytes.\(^3\)\(^4\) This clonal nature of CML has been mainly established by cytogenetic and glucose-6-phosphate dehydrogenase (G6PD) isoenzyme analyses.

The extent to which T lymphocytes are involved in the malignant process is still a matter of controversy. The majority of peripheral blood T cells responding to mitogen have not shown the Philadelphia (Ph) chromosome and are polyclonal by G6PD analyses.\(^5\)\(^6\) On the other hand, rare reports on T cell blast crisis,\(^7\)^{12} Ph-positive T cells obtained from multilinage colonies,\(^7\)\(^8\) or clonal G6PD patterns in T cells of CML patients\(^7\)\(^8\) indicate that the T lineage may be, albeit rarely, involved in Ph-positive CML.

In this report we addressed this topic by an alternative approach that is neither dependent on in vitro stimulation of respective cell populations nor restricted to the limited number of patients suitable for G6PD analyses. Proceeding from (1) the molecular hallmark of Ph-positive CML, ie, a rearrangement between c-abl and bcr genes,\(^9\)\(^10\) and (2) T cell receptor sequences as a unique clonality marker\(^16\) we investigated T cell and non-T cell fractions of 12 Ph-positive CML patients by Southern blot analysis.

MATERIALS AND METHODS

Patients. After informed consent was given peripheral blood samples were obtained from 12 Ph-positive CML patients. Some clinical data are listed in Table 1. Ten patients were in chronic phase at the time of study, from 1 to 59 months after diagnosis. Patient 4 had been in an accelerated state for 3 months. Cytogenetic analyses showed a standard translocation (9;22) in these 11 cases. Patient 11 developed lymphoblastic blast crisis 3 weeks prior to the investigation, and a bone marrow specimen showed 50% blasts of common acute leukemia antigen (CALLA)\(^+\) phenotype and a genotype characterized by mononuclear Ig+ rearrangement as well as CTF\(_d\) germline configuration; besides showing 40% of the metaphases exhibiting a t(9;22), 60% of the blasts revealed additional chromosome aberrations, ie, trisomies 8 and 12.

Isolation of cell samples. Peripheral blood mononuclear cells (PBMC) were isolated by gradient centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala Sweden). PBMC (1.2 to 1.8 \(\times\) 10\(^8\)/mL) were mixed with an equal volume of neureminidase-treated (0.2 \(\mu\)l \(Vibrio cholerae\) neuraminidase/mL, Behringwerke, Marburg, FRG) sheep erythrocytes (5% in RPMI). Thereafter the cells were centrifuged on Ficoll-Paque for 30 minutes at 1,800 rpm at room temperature. Interphase cells were washed twice and applied as the non-T cell fraction in Southern blot analysis. Contamination of this cell fraction with T lymphocytes never exceeded 2% as confirmed by staining with monoclonal antibody (MoAb) T11 (Ortho Pharmaceutical, Raritan, NJ).

After lysis of rosetted sheep erythrocytes, T cells obtained from the pellet were analyzed for purity by T11 staining. In cases exhibiting <97% purity, cell fractions were incubated with MoAb VIM-DS (20 \(\mu\)g/mL, kindly provided by W. Knapp, Vienna), for 45 minutes at 4°C and consequently exposed to undiluted rabbit complement (Behringwerke) for 90 minutes at 37°C. After this procedure, the purity of T cell samples exceeded 98% as confirmed by indirect immunofluorescence staining with MoAb T11.

Southern blot analysis. DNA was extracted from T and non-T cell populations by standard techniques. Ten micrograms of DNA was digested with appropriate restriction enzymes (Boehringer, Mannheim, FRG), electrophoresed on a 0.7% agarose gel, and blotted and hybridized as described.\(^15\) A-DNAs were included as molecular weight standards (not shown). BglI-digested DNA was hybridized to a 2-kb BglI/HindIII 5' bcr probe\(^17\) that detects a 5-kb germline fragment, and EcoRI or BamHI digests were hybridized to CTF\(_d\) sequences\(^18\) detecting 4.2- (T\(_d\)) and 12-kb (T\(_b\)) or 23 kb (T\(_b\) + T\(_d\)) germline fragments, respectively. Filters were exposed to XAR-2 films (Kodak, Rochester, NY) for 12 hours at —70°C with intensifying screens.

RESULTS

Southern blot analyses revealed a rearrangement of the bcr gene in non-T cell populations of all Ph-positive CML patients (Fig 1, lane a). However, respective T cell fractions lacked rearranged bcr sequences (Fig 1, lane b). Long time exposures (up to 3 weeks) of the filters also failed to exhibit rearranged bcr bands in all T cell populations (not shown). Taking into account the detection limits of Southern blot analyses and the fact that T cell lineages are not derived from pluripotent stem cells involved in leukemogenesis, we conclude that T lymphocytes are not clonally involved in CML.

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analyses (~1% clonal cells) the presence of a Ph translocation is virtually ruled out for more than 99% of the T cells studied in each case. In case 11, a faint hybridization signal of the rearranged fragment in the T cell sample represents in all likelihood a contamination with non-T cells (3%) since these cells had not been lysed after E rosetting. Moreover, the alternative explanation that a minor subset of T cells is characterized by a bcr rearrangement appears to be less likely because Tβ gene analyses (see the following paragraphs) demonstrated a clonal relationship of all T cells.

Southern blots showed a germline configuration of T cell receptor β chain genes in all non-T samples, as expected (Fig 2, lane a). T cell populations of 11 cases exhibited only the 4.2-kb germline band, whereas the 12-kb fragment was absent. BamHI digests failed to detect rearranged fragments (not shown). This configuration is observed in polyclonal T cell populations.14,15 The reason for the loss of the 12-kb band in polyclonal T cells as compared with the 4.2-kb band is evident from an EcoRI restriction site just before CI32 sequences. Thus the site of the 4.2-kb EcoRI fragment bearing the C132 gene does not move, even in the case of Cβ2 rearrangements.

Surprisingly, a monoclonal Tβ gene rearrangement was observed in T cells of patient 11 (Fig 2, lane 11b); this result was confirmed for BamHI digests (not shown). Leukemic blasts of this patient showed a B cell phenotype and genotype as well as the rearranged bcr fragment (Fig 1, lane 11a). On the contrary, T cells showed neither bcr nor IgCt rearrangements. Moreover, the clonal T genotype contrasts with the phenotypic analysis of this T cell fraction that shows no restriction to a specific T cell subset (terminal deoxynucleotidyl transferase [TdT]-, T3+T4+/T8+ in normal ratio).

**DISCUSSION**

Ph-positive CML is molecularly characterized by a rearrangement of the c-abl oncogene and a gene provisionally called bcr.15,20 As a consequence of this genomic recombination on the Ph chromosome, CML cells transcribe a chimeric 8.5-kb RNA species consisting of both 5′ bcr and c-abl sequences.21,22 This molecule is translated into a p210 c-abl protein that differs from the normal p145 abl counterpart as to associated tyrosine kinase activity.24

In this study we used sequences of the bcr gene as a specific molecular marker for Ph-positive CML and investigated a possible involvement of T cells in leukemic cell populations. Our results indicate that T cells of all 12
patients lack Ph-specific \( bcr \) rearrangements. This is in agreement with previous reports based on G6PD or cytogenetic analyses.\(^3,5,14\) However, since Southern blot analysis will only detect clonal populations of \( \geq 1\% \), we cannot totally rule out the existence of minor, affected T cell clones.

Several observations suggest that the generation of Ph-positive CML is a multistep process in which a yet- unidentified first event causes the proliferation of pluripotent stem cells and a consecutive step is identified by genomic alterations associated with the Ph translocation.\(^2,5\) To rule out the possibility that the T cell population of the 12 CML patients shows no \( bcr \) rearrangement but is nevertheless clonally related, we performed T cell receptor \( \beta \) chain analyses on respective samples. With case 11 being the only exception, all T cell fractions showed a polyclonal pattern.

The \( \beta T \) gene rearrangement of case 11 cannot readily be explained. The clonal genotype of these T cells, despite phenotypic heterogeneity, would suggest that an immature thymocyte, already characterized by a specific \( \beta T \) gene rearrangement, is nevertheless able to differentiate into different functional T cell subsets. From the molecular analyses reported here and in the absence of G6PD studies, we cannot settle the relationship between this T cell clone and the blast cell population. One possibility would be that both clones originated from a common, pluripotent stem cell. However, it is difficult to understand how, during the evolution of the primary stem cell clone, two different subclones developed and why the non-T clone led to the clinical manifestation of CML whereas the T cell clone appears to be biologically inert.

One possible explanation for the polyclonality of T lymphocytes in the majority of investigated cases could be that they are long-lived and antedate the development of CML. However, the fact that our studies were performed up to 5 years after the onset of the leukemia favors another view. Thus the demonstration of polyclonal, \( bcr \) rearrangement–negative T cells may reflect persistence of stem cells committed to differentiate only into T cells, as already suggested by Fialkow et al.\(^14\)

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