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Clonal instability preceding lymphoid blastic transformation of chronic myeloid leukemia

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We have sought the presence of rearrangements of the immunoglobulin heavy chain gene locus in 13 patients with chronic myeloid leukemia (CML) in lymphoid blastic transformation (L-BT) using the polymerase chain reaction (PCR). The lymphoid nature of the transformation was confirmed by immunophenotyping and/or Southern blot hybridization with a J_H probe. Clonal rearrangements were detected in 85% of cases and two or more rearrangements were visible in 64% of informative cases. The pattern of V_H gene family utilization revealed an apparent reduction in V_H 4 family gene usage but otherwise reflected the known proportion of each gene family in the germline repertoire. In six cases the third complementary determining regions (CDR3) of the predominant blast crisis clone/s were sequenced revealing minimal evidence of somatic mutation. No clonal changes were detected in the chronic phase leukemia cells collected more than 6 months before the onset of L-BT in three of these patients. Of the other three patients studied in chronic phase from 1 to 6 months before L-BT, two showed clonal rearrangements which differed in size from those present at L-BT. In one patient a V_H3 to V_H5–D_H–J_H substitution had occurred at least 3 months prior to L-BT. In the other patient, however, the sequence of the rearrangement present 5 months prior to L-BT was unrelated to the rearrangements at the time of L-BT indicating a pattern of clonal succession. We conclude that: (1) IgH gene rearrangements are detectable in the majority of patients with L-BT using PCR and the lymphoid lineage of blastic CML is most readily confirmed using consensus primers to the framework 3 region; (2) somatic mutation is uncommon; and (3) B lymphoid clones distinct from those identified later may be detected before overt lymphoid BT. The identification of such 'abortive' clones is evidence for clonal instability before the onset of transformation and might have prognostic value.

Keywords: clonal instability; lymphoid blastic transformation; CML; PCR; immunoglobulin gene

Introduction

Lymphoid blastic transformation (L-BT) accounts for approximately one third of the blast crises occurring in progressive chronic myeloid leukemia (CML). There is a reasonable likelihood of achieving a second chronic phase following L-BT with appropriate combination chemotherapy,[1] which may allow more successful utilization of allogeneic transplantation when compared to using the same treatment during blast crisis.[2,3] L-BT is phenotypically similar to acute lymphoblastic leukemia (ALL).[4] Furthermore, analysis of the immunoglobulin heavy chain (IgH) gene loci by Southern blotting[5] suggests that, as in ALL, rearrangement of one or more of the genes may serve as molecular markers of the transformed clone or clones.

In ALL IgH PCR studies with consensus primers designed to amplify the conserved regions flanking the third complementarity determining region (CDR3) of the IgH gene (Figure 1) are informative in the majority of cases.[6] This contrasts with the situation in follicular lymphoma and myeloma where strategies utilizing Framework 3 primers have met with only limited success,[7] possibly as a result of somatic mutations, a phenomenon seen only rarely in ALL.[8] We have used consensus primers for the Framework 1 and Framework 3 regions of the IgH gene locus as previously described[7,9–11] (Figure 1) to determine if clonal rearrangements of the IgH gene are diagnostic of L-BT. We have also examined chronic phase leukemia cell DNA from some of the same patients to determine if IgH gene rearrangements can be detected prior to the onset of L-BT.

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Materials and methods

Thirteen patients with a diagnosis of L-BT of Ph chromosome-positive CML, made at the Hammersmith Hospital between October 1989 and July 1995, on whom adequate cryopreserved BT material (11 peripheral blood/two bone marrow) was available were studied. The diagnosis of L-BT was based on the combined results of the morphological, cytochemical and immunophenotypic (not available in two cases) findings at the time of transformation. In six cases, where peripheral blood was available from the time of BT, one or more samples of peripheral blood mononuclear cells had been cryopreserved prior to L-BT and were available for study. Cytogenetic analysis (minimum of 20 metaphases) prior to and at the time of L-BT did not reveal clonal involvement of chromosome 14 in any of the cases studied. Material from 10 patients with myeloid BT and peripheral blood from 10 normal volunteers was also analyzed. Informed consent was obtained in all instances.

Mononuclear cells/DNA preparation/Southern blot analyses

Peripheral blood or bone marrow mononuclear cells were obtained by centrifugation on Lymphoprep (Nycomed, Oslo, Norway), pelleted and frozen at –70°C prior to use. DNA was then extracted and purified and Southern blot analyses were performed using a J_H probe following digestion with *Hin*dIII and *Bgl*II as previously described.[12]

Polymerase chain reactions

PCR was performed on all L-BT, myeloid BT, CP and normal material using nine different primer combinations: NV2/NJ2, FR3A/JN2 and V_H (1,2,3,4a,4b,5 and 6)/CJH.[9–11] The reactions with NV2/NJ2 and FR3/NJ2 were set up in 20 μl volumes containing 10 mm Tris pH 8.3, 50 mm KCl, 1.5mm $MgCl_2$, 250 μm

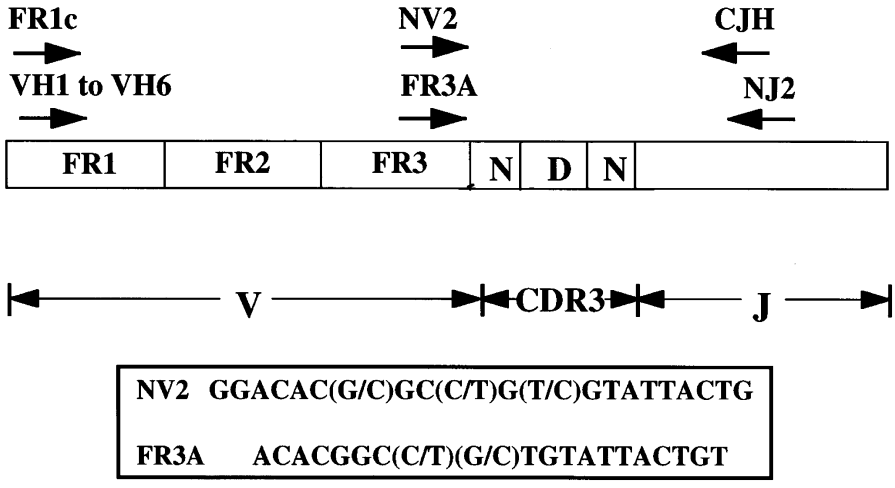

Figure 1 Schematic representation of a rearranged IgH chain gene showing the annealing sites of the primers used. The third complementarity determining region (CDR3) is an area of hypervariability comprised of a diversity gene and two unique sequences of nucleotides (N-regions) which connect it to the 5′ variable gene and 3′ joining gene. Highly conserved regions within FR1, FR3 and the joining gene of the rearranged IgH chain gene allow the use of consensus primers. The sequences of the two primers complimentary to the FR3 region are shown. FR, Framework; CDR, complementarity determining region; N, N-region nucleotides; D, diversity gene; V, variable gene; and J, joining gene.

Table 1 Patient and disease characteristics

Patient No.	*Sex*	*Age (years)*	*WC*[b]	*BC (%)*	*CP duration (months)*	*Surface antigens*[a]									
						CD2	*CD7*	*CD10*	*CD13*	*CD14*	*CD19*	*CD33*	*CD34*	*TdT*	*HLA-DR*
1	F	53	8.0	50	49	21	21	92	1	3	84	11	70	73	90
2	M	18	3.3	60	60	ND	ND	80	70	ND	70	50	55	ND	ND
3	M	50	62.7	50	20	3	4	97	3	2	96	15	2	96	98
4	F	32	96.6	50	5	20	7	96	20	2	93	23	75	86	95
5	M	28	55.5	70	133	3	2	75	15	3	25	ND	ND	95	ND
6	M	32	9.0	22	39	ND	ND	23	20	1	3	6	53	18	84
7	M	45	65.6	54	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	M	24	13.5	30	77	8	5	76	7	4	78	3	ND	93	83
9	M	34	45.6	20	67	12	5	54	3	2	49	1	ND	86	80
10	M	37	144.0	25	20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	F	43	182.7	30	1	9	3	80	2	ND	84	9	33	87	92
12	M	17	42.6	90	10	3	2	82	3	4	83	5	69	56	81
13	M	72	62.7	40	34	9	47	86	75	ND	62	23	2	60	ND

[a]Number of positive cells expressed as a percentage.
[b]$n \times 10^9$/l.
WC, white cell count; BC, peripheral blood blast percentage; CD, cluster of differentiation; CP duration, duration of chronic phase prior to blastic transformation; NA, not available; ND, not determined.

each dATP, dCTP, dGTP and dTTP, 0.5 μm of each primer, 0.2 U Taq polymerase and 0.5–1.0 μg of genomic DNA template. Amplification was carried out on a programmable heating block (MJ Research, Watertown, MA, USA) by 36 cycles of 96°C for 30 s, 60°C for 50 s and 72°C for 1 min followed by a 10 min extension at 72°C. The V_H family-specific primers (V_H1 to V_H6) each in combination with CJH (seven separate PCR reactions) were used at a concentration of 0.3 μm and an annealing temperature of 62°C but conditions were otherwise identical. PBMC DNA from a normal individual was included in each experiment to show that adequate amplification and radioactive labelling had occurred.

In each case a 5 μl aliquot of reaction product was electrophoresed on a 2% agarose gel to confirm adequate amplification. A 1 μl aliquot of each was then reamplified with the same primers and under the same conditions incorporating ^{32}P dCTP. The products were then electrophoresed on 6% (V_H1 to V_H6/CJH) or 8% (NV2/NJ2 and FR3/NJ2) polyacrylamide gels and visualised after overnight exposure to radiographic film at –70°C. All PCR reactions and polyacrylamide electrophoreses were performed at least twice to ensure reproducibility of the results.

Sequencing

Following amplification with the NV2/NJ2 primers the band(s) on the polyacrylamide gels corresponding to clonal CDR3 rearrangements were excised (both bands in patient No. 12 L-BT, otherwise the predominant band if more than one rearrangement was present). The DNA was eluted and reamplified using the same set of primers. The products were purified using polyethylene glycol precipitation, visualised on 2% agarose gels to confirm purity and then sequenced on an

ABI 373A automated DNA sequencer with the ABI dye termination kit (Perkin Elmer, Foster City CA, USA). Identification of the V_H gene family corresponding to the sequenced CDR3 regions was achieved by amplification at the corresponding time points by 1 of the 6 V_H gene family-specific primers. In patient No. 4 the predominant V_H gene family band was assigned to the sequenced CDR3 region.

CDR3-specific PCR

A primer (J498) complementary to the sequenced D–N–J region of the CDR3 rearrangement detected during the chronic phase of patient No. 12 was designed. PCR was then carried out on the patient's sequential CP and L-BT material using primer J498 in combination with both the V_H1 gene family-specific primer and with a V_H gene family consensus primer FR1c.[7] The products were then visualized on polyacrylamide gels as previously described.

Results

Lymphoid blastic transformation

No clonal rearrangements were detected in any of the myeloid BT patients or normal individuals analyzed. Immunophenotyping and Southern blotting results for patients in L-BT are shown in Tables 1 and 2, respectively. In two cases (Nos 2 and 13) evidence of bi-lineage transformation was present with co-expression of CD10/CD19 and CD13/CD33. IgH rearrangement was seen in all but one of the 10 cases analyzed by Southern blotting (Table 2), a germline configuration being found in one of the cases with bi-lineage transformation.

Overall the number of rearranged bands by Southern blot analyses correlated well with the PCR results. The combined results of the PCR analyses at the time of L-BT are shown in Table 2. The detection rate with each of the Framework 3 primer combinations was identical, with the same 11 of 13 (85%) patients showing one or more rearrangements. Using the 7 V_H family-specific primers one case previously scored as negative with the Framework 3 combinations (No. 10) was found to be positive; conversely, the rearrangement observed with the FR3 PCR assay in patient No. 7 was not detected with the V_H family-specific primers giving the same overall detection rate of 85%. More than one PCR detectable rearrangement was present in 64% and >2 in 27% of the informative cases. V_H3 and V_H1 were the most commonly detected variable genes. In general, the variable gene utilization, apart from an apparent under-utilization of V_H4, reflected the proportions of each V_H family within the germline repertoire. The predominant clone(s) at the time of L-BT were sequenced in six patients (Table 3). In all instances comparison with previously published germline diversity and joining gene sequences revealed no evidence of somatic mutation.[8,13–18]

Chronic phase

Chronic phase material was available for analysis at various intervals prior to the development of L-BT from six patients: No. 2 (22 and 33 months prior), No. 3 (3 months prior), No. 4 (3 and 4 months prior), No. 6 (37 and 38 months prior), No. 9 (60 and 61 months prior) and No. 12 (1, 5 and 6 months prior). Four of these patients (Nos 2, 4, 6 and 9) had no clonal rearrangements detected. Clonal rearrangements were detected by PCR but not by Southern blot analysis in patient

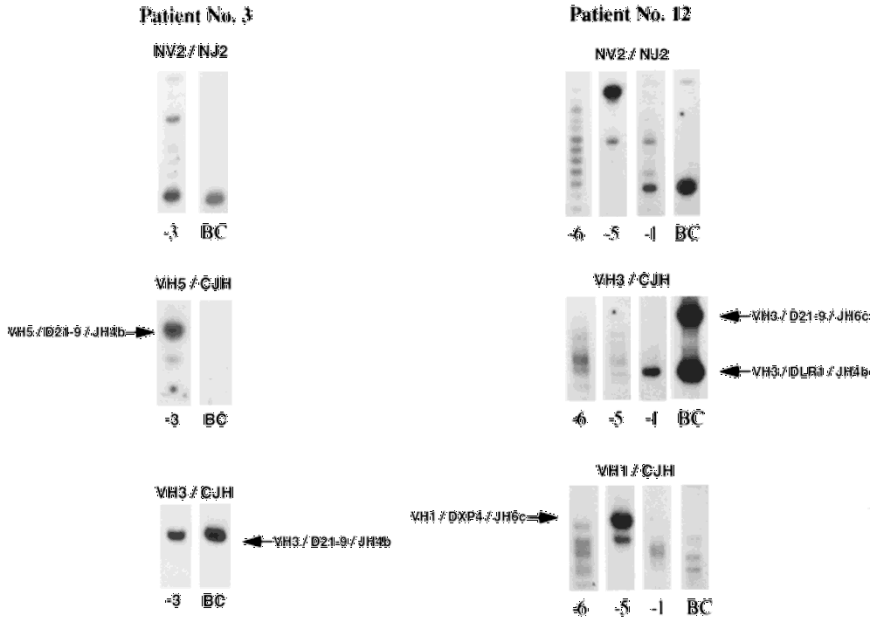

Figure 2 Polyacrylamide gel electrophoresis of L-BT and chronic phase IgH rearrangements present in No. 3 and No. 12. *Patient No 3*: CDR3 amplification with NV2/NJ2 (top left panel) shows two rearrangements 3 months prior to L-BT one of which subsequently disappears. Use of the more specific V_H gene family-specific primers (middle and bottom left panel) and subsequent sequencing was consistent with a V_H5 to V_H3 gene substitution occurring more than 3 months prior to the diagnosis of L-BT. *Patient No. 12*: Th V_H1 rearrangement detected and sequenced from 5 months prior to L-BT is no longer detectable 1 month prior to or at the time of L-BT despite the presence of residual amplifiable V_H1 utilizing lymphopoiesis (bottom right panel). The two rearrangements at the time of BT (right top and middle right panel) were unrelated to the preceding chronic phase rearrangement. The numbers below each lane indicate the number of months prior to transformation.

Table 2 PCR and Southern blot results

Patient No.	*PCR*[a]									*Southern blot*
	NV2	*FR3A*	*VH1*	*VH2*	*VH3*	*VH4a*	*VH4B*	*VH5*	*VH6*	
1	1	1	2	0	0	0	0	0	0	R/D
2	1	1	0	0	1	0	0	0	0	G
3	1	1	0	0	1	0	0	0	0	R_1/R_2
4	3	3	2	1	1	0	1	0	0	R_1/R_2
5	0	0	0	0	0	0	0	0	0	ND
6	1	1	2	0	0	0	0	0	0	ND
7	3	2	0	0	0	0	0	0	0	$R_1/R_2/R_3$
8	3	2	0	2	3	0	0	1	0	$R_1/R_2/R_3$
9	1	1	0	0	1	0	0	0	0	R/G
10	0	0	3	0	0	0	0	0	0	R_1/R_2
11	1	1	0	0	1	0	0	0	0	ND
12	2	2	0	0	2	0	0	0	0	R_1/R_2
13	1	1	1	0	1	0	0	0	0	R_1/R_2

[a]Number of clonal rearrangements detected with each of the 9 primer combinations used.
R, rearranged; G, germline; D, deleted; ND, not determined.

No. 3 3 months prior to L-BT and in patient No. 12 5 months and again 1 month prior to L-BT (Figure 2). In both instances the rearrangements differed in size from those detected at the time of L-BT. In patient No. 3 the chronic phase rearrangement (V_H5/D21-9/J_H4b) showed partial identity with the rearrangement present at the time of L-BT (V_H3/D21-9/J_H 4b). In patient No. 12, however, the predominant rearrangement 5 months prior to L-BT was clearly not related to either of the rearrangements seen at the time of L-BT, even though it utilized the same joining region gene. The band detected 1 month prior to L-BT was the same size as the V_H3/DLR1/J_H4b rearrangement and was considered to represent early detection of this rearrangement. PCR with the CDR3 primer specific for the clone detected 5 months prior to L-BT in combination with either V_H1 or FR1c showed amplification at 5 months prior to L-BT only (Figure 3).

Discussion

We have shown that the lymphoid nature of cells from patients considered by immunophenotyping to be in lymphoid transformation of CML can in most cases be confirmed reliably by PCR amplification on the IgH chain gene locus using consensus primers. The detection rates with FR3 primers and with the FR1 V_H family primers were identical (85%) and similar to results reported in patients with ALL.[6] The FR3 PCR findings correlated well with the Southern blot results showing a comparable detection rate (85% *vs* 90%) and sensitivity. Use of the 7 FR1 V_H family primers resulted in the detection of a greater number of rearrangements (Figure 4) due to the greater sensitivity of the method[19] but did not improve the overall patient positivity rate compared to either of the FR3 primers. The use of either of the FR3 primers is technically simpler than

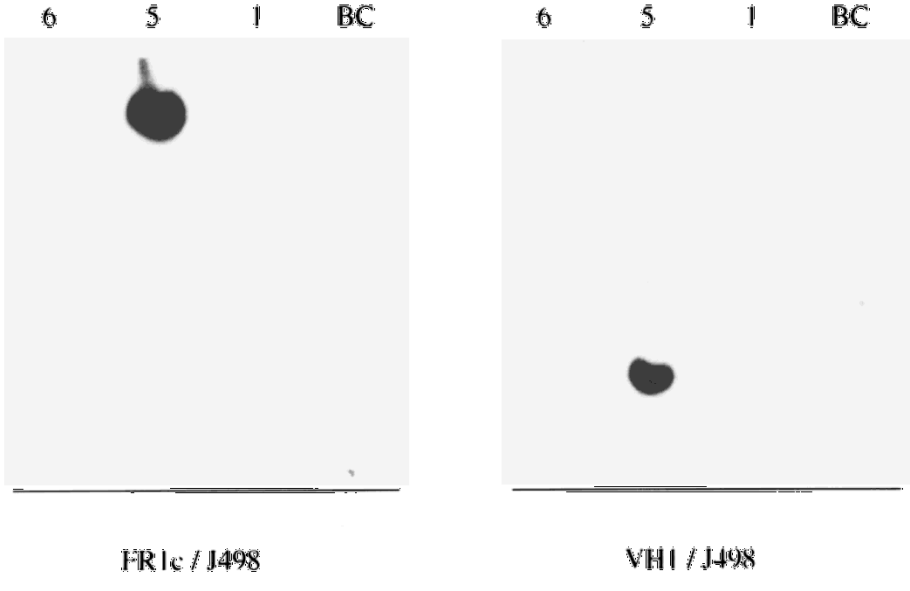

Figure 3 Polyacrylamide gel electrophoresis of amplified L-BT and chronic phase material from patient No. 12 using a CDR3 specific primer (J498) complementary to the IgH rearrangement detectable 5 months prior to L-BT. Amplification with both FR1c/J498 and V_H1/J498 confirms the presence of a clonal IgH rearrangement 5 months prior to L-BT that is not detectable at any other time-point tested.

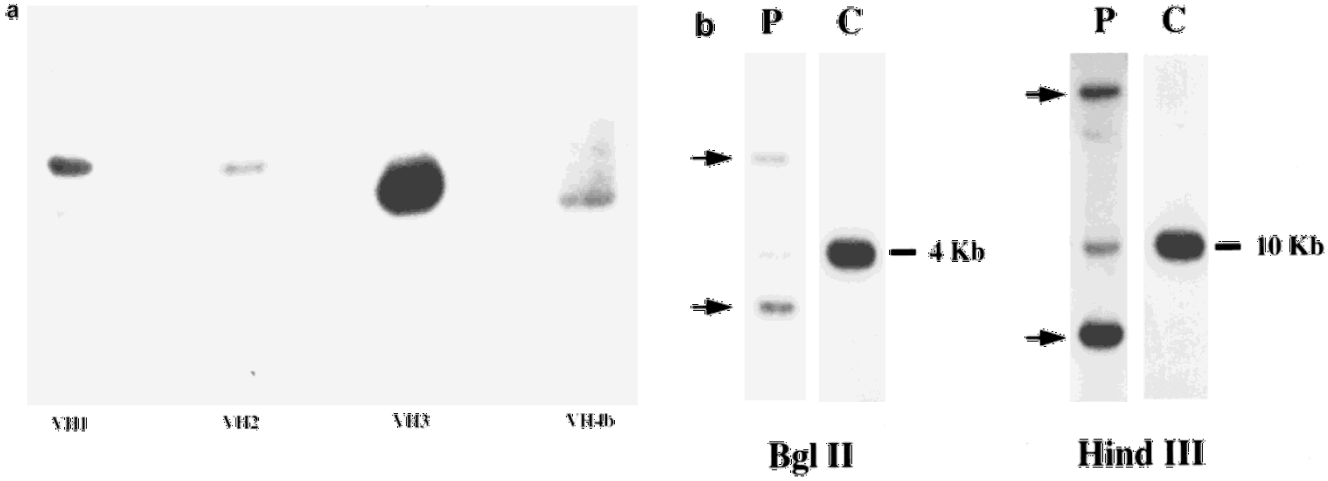

Figure 4 (a) Composite figure of the five PCR detectable IgH gene rearrangements at the time of L-BT in patient No. 4 using the 7 V_H family gene specific primers. (b) Southern blot analysis at the same time-point with the arrows to the left indicating the two rearranged bands detectable. An indentical R1/R2 pattern was observed following digestion of genomic DNA with *Xba, Eco* and *Bam* (not shown). The Southern analysis is consistent with a monoclonal process whereas the more sensitive PCR results are consistent with an oligoclonal process.

either FR1 amplification (one reaction *vs* up to seven reactions) or Southern blotting and would therefore be most appropriate as an initial diagnostic procedure. The detection of an R1/R2 pattern by Southern blotting but a failure of PCR amplification was noted in patient No. 10. The reason for this is uncertain but could be explained by the presence of somatic mutations at the FR3 anealing sites.

After examining variable region family gene usage in both ALL and CLL, Deane and Norton[9] showed a possible bias in the usage of the gene families V_H5 and V_H6 but overall a predominance of the most complex V_H gene family, V_H3. Our results confirm the latter observation – 42% (11 of 26) of the identified L-BT rearrangements utilized V_H3, but, only one case utilized a V_H4 gene. (The V_H4 gene family represents approximately 25% of the known functional genes in the germline repertoire.) Whether this represents a true bias in gene utilization is uncertain as a greater number of patients would need to be examined.

The sequences of the predominant IgH rearrangement in five patients and of both rearrangements in patient No. 12 at the time of diagnosis of L-BT were determined. The diversity and joining genes utilized were readily identifiable from previously described germline sequences[8,13–18] and on comparison showed no evidence of somatic mutation, as has been described in ALL.[8] This likely explains the low PCR false negativity rate, in contrast to the situation in myeloma and follicular NHL.[7]

We have found two or more rearrangements in over half of the informative cases analyzed which is consistent with the findings in ALL.[20,21] Steenbergen *et al*[22] reported a case of lymphoid blastic transformation of CML in which both related and unrelated IgH gene rearrangements were detectable in the same patient. They attributed this to transformation occurring in a B cell precursor prior to and competent for IgH gene rearrangement. An alternative hypothesis, and one which would also explain the presence of unrelated lymphoid blastic clones in sequential transformations[23] can be proposed. That is, that in a subgroup of CML patients, primitive BCR-ABL-positive cells committed to B lymphoid differentiation acquire genetic abnormalities producing significant genomic instability within a pool of self-renewing B lymphoid progenitors. Further mutations(s) representing the final transformation event(s) could then occur in more than one of these unstable progenitors giving rise to an oligoclonal rather than a monoclonal L-BT.

An unexpected finding was the detection of clonal IgH rearrangements in peripheral blood CP cells from two of three patients within 6 months of the onset of blastic transformation that were different from those found at the time of established L-BT (Figure 2). At the time of detection neither patient had any systemic symptoms or signs such as bone pain, fevers or resistant splenomegaly suggestive of disease progression and the peripheral blood appearance was consistent with chronic phase. Patient No. 3 had cytogenetic evolution at the time of transformation which was not evident on cytogenetic analysis of the bone marrow 3 months prior to the diagnosis of L-BT. Cytogenetic evolution was not present at the time of L-BT in patient No. 12. The analysis of the CDR3 sequences suggests that different underlying mechanisms were responsible for the findings in the two patients.

In patient No. 3 the rearrangement V_H3/D21-9/J_H4b (Figure 2) was identified at the time of L-BT. Three months prior to L-BT two rearrangements were visible, one of which was identical in size to V_H3/D21-9/J_H4b. Sequencing showed that the other larger rearrangement was V_H5/D21-9/J_H4b (Figure 2). In addition to identical D-J_H utilization and the N region between the D_H and J_H segments there was also identity of the three most 3′ V_H-D join N-nucleotides (Table 3) between the CP and L-BT rearrangements. The most likely explanation for the disappearance of the V_H5/D21-9/J_H4b rearrangement is clonal evolution secondary to a V_H to V_H–D–J_H substitution occurring more than 3 months prior to L-BT utilizing the conserved 3′ V_H heptamers TACTGTG[24] present in both rearrangements (not shown). A previously germline V_H3 gene would have replaced the more J_H-proximal V_H5 gene and in the same rearrangement process a 5′ 20 bp deletion of the CP V_H–D join could have occurred.

The degree of identity between the CP and L-BT rearrangements argues against the alternative explanation, namely two independent V_H to D–J_H rearrangements occurring in a transformed D21-9/J_H4b rearrangement B cell precursor.

In patient No. 12 both the Southern blot and the V_H3 family primer PCR amplification (Figure 2) revealed two bands at the time of L-BT with no evidence of a germline band on the

Table 3 Sequences of blast crisis and two preceding chronic phase CDR3 rearrangements

Patient No.	*Status*	*V_H*	*N1*	*D_H[b]*	*N2*	*J_H[c]*
2	L-BT	VH3	GCCCTGGGGCC	TATAGCAGCAGCTGCT	GTGGCT	TACGGT-JH6b
				DN1		
3	L-BT	VH3	CGT	TGATAGTA	CGG	ACTACT-JH4b
				D21-9		
3	CP[a]	VH5	CGAGGGACGAAGGAGGAAGACGT	TGATAGTA	CGG	ACTACT-JH4b
				D21-9		
4	L-BT	VH3	AACGTGC	TACGATTTTTGGAGTGGTT	GAGA	TACTAC-JH6b
				DXP4		
6	L-BT	VH1	CTCACCTA	TGGTAGCTGCTAC	CTTTTC	CTTTGA-JH4b
				D2		
9	L-BT	VH3	TCCCCGGGAGGGG	GGATATTGTAGTGGTGGTAGCTGC	AC	GCTGAA-JH1
				D2		
12	L-BT	VH3	TCCCCACTAG	ATTGTACTAATGGTGTATGCT	—	GGGGCC-JH4b
				DLR1		
12	L-BT	VH3	AGCAAAACCACCCATC	TACTATGATAGTAGTGGTTATTACT	GCTA	ACTACT-JH6c
				D21-9		
12	CP	VH1	GATGGGT	CATTACGATTTTTGGAGTGG	CCCCC	ATACTA-JH6c
				DXP4		

[a]The 20 base pairs deleted in the V_H3 to V_H5/D21-9/J_H4b substitution are underscored.
[b]Germline diversity gene from which rearranged segment is derived is shown in bold lettering.
[c]Only the six most 5′ bases of the rearranged J_H genes are shown.
L-BT, lymphoid blastic transformation; CP, chronic phase; V_H, heavy chain variable gene; D_H, heavy chain diversity gene; J_H, heavy chain joining gene; N1 and N2, N region nucleotides.

Southern blot (not shown). Taken together this is consistent with bi-allelic IgH gene rearrangement within a single transformed clone at the time of L-BT. Using the V_H1 family primer two rearrangements were also detectable 5 months prior to the onset of L-BT. Sequencing revealed the predominant CP rearrangement to be V_H1/DXP4/J_H6c. Despite the obvious amplification of residual V_H1 utilizing lymphopoiesis both 1 month prior to and at the time of L-BT no band of a size corresponding to the V_H1/DXP4/J_H6c clone was detectable. Furthermore, the two rearrangements at the time of L-BT, V_H3/D21-9/J_H6c and V_H3/DLR1/J_H4b were completely unrelated to the preceding chronic phase rearrangement (Table 3). In view of these observations, the proposed mechanism underlying the clonal evolution in patient No. 3 could not have been responsible in this instance and clonal succession is a more appropriate description. This was confirmed by the CDR3-specific PCR (Figure 3) which was positive 5 months prior to the L-BT but at no other time point analyzed.

The ability of the V_H3 clone in patient No. 12 to produce a clinical picture consistent with L-BT and the inability of the V_H1 clone to do so, imply the presence of important biological difference(s) between the two. Whereas both were capable of clonal expansion the latter failed to progress and generate a clinical L-BT. Indeed, after only a transient appearance, the V_H1 clone fell below the level of detection of the CDR3-specific PCR assay, possibly undergoing 'clonal deletion'. This suggests the probable absence within the V_H1 clone of a genetic abnormality essential for indefinite clonal survival. Such an abnormality must have been present in the V_H3 clone and enabled this cell population to expand and establish a true blastic transformation.

Examples of clonal succession occurring in sequential blastic transformations have been described previously.[23,25–28] In all of these instances the original transformed clone was eliminated by chemotherapy enabling the subsequent emergence of secondary clones. On occasion these secondary clones coexisted with the original dominant BT clone prior to myeloablative therapy.[27,28] The present study is however the first to provide evidence that in some CML patients a state of 'clonal instability' may exist during an otherwise typical CP with spontaneous clonal succession preceding the emergence of a dominant blastic clone. Indeed, this is not inconsistent with the hypothesis we have proposed as a possible explanation for the presence of unrelated IgH gene rearrangements at the time of diagnosis of L-BT. Kitchingham[29] proposed that the VDJ recombinase system may be especially active in the target cells at the time of disease onset in a high percentage of ALL patients leading to genomic instability. It is possible that a similar mechanism operates in patients with CML heralding the onset of blast crisis.

In conclusion, we have demonstrated the reliability and specificity of the IgH PCR assay in confirming the lineage of blastic transformation of CML. In some patients clonal IgH rearrangements detectable in the peripheral blood during apparently stable chronic phase may be a sensitive marker of clonal instability and impending disease progression. It is uncertain if the same results would be obtained using bone marrow. We are currently investigating whether such 'abortive' rearrangements are detected only in the peripheral blood of patients destined to develop lymphoid rather than myeloid BT and whether their timing is such that this technique could be used to monitor chronic phase patients.

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