Clonality Analysis of Hematopoiesis in Essential Thrombocythemia: Advantages of Studying T Lymphocytes and Platelets

By Nahed El-Kassar, Gilles Hetet, Jean Brière, and Bernard Grandchamp

Essential thrombocythemia (ET) is a myeloproliferative disorder characterized by a sustained elevation of the platelet count in the absence of other causes of thrombocytosis. ET is difficult to diagnose, and the demonstration of clonal hematopoiesis may be of value. However, clonality analysis of hematopoietic cells based on the study of the X-chromosome inactivation pattern is complicated by the observation that some normal females present skewed lyonization. Moreover, DNA methylation of X-linked genes in hematopoietic cells may differ from that in other tissues. Appropriate controls for skewed lyonization are therefore critical for the study of clonality. We developed two techniques based on X-chromosome inactivation and polymerase chain reaction (PCR) analysis of polymorphisms, to study clonality in ET patients. Reverse transcriptase-PCR analysis of IDS, P55, and G6PD mRNAs was used to examine the different hematopoietic cell lineages including platelets in patients heterozygous for these polymorphisms and analysis of the HUMARA gene methylation pattern permitted us to study clonality in all nucleated cell fractions of the other patients. Using both types of assay and T lymphocytes as a control tissue for lyonization, clonal hematopoiesis was demonstrated in 28 patients. In 14 patients, the granulocytes were polyclonal; among these patients, platelets were monoclonal in 3 cases, polyclonal in 7 cases, and in the remaining 4 cases this fraction could not be studied because the patients were homozygotes for all RNA markers. No conclusion about clonality could be drawn in 6 cases. Polyclonal hematopoiesis was found in all the cases of reactive thrombocytosis. These findings confirm the high frequency of monoclonal hematopoiesis in ET, the utility of studying platelets, and the possibility of using T lymphocytes as a control tissue for X-chromosome inactivation patterns.

© *1997 by The American Society of Hematology.*

AN INCREASE in the platelet count is common in patients with myeloproliferative diseases (MPD); it may also occur as a reactive phenomenon in patients with acute or chronic inflammatory conditions, and in iron deficiency. Patients with thrombocytosis who lack all these criteria are diagnosed as having essential thrombocythemia (ET), following the criteria of the Polycythemia Vera Study Group (PVSG).[1] A large number of studies have attempted to identify positive diagnostic critera for ET. Platelet size heterogeneity, platelet aggregation and platelet nucleotide ratio (ATP/ADP) are higher in ET than in reactive thrombocytosis, but these abnormalities cannot be used for positive diagnosis of ET as they are neither very sensitive nor highly specific.[2] Culture of peripheral blood (PB) or bone marrow may then be helpful.[3] Spontaneous growth of erythroid burst-forming units or megakaryocyte colony-forming units supports the diagnosis of ET. Unfortunately these changes are not found in all patients and culture conditions are difficult to standardize.[2,4]

The most promising developments for diagnosis of ET are attempts to show the presence of clonal hematopoiesis by studying the X-chromosome inactivation pattern in female patients. Either the maternal or the paternal X-chromosome is randomly inactivated in each cell at an early stage of embryogenesis.[5] Therefore, clonal tissue will consist of a population of cells all containing the same active X-chromosome, whereas one of the X-chromosomes is randomly inactivated in each cell in nonclonal tissue. Methods for determining X-chromosome inactivation have been devised at the protein, DNA and RNA levels. Evidence for the underlying clonality of hematopoiesis in ET was originally obtained by studying the glucose-6-phosphate dehydrogenase (G6PD) isoenzyme pattern.[6-8] However, this method has limited applications as G6PD polymorphism is informative in only a small minority of females. A more widely applicable technique makes use of the differences in DNA methylation pattern between active and inactive copies of X-chromosome genes.[9] The two alleles from a polymorphic gene can be distinguished either by specific restriction endonuclease sites, eg, phosphoglycerate kinase[10] and hypoxanthine phosphoribosyl transferase,[9] or by the variable number of tandem repeat sequences, eg, the 26-bp repeat sequence in the DXS255 locus recognized by the probe M27 β,[11,12] or the CAG short tandem repeat (STR) in the androgen receptor gene (AR, HUMARA).[13-15] Using such approaches, we and others have shown that clonal hematopoiesis is a frequent but not a constant feature of ET.[14,16-20] Prchal et al[21,22] have developed another clonality assay using the ligase detection reaction, to identify the transcribed alleles of X-chromosome genes by studying mRNA polymorphisms from G6PD (C/T at cDNA 1311) and P55 (palmitoylated erythrocyte membrane protein, G/T at cDNA 358) genes.[21,22] They recently showed clonality of hematopoiesis in one patient with ET, on granulocytes, reticulocytes, and platelets, whereas T lymphocytes, B lymphocytes, and CD34 medullar progenitors were polyclonal.

Previous studies in our laboratory have shown a high prevalence of nonrandom X-chromosome inactivation in hematopoietic cells of ET patients[14] suggesting that many patients with this disease have clonal hematopoiesis. Analysis of G6PD transcripts (C/T at cDNA 1311) in platelets of these patients indicated that the contribution of clonal hematopoiesis in different hematopoietic lineages may be variable and, in some cases can be detected only in the megakaryocytic lineage.

To extend our initial study and to further evaluate the clonality of platelets in ET, we developed reverse tran-scriptase-polymerase chain reaction (RT-PCR) assays to determine the X-chromosome inactivation pattern from the allelic expression of three polymorphic genes: iduronate-2-sulfatase (IDS),[23] P55 and G6PD. Clonality results based on the detection of these mRNA transcripts were compared to those deduced from the methylation pattern of the HUMARA gene in nucleated cells. We used the T-lympho-cyte fraction as a control tissue for the X-chromosome inacti-vation patterns, as most previous studies have shown that they are not involved in this disease.[7,20]

MATERIALS AND METHODS

Subjects. Blood samples were obtained from 48 females with ET, 51 normal controls, and 8 patients with reactive thrombocytosis with their informed consent.

ET patients aged from 13 to 80 years (mean, 53 years) at the time of diagnosis were selected according to PVSG criteria[1] and had no known cause for reactive thrombocytosis for at least 6 months after the diagnosis. Clinical symptoms and platelet counts at diagnosis, treatment, and the follow-up period at the time of clonality analysis are shown (see Tables 4 and 5).

Normal controls were aged from 22 to 87 years (mean, 36.5 years) and patients with reactive thrombocytosis were aged from 33 to 75 years (mean age: 53 years). The mean platelet count in this latter group was 810×10^9/L (600 to 1,047). One patient had lung cancer, 3 had iron deficiency, and 4 had infectious diseases.

Cell fractionation. PB (50 mL) was collected on citrate or EDTA. DNA was extracted from 5 mL with the Genome Kit (Bio 101, Ozyme, France). Forty-five milliliters of blood was used to isolate platelets, granulocytes, and lymphocytes. After centrifugation at 150g, platelets were collected from the upper half of the plasma layer. The remaining blood was mixed with a Dextran solution (Phar-macia Biotech, Uppsala, Sweden) and left for 45 minutes. The upper layer was centrifuged through an Isopaque gradient (GIBCO-BRL, Gaithersburg, MD)[1] at 300g for 20 minutes, then, mononuclear cells and granulocytes (95% by light microscopy after May-Grünwald-Giemsa staining) were obtained from the interphase and pellet, re-spectively. T lymphocytes were collected from interphase cells using immunomagnetic beads (Dynabeads, Dynal, France), coated with monoclonal antibodies (MoAbs) CD2 and CD4 MoAbs. DNA was extracted from granulocyte and mononuclear cell fractions by using the Isoquick kit (ORCA Research, Bothell, WA).

Genotyping of G6PD, IDS, and P55 polymorphisms. The IDS gene contains a silent polymorphism at nucleotide 438 (C/T).[23] We found that this gene is transcribed in different blood fractions, includ-ing platelets. The G6PD gene shows a C/T polymorphism at position 1311,[21] and the P55 gene shows a G/T polymorphism at position 358[22] (Table 1).

Genomic DNA extracted from whole blood was used for genotyp-ing. DNA fragments containing the polymorphic bases were ampli-fied by PCR. Primers and PCR conditions are shown in Table 1. In each case, the sequence of one primer introduced a mismatch to create a restriction site specifically in PCR products from one of the two alleles (Table 1). Restriction fragments were separated by gel electrophoresis and visualized by UV illumination after ethidium bromide staining. In heterozygous females, 2 bands were observed: 151 and 126 for IDS; 183 and 162 pb for G6PD; whereas only one band was found in homozygous females. For P55, bands of 80, 78, and 34 bp were found in cases with homozygous G; 80, 57, 34, and 21 in cases with homozygous T and 80, 78, 57, 34, and 21 in heterozygotes.

Clonality study by mRNA transcript analysis. RNA was ex-tracted from cell fractions, including platelets, according to Chom-zinski et al.[24] After reverse-transcription from 1 μg of RNA using random priming,[25] cDNAs were amplified and the resulting frag-ments were digested and analyzed on agarose gel as described above. Fragment lengths, polymerase chain reaction (PCR) conditions, primer sequences, and enzymes are shown in Table 2. After migra-tion, two bands were seen in polyclonal patients: 158 and 133; 79 and 58; 138 and 122; for IDS, G6PD, and P55, respectively, whereas one single band was present in monoclonal patients.

Clonality analysis using HUMARA trinucleotide repeat polymor-phism. This method has previously been described in detail.[14] Briefly, DNA was amplified using two primers flanking the STR in the HUMARA gene. One primer was labeled at the 5′ end with fluorescein. The PCR products were analyzed and quantified using an automated DNA sequencer (ALF; Pharmacia).

RESULTS

Patterns of X-chromosome inactivation from total blood using DNA and RNA-based clonality assays. The overall heterozygosity of the markers was 81%, 51%, 37%, and 17%, for HUMARA, IDS, P55, and G6PD, respectively. At least one mRNA marker was informative in 72% of the cases. Thirty-four controls, 28 ET patients and 4 patients with reactive thrombocytosis were informative for both DNA and RNA markers. In normal controls, nonrandom lyoniza-tion was found in 9% of cases by DNA analysis (4 out of 44 informative patients) and in 2.4% of cases by mRNA analysis (1 out of 41 informative patients) (see Table 4). The patients with reactive thrombocytosis showed a polyclonal pattern of X-chromosome inactivation as determined by DNA analysis (in 2 patients), RNA analysis (in 2 patients), or both (in 4 patients). Among ET patients, 74% and 68% showed a nonrandom inactivation pattern by DNA and mRNA analysis, respectively. Results were usually concor-dant between both types of markers. However, some discrep-ancies were observed: in normal controls, a nonrandom methylation pattern at the HUMARA locus was observed in 3 cases, whereas the expression of X-linked genes was bal-anced (Table 3); in the patient group, the opposite was ob-served in 2 cases (Table 3).

Clonality analysis on cell fractions. In the ET group, cell fractions containing T lymphocytes, granulocytes, and platelets were studied using RNA markers when at least one of these markers was informative. When no marker was informative, the same fractions but platelets were studied using the HUMARA DNA polymorphism.

In 2 out of 48 patients studied by DNA and RNA analysis, discordant results obtained from unfractionated blood (Table 3) were confirmed on cell fractions, running out conclusions about clonality. A nonrandom X-chromosome inactivation pattern was observed in all fractions using both techniques in 2 patients (patients 1 and 2; Tables 4 and 5), and using DNA analysis only in 2 other patients (patients 32 and 33; Table 5). In 28 patients, clonality of the granulocyte fraction (patients 34 through 42; Table 4 and patients 33 through 41; Table 5) was observed in the presence of polyclonality of T lymphocytes. Among these patients, the clonality of platelets was shown in all cases informative for RNA markers (pa-tients 3 through 21; Table 4) (Fig 1). Interestingly, in 3

Table 1. DNA Genotyping: Primer Sequences, PCR Conditions, and Sizes of PCR Products

Gene	Primers	Sequence 5′→3′	Exon	Hybridization T°	Cycles No.	[MgCl₂] mmol/L	Size of PCR Product	Enzymes
IDS	IDS 3b	CCAAAGAAGG GAGGGTCCAC	Intron 3	48	35	1.5	151	*Hpa*II
	(sense)							
	IDS 4	AGACCAGCTAT ACGGAGAATCA **C***C	4					
	(antisense)							
P55	P55 DNAs	ATCTCAAATTC CTCCTCAAA	Intron 2	44	35	2.0	192	*Dde* I
	(sense)							
	P55 DNAas	TGTTTTTCATTC AGCTTC**T***G	3					
	(antisense)							
G6PD	G6PD-E10	GCTGGACCTGA CCTACGGCAAC A	10	59	35	3.0	183	*Pvu* I
	(sense)							
	G6PVPDPVU1	GAAGACGTCCA GGATGAGGCG **A***TC	11					
	(antisense)							

* Indicate the mismatched nucleotides introduced by the primer to create restriction sites.

additional patients, platelets were clonal despite polyclonality of all other hematopoietic cell fractions (patients 22 through 24; Table 4). Therefore, 31 patients had evidence of clonality in at least one hematopoietic lineage. In contrast, 11 patients had polyclonality of all studied lineages (patients 25 through 31; Table 4 and 43 through 46; Table 5), including platelets in 7 cases where RNA markers could be studied (patients 25 through 31; Table 5). An example of each clonality pattern is presented in Fig 2.

Normal controls with a nonrandom pattern of X-chromosome inactivation in total blood displayed the same pattern in all fractions (data not shown).

Comparison of clinical and biological features of ET patients with monoclonal and polyclonal hematopoiesis. We observed no correlation between the severity of the disease, as assessed by the prevalence of ischemic and hemorrhagic signs (Fisher's test, $P = .33$), the number of treated patients ($P = .28$), or the duration of the disease ($P = .09$) on the one hand, and the pattern of clonality on the other hand. The mean platelet count at diagnosis was higher in the group of patients with monoclonal hematopoiesis (mean = 1,070 × 10⁹/L; range 631 to 2, 160 × 10⁹/L) than in the group of patients with polyclonal hematopoiesis (mean = 845 × 10⁹/ L, range 600 to 1,098 × 10⁹ /L) ($P = .04$). We also found a significant difference in mean age between the patients with monoclonal hematopoiesis (56 years; range 16 to 80),

Table 2. RT-PCR Analysis of RNA Transcripts: Primer Sequences, PCR Conditions, and Sizes of PCR Products

Gene	Primers	Sequence 5′→3′	Exon	Hybridization T°	Cycles No.	[MgCl₂] mmol/L	Size of PCR Product	Enzymes
IDS	IDS 3a	TGTACGACTTCAACT CCTACTGGA	3	47	35	2.5	158	*Hpa*II
	(sense)							
	IDS	AGACCAGCTATACG GAGAATCA**C***C	4					
	(antisense)							
P55	P55 H	AGAGCCCATGGGAA TC**G***C	3-4	51	35	1.5	138	*Hha* I
	(sense)							
	P55 I	ACATTTGTGCCATTG ATTTCTAGG	4					
	(antisense)							
G6PD	G6PD-E10	GCTGGACCTGACCTA CGGCAACA	10	59	35	3.0	79	*Pvu* I
	(sense)							
	G6PVPDPVU1	GAAGACGTCCAGGA TGAGGCG**A***TC	11					
	(antisense)							

* Indicate the mismatched nucleotides introduced by the primer to create restriction sites.

Table 3. Comparison of the Results of X-Chromosome Inactivation Pattern Between RNA and DNA Markers in Total Blood

		RNA	
		Random	Nonrandom
Normal Controls (n = 34)			
DNA	Random	30	0
	Nonrandom	3	1

		RNA	
		Random	Nonrandom
Patients (n = 28)			
DNA	Random	5	2
	Nonrandom	0	21

Thirty-four normal controls and 28 patients were informative for the HUMARA polymorphism and at least one of the three RNA markers. The X-chromosome inactivation pattern was scored as random or nonrandom and the results obtained using RNA and DNA markers are compared.

and the patients with polyclonal hematopoiesis (37 years; range 24 to 76) (P = .01).

Interestingly, 3 ET patients with polyclonal hematopoiesis had clinical signs of MPD: splenomegaly had been present in patient 25 since disease onset. Severe peripheral vascular ischemia was present in patients 29 and 31 (aged 30 and 76 years, respectively). The other 4 patients in this group had no clinical signs of MPD. To search for familial thrombocytosis, the platelet counts of the parents, the 2 children of patients 29 and the 3 children of patient 30 were determined and were found to be normal.

DISCUSSION

Like other MPD, ET is thought to be a clonal disorder. In a previous study, we described a technique based on HUMARA CAG polymorphism. This technique permitted clonality analysis in 81% of females, but did not allow us to study the clonality of hematopoiesis in platelets. This was overcome by making use of techniques based on the detection of RNA transcripts. In addition to G6PD and P55 transcript analysis described by Prchal,[21,22] the study of IDS

Table 4. Clinical and Laboratory Findings in ET Patients and Results of Clonality Analysis Using mRNA Genes

No.	Age at Diagnosis	Follow-up Time	Treatment	C.S	Unfractionated Blood	T-Lymphocyte Fraction	Granulocyte Fraction	Platelets	Platelet Counts at Diagnosis $\times 10^9$/L
1*	36	53	Hydrea	+	MO	MO	MO	MO	1,100
2*	34	148	Hydrea	+	MO	MO	MO	MO	889
3*	37	77	Interferon	−	MO	PO	MO	MO	2,160
4*	80	18	Hydrea	+	MO	PO	MO	MO	812
5	21	1	0	−	MO	PO	MO	MO	1,886
6	50	57	Hydrea	+	MO	PO	MO	MO	1,700
7*	33	34	Hydrea	−	MO	PO	MO	MO	1,750
8*	65	49	Hydrea	−	MO	PO	MO	MO	803
9*	55	100	Hydrea	+	MO	PO	MO	MO	800
10	75	52	Hydrea	−	MO	PO	MO	MO	710
11	57	98	Hydrea	−	MO	PO	MO	MO	800
12	71	1	0	−	MO	PO	MO	MO	1,150
13	69	2	0	−	MO	PO	MO	MO	900
14	69	12	Hydrea	+	MO	PO	MO	MO	2,000
15	39	1	0	−	MO	PO	MO	MO	1,400
16	77	1	0	+	MO	PO	MO	MO	650
17	69	48	0	−	MO	PO	MO	MO	787
18*	65	38	Hydrea	−	MO	PO	MO	MO	745
19*	48	24	Hydrea	−	MO	PO	MO	MO	1,069
20	63	123	Hydrea	−	MO	PO	MO	MO	950
21*	59	120	Hydrea	−	MO	PO	MO	MO	768
22	38	1	0	−	PO	PO	PO	MO	674
23	63	1	0	+	PO	PO	PO	MO	1,200
24*	71	1	0	−	PO	PO	PO	MO	751
25	24	124	0	−	PO	PO	PO	PO	950
26*	25	59	0	−	PO	PO	PO	PO	751
27	25	336	0	−	PO	PO	PO	PO	780
28	43	132	0	−	PO	PO	PO	PO	600
29	30	85	Hydrea	+	PO	PO	PO	PO	648
30	37	108	Interferon	−	PO	PO	PO	PO	1,098
31	76	50	Hydrea	+	PO	PO	PO	PO	660

Follow-up (months) at the time of clonality analysis.

Abbreviations: C.S, ischemic or hemorrhagic symptoms; MO, monoclonal; PO, polyclonal; RNA genes, G6PD or IDS or P55.

* Patients reported in our previous study.[14]

Table 5. Clinical and Laboratory Findings in ET Patients and Results of Clonality Analysis Using the AR Gene

No.	Age at Diagnosis	Follow-up Time	Treatment	C.S	Unfractionated Blood	T-Lymphocyte Fraction	Granulocyte Fraction	Platelet Counts at Diagnosis $\times 10^9$/L
1*	36	53	Hydrea	+	MO	MO	MO	1,100
2*	34	148	Hydrea	+	MO	MO	MO	889
32	57	7	Hydrea	−	MO	MO	MO	1,300
33*	68	56	Hydrea	−	MO	MO	MO	962
34	70	48	0	+	MO	PO	MO	693
35*	46	12	Hydrea	+	MO	PO	MO	1,060
36	16	70	Hydrea	−	MO	PO	MO	907
37*	43	19	0	+	MO	PO	MO	1,500
38*	68	1	0	−	MO	PO	MO	843
39	42	2	0	+	MO	PO	MO	990
40*	63	36	Hydrea	−	MO	PO	MO	1,025
41*	47	42	Hydrea	+	MO	PO	MO	1,067
42*	61	84	Hydrea	−	MO	PO	MO	631
43	37	1	0	−	PO	PO	PO	880
44	16	2	0	−	PO	PO	PO	900
45	13	14	Interferon	−	PO	PO	PO	1,400
46*	65	94	Hydrea	−	PO	PO	PO	624

Follow-up (months) at the time of clonality analysis.

Abbreviations: C.S, ischemic or hemorrhagic symptoms; MO, monoclonal; PO, polyclonal; AR, androgen receptor gene.

* Patients reported in our previous study.[14]

polymorphic mRNAs permitted clonality analysis of platelets in 72% of females.

Because the X-chromosome inactivation phenomenon occurs in different tissues at different times during the embryonic period,[26] the clonality of hematopoiesis cannot be confirmed without finding polyclonality in one or more hematopoietic lineage, thus ruling out a skewed lyonization.[27] T cells appear to be the best choice as a control tissue

Fig 1. RT-PCR analysis expression of P55, IDS, and G6PD genes in patients with monoclonal hematopoiesis. RNA of heterozygous patients was submitted to RT-PCR. Amplified products were digested with *Hha* I, *Hpa*II and *Pvu* I, respectively, and analyzed by gel electrophoresis and ethidium bromide staining. Three to 24 correspond to the patient numbers in Table 4. U, undigested PCR products; P, platelets; T, T lymphocytes. Arrows indicate the size of the fragments in base pairs.

in myeloid malignancies like acute myeloid leukemia,[27] and we tested for their absence of involvement in ET. Our present results confirm that T lymphocytes derive from polyclonal hematopoiesis in most ET patients.[7,20] Although 88% of patients with a nonrandom X-chromosome inactivation pattern in granulocytes and/or platelets showed a random pattern in T lymphocytes, only 12% of them probably had skewed lyonization, as the same nonrandom pattern was observed in all hematopoietic lineages. However, as T cells and myeloid cells are derived from a common stem cell, we cannot formally exclude the involvement of pluripotent stem cells in these patients.

This study shows that the majority of patients with ET have monoclonal hematopoiesis (74%) detectable at least in platelets. The present data confirm and extend previous observations indicating a variable contribution of monoclonal hematopoiesis to different lineages.[14,18-20,22] Interestingly, in some patients, monoclonality of hematopoiesis is restricted to platelets, despite polyclonality in the other lineages. Surprisingly, a minority of patients with clinical and biological features of ET (n = 7) had polyclonal hematopoiesis in all cell lineages. Reactive thrombocytosis in these patients was formally excluded by the long clinical history of the disease (see Results). Although we cannnot formally exclude the possibility of a monoclonal cellular fraction mixed with cells from persistent normal hematopoiesis in these patients, visual inspection of two bands corresponding to amplified fragments derived from both alleles shows whether or not the allelic ratio differs between lineages (Fig 1). The absence of an allelic ratio variation between T lymphocytes and other lineages excludes the possibility that a significant proportion of clonal cells exists in the granulocyte or platelet fractions in these patients.

The variable involvement of different lineages in patients with monoclonal hematopoiesis could result from two possi-
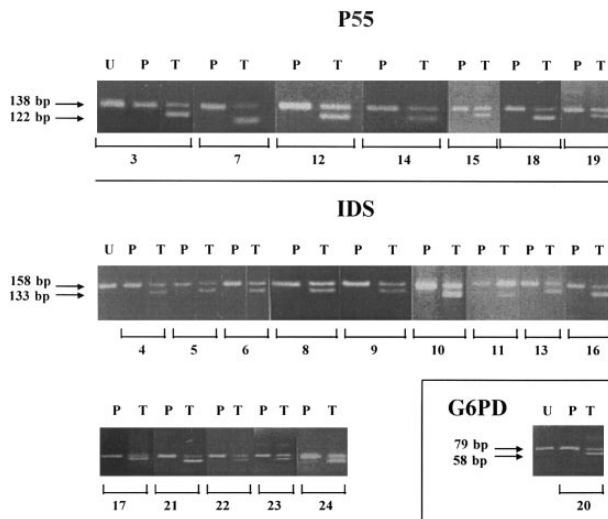
Fig 2. RT-PCR analysis of allelic expression of the P55 gene. RNA of heterozygous patients was submitted to RT-PCR. Amplified products were digested with *Hha* I and analyzed by gel electrophoresis and ethidium bromide staining. (A) Illustrates the results obtained from a patient with monoclonal hematopoiesis in platelets and granulocytes; (B) patient with monoclonal hematopoiesis only in platelets; (C) patient with polyclonal hematopoiesis; (D) patient with a nonrandom X-chromosome inactivation pattern. U, undigested PCR products; 1, platelets; 2, T lymphocytes; 3, granulocytes. Arrows indicate the size of the fragments in base pairs.
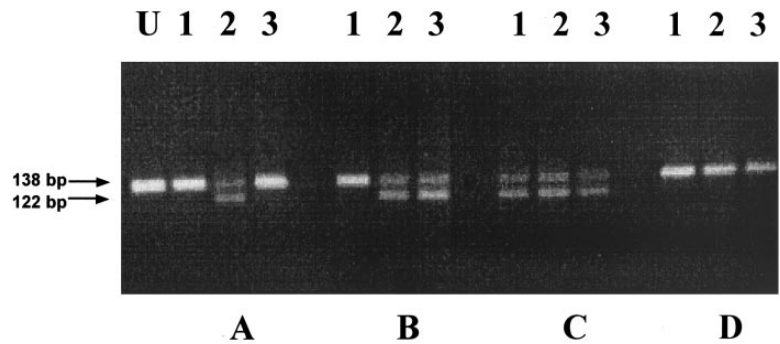
ble events: the transformation event may affect stem cells or progenitors at different levels, or the abnormal clone could have a variable selective advantage over normal hematopoiesis in the different patients.

Thrombocytosis describes any condition where there is a significant elevation of the platelet count, and occurs in a number of disorders. The most common forms are secondary or reactive thrombocytosis. Primary or essential thrombocythemia was considered to be a myeloproliferative and clonal disorder. However, like primary polycythemia, ''primary thrombocytosis,'' defined as ET, is probably a heterogeneous disease and may be classified in different subcategories on the basis of clonality analysis. ET patients with monoclonal hematopoiesis may have an acquired abnormality of hematopoietic progenitor cells resulting in an autonomous or enhanced response of megakaryocyte progenitors to normal growth factors, as described in polycythemia vera.[28] In contrast, in ET patients with polyclonal hematopoiesis, an inherited mutation enhancing the growth of the megakaryocytic lineage (in the thrombopoietin-receptor gene for example) or inappropriate secretion of thrombopoietin might be responsible for the increased platelet count.

Clonality analysis in granulocytes and/or in platelets may be useful for distinguishing between ET and reactive thrombocytosis, as the majority of patients have monoclonal hematopoiesis, whereas all patients with benign thrombocytosis had polyclonal hematopoiesis. However, ET cannot be ruled out in patients with polyclonal hematopoiesis, as some of them have the same clinical manifestations as patients with monoclonal hematopoiesis.

ACKNOWLEDGMENT

REFERENCES

1. Murphy S, Iland H, Rosenthal D, Laszlo J: Essential thrombocythemia: An interim report from the Polycythemia Vera Study Group. Blood 23:177, 1986

2. Dudley JM, Messinezy M, Eridani M, Holland LJ, Lawrie A, Nunan TO, Sawyer B, Savidge GF, Pearson TC: Primary thrombocythemia: Diagnosis criteria and a simple scoring system for positive diagnosis. Br J Haematol 71:331, 1989

3. Han ZC, Brière J, Abgrall JF, Sensèbe L, Nedellec G, Parent D, Guern G: Spontaneous formation of megakaryocyte progenitors (CFU-MK) in primary thrombocythaemia. Acta Haematol 78:51, 1987

4. Pearson TC: Primary thrombocythemia: Diagnosis and management. Br J Haematol 78:145, 1991

5. Lyon MF: Gene action in the X chromosome of the mouse. Nature 190:372, 1961

6. Fialkow PJ, Faguet GB, Jacobson RJ, Vaidya K, Murphy S: Evidence that essential thrombocythemia is a clonal disorder with origin in a multipotent stem cell. Blood 58:916, 1981

7. Gaetani GF, Ferraris AM, Galiano S, Giuntini P, Canepa L, Urso M: Primary thrombocythemia: Clonal origin of platelets, erythrocytes and granulocytes in A Gd^B/Gd^{Mediterranean} subjects. Blood 59:76, 1982

8. Raskind WH, Jacobson R, Murphy S, Adamson JW, Fialkow PJ: Evidence for the involvement of B lymphoid cells in polycythemia vera and essential thrombocythemia. J Clin Invest 75:1388, 1985

9. Vogelstein B, Fearon ER, Hamilton SR, Feinberg AP: Use of restriction fragment length polymorphisms to determine the clonal origin of human tumors. Science 227:642, 1985

10. Gilliland DG, Blanchard KL, Levy J, Perrin S, Bunn HF: Clonality in myeloproliferative disorders. Proc Natl Acad Sci USA 88:6848, 1991

11. Jinnai I, Yoshida S, Toyoda A, Murohashi I, Bessho M, Hirashima K, Nagai K, Tomonaga M, Boyd Y: A clonal study of hematopoiesis using the M27 b probe: Aberrant band patterns caused by incomplete digestion of a methyl-sensitive enzyme in the inactive X-chromosome. Leukemia 7:1432, 1993

12. Fey MF, Liechti-Gallati S, Von Rohr A, Borisch B, Theilkâs L, Schneider V, Oestreicher M, Nagel S, Ziemiecki A, Tobler A: Clonality and X-inactivation patterns in hematopoietic cell populations detected by highly informative M27b DNA probe. Blood 83:931, 1994

13. Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW: Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet 51:1229, 1992

14. El Kassar N, Hetet G, LI Y, Brière J, Grandchamp B: Clonal analysis of haematopoietic cells in essential thrombocythaemia. Br J Haematol 90:131, 1995

15. Ann K, Ito M, Misawa M, Ohe Y, Kai S, Kohsaki M, Hara H: Clonal analysis of peripheral blood and haemopoíetic colonies in patients with aplastic anaemia and refractory anaemia using the polymorphic short tandem repeat on the human androgen-receptor (HUMARA) gene. Br J Haematol 89:838, 1995

16. Lucas GS, Padua RA, Masters GS, Oscier DG, Jacobs A: The application of X-chromosome gene probes to the diagnosis of myeloproliferative disease. Br J Haematol 72:530, 1989

17. Anger B, Janssen JWG, Schrezenmeier H, Hehlmann R, Heimpel H, Bartram CR: Clonal analysis of chronic myeloproliferative disorders using X-linked DNA polymorphisms. Leukemia 4:258, 1990

18. Janssen JWG, Anger BR, Drexler HG, Bartram CR, Heimpel H: Essential thrombocythemia in two sisters originating from different stem cell levels. Blood 75:1633, 1990

19. Turhan AG, Cashman JD, Eaves CJ, Humphries RK, Eaves AC: Variable expression of features of normal and neoplastic stem cells in patients with thrombocytosis. Br J Haematol 82:50, 1992

20. Tsukamoto N, Morita K, Maehara T, Okamoto K, Sakai H: Clonality in chronic myeloproliferative disorders defined by X-chromosome linked probes: Demonstration of heterogeneity in lineage involvement. Br J Haematol 86:253, 1994

21. Prchal JT, Guan YL: A novel clonality assay based on transcriptional analysis of the active X-chromosome. Stem Cells 11:62, 1993

22. Prchal JT, Liu Y, Prchal JF, Hoffman R, Tushinski R: Are normal stem cells present in myeloproliferative syndrome? Blood 84:207, 1994 (abstr, suppl 1)

23. Bunge S, Steglich C, Zuther C, Beck M, Morris CP, Schwinger E, Schinzel A, Hopwood JH, Gal A: Iduronate-2-sulfatase gene mutations in 16 patients with mucopolysaccharidosis type II (Hunter syndrome). Hum Molec Genet 2:1871, 1993

24. Chomzynski P, Sacchi N: Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156, 1989

25. Delfau MH, Kerkaert JP, Collyn d'Hooghe G, Fenaux P, Lai JL, Jouet JP, Grandchamp B: Detection of minimal residual disease in chronic myeloid leukemia patients after bone marrow transplantation by polymerase chain reaction. Leukemia 4:1, 1990

26. Tan SS, Williams EA, Tam PPL: X-chromosome inactivation occurs at different times in different tissues of the post-implantation mouse embryon. Nature Genet 3:170, 1993

27. Gale R, Wheadon H, Boulos P, Linch DC: Tissue specificity of X-chromosome inactivation patterns. Blood 83:2899, 1994

28. Prchal JT, Prchal JF: Evolving understanding of cellular defect in polycythemia vera: Implications for its clinical diagnosis and molecular pathophysiology. Blood 83:1, 1994