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PROLIFERATION, DIFFERENTIATION, AND POSSIBLE RADIATION-INDUCED CHROMOSOME ABNORMALITIES IN CIRCULATING HEMOPOIETIC STEM CELLS

循環血中造血幹細胞の増殖，分化能及び放射線誘発染色体異常

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SUMMARY

The effects of atomic bomb radiation on hemopoietic stem cells were studied cytogenetically and from the aspect of differentiation and proliferation, using single colonies derived from human hemopoietic stem cells. The subjects studied were A-bomb survivors in the high dose exposure group (T65D 100+rad) with a high incidence (10% or more) of radiation-induced chromosome abnormalities in their peripheral lymphocytes, and their controls. Examinations were performed on 21 A-bomb survivors (10 males and 11 females) and 11 controls (5 males and 6 females).

Colony formation of hemopoietic stem cells (granulocyte/monocyte-colony-forming cells, GM-CFC and burst-forming unit-erythrocytes, BFU-E) was made by the methylcellulose method patterned after the methods of Iscove et al and Ogawa et al using 5-10 ml of peripheral blood. Chromosome specimens were prepared from single colonies by the micromethod which we have reported elsewhere.

要 約

ヒト造血幹細胞由来単一コロニーを用いて，造血幹細胞に対する原爆放射線の影響を細胞遺伝学並びに分化・増殖能の面から検討した．対象は，末梢血リンパ球に放射線誘発性の染色体異常を高率（10%以上）に有する高線量被爆者群（T65D 100rad 以上）とその対照群である．検討し得た対象例数は，被爆者群が21名（男10，女11），対照群が11名（男5，女6）である．

造血幹細胞（顆粒球/単球コロニー形成細胞，GM-CFC 及びburst 形成単位赤血球，BFU-E）のコロニー形成は末梢血 5～10ml を用いて，Iscove ら及び Ogawa らの方法に準じたメチルセルロース法によって行った．単一コロニーからの染色体標本の作製は，既に我々が報告した micromethod によった．

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The total number of colonies analyzed in the exposed group was 131 GM-CFC and 75 BFU-E. Chromosome abnormalities were observed in 15 (11.5%) and 9 (12.0%) colonies, respectively. In the control group, the total number of colonies analyzed was 61 GM-CFC and 41 BFU-E, but none of the colonies showed chromosome abnormalities. A highly significant difference in chromosome abnormalities was demonstrated by an exact test with a probability of 0.3% for GM-CFC and 1.7% for BFU-E. The karyotypes of chromosome abnormalities obtained from the colonies of hemopoietic stem cells in the exposed group were mostly translocations, but deletion and marker chromosomes were also observed. In two individuals, such karyotypic abnormalities as observed in the peripheral lymphocytes were seen also in the hemopoietic precursor cells. This finding suggests that radiation may produce an effect even on relatively undifferentiated hemopoietic stem cells.

INTRODUCTION

Longitudinal cytogenetic studies of peripheral lymphocytes in A-bomb survivors have demonstrated radiation-induced chromosome abnormalities even after more than 30 years.[1] Some cases have even shown clone formation. Radiation-induced chromosome abnormalities have also been observed in bone marrow cells of A-bomb survivors.[2-4] These findings suggest the possibility that radiation-induced chromosome abnormalities and abnormal proliferation and differentiation continue to be present in the hemopoietic stem cells of A-bomb survivors.

Recently, with the advancement of studies on hemopoietic stem cells, it has become possible to identify GM-CFC and BFU-E in the peripheral blood. GM-CFC and BFU-E are hemopoietic precursors of granulocyte/monocytes and erythrocytes, respectively. Each of these colonies observed in in vitro hemopoietic cell colony formation represents a group of cells derived from a single hemopoietic stem cell.[5,6] Peripheral lymphocytes may possibly be derived from lymphoid stem cells as well. It is still unknown whether there exist in human adults totipotential stem cells which are the common stem cells of lymphoid, granuloid, erythroid, and megakaryocytic cells.[7-9] If radiation-induced chromosome aberrations as observed in peripheral lymphocytes are derived from abnormalities in such totipotential stem cells, the same abnormalities may possibly be detected in myeloid cells.

被爆者群で解析し得たコロニー数はGM-CFCで延べ131，BFU-Eで延べ75であり，そのうち染色体異常が認められたのは各々15(11.5%)及び9(12.0%)であった．一方，対照群では解析し得たコロニー数はGM-CFCで延べ61，BFU-Eで延べ41であったが，染色体異常はいずれのコロニーにも認められなかった．精密なテストにより染色体異常にGM-CFCで0.3%，BFU-Eで1.7%の確率で極めて有意な差が認められた．被爆者群の造血幹細胞コロニーで得られた染色体異常の核型の多くは転座型であったが，その他に欠失，マーカー染色体も観察された．また2個体において，造血前駆細胞に末梢血リンパ球にみられたような核型異常が観察された．この事実は放射線の影響がより未分化な造血幹細胞にも及んでいることを示唆している．

緒　言

原爆被爆者の末梢血リンパ球における経時的な細胞遺伝学的調査によると，放射線誘発性の染色体異常が30数年を経てもなお観察されている．[1] しかもその中には，クローンを形成している例もみられる．同様に被爆者の骨髄細胞にもまた，原爆放射線誘発性の染色体異常が認められる．[2-4] これらの事実は，原爆被爆者の造血幹細胞において放射線誘発性の染色体異常や，増殖・分化能における異常が存続している可能性を示唆している．

近年，造血幹細胞の研究が進歩し，顆粒球/単球や赤血球の造血前駆細胞であるGM-CFCやBFU-Eの末梢血中での同定も可能となった．このin vitroでの造血コロニー形成法でみられる個々のコロニーは，それぞれ単一造血幹細胞に由来する子孫としての細胞集団を表している．[5,6] 一方，末梢血リンパ球にもリンパ球系幹細胞が考えられている．リンパ球系，顆粒球系，赤血球系及び巨核球系細胞の共通の幹細胞であるtotipotential stem cellがヒト成人にも存在するかどうかは，未解決の問題である．[7-9] もしも末梢血リンパ球にみられる放射線誘発性の染色体異常が，このようなtotipotential stem cellに生じた異常に由来するならば，骨髄系細胞にも同一の異常が認められると考えられる．

Further, it is possible that such chromosome aberrations have a role in the developmental mechanism of leukemia and other related diseases and also in other late radiation effects. In this respect, it is important to elucidate the behavior of hemopoietic stem cells with chromosome aberrations. The present study was conducted in an attempt to ascertain these possibilities using a newly developed in vitro method of hemopoietic cell colony formation and a method of preparation of chromosome specimens from single colonies.

MATERIALS AND METHODS

The subjects consisted of high dose A-bomb survivors selected from the Adult Health Study sample who were under 20 years of age at the time of the bomb (ATB), whose estimated radiation dose (T65D) was 100 rad or more and whose chromosome study by culture of phytohemagglutinin (PHA)-stimulated peripheral leukocytes disclosed a high percentage (10% or more) of chromosome aberrations, and their controls. The controls were those in the same age-group, whose estimated dose was 0 rad and whose percentage of chromosome aberrations was 0%-1%. Examined in this study were a total of 39 individuals comprising of 16 males and 23 females. Mononuclear cells were collected by Ficoll-Metrizoate's density gradient centrifugation method from 5-10 ml of heparinized peripheral blood. The mononuclear cells were washed three times with alpha-medium (Flow Laboratories).

Formation of Colonies

The methylcellulose method, patterned after Iscove et al[10] was used for formation of GM-CFC-derived colonies. To obtain $4\text{-}5\times10^5$ cells/ml in the final concentration, peripheral blood mononuclear cells were mixed with media containing 20% fetal calf serum (FCS, Hyclone, Sterile System Inc., Logan, UT), 0.88% methylcellulose, and 10% giant cell tumor-conditioned medium (GCT-CM, GIBCO), and 1 ml of the mixed solution was dispensed to four to eight plastic petri dishes (Lux).

For formation of BFU-E-derived colonies, the methylcellulose method patterned after Ogawa et al[11] was used. Cell suspensions were mixed with media containing 30% FCS, 1% bovine serum albumin (Sigma Chemical Co., St. Louis, MO), 2 U/ml erythropoietin (EPO; Step III,

更に，このような染色体異常は白血病やその関連疾患の発生機構や，その他の放射線晩発効果に対し何らかの役割を果たしている可能性がある．これらの点を見極めるために，染色体異常を有する造血幹細胞がいかなる動向を示すかを明らかにすることは重要である．本研究では，新しく開発された in vitro での造血コロニー形成法並びに単一コロニーからの染色体標本作製法を用いて，これらの可能性の解明を試みた．

材料及び方法

対象は成人健康調査集団から選定した高線量被爆者群とその対照群からなる．前者は被爆時年齢が20歳未満で，推定被曝線量（T65D）が 100 rad 以上，植物性赤血球凝集素（PHA）添加末梢血白血球培養の染色体検査での異常頻度が10%以上の者とした．後者は同じ年齢群で推定被曝線量は 0 rad，末梢血における染色体の異常率が 0 ～ 1 %の者である．今回検討された対象例数は39名で，そのうち男性は16名，女性は23名である．方法は，被検者より得られたヘパリン処理末梢血液 5 ～10ml から，Ficoll-Metrizoate 法による比重遠心法により単核細胞を集めた．Alpha-medium（Flow 研究所）で 3 回洗った後，細胞浮遊液とした．

コロニー形成法

GM-CFC 由来のコロニー形成は Iscove ら[10] の方法に準じたメチルセルロース法で行った．終濃度がそれぞれ 4 ～5 ×10^5 個細胞/ml となるように，末梢血単核細胞に20%胎牛血清（FCS; Hyclone, Sterile System 社，Logan, UT），0.88% メチルセルロース及び 10% giant cell tumor-conditioned medium（GCT-CM, GIBCO 社）を調製混合し，その混合液 1 ml ずつをプラスチックペトリ皿（Lux）4 ～8 個へ分注した．

BFU-E 由来のコロニー形成は Ogawa ら[11] の方法に準じたメチルセルロース法で行った．同様に終濃度がそれぞれ 4 ～5 ×10^5 個細胞/ml，30% FCS，1 % 牛血清アルブミン（BSA; Sigma Chemical 社，St. Louis, MO），エリスロポエチン（Epo; Step Ⅲ，

Connaught Laboratories, Toronto, Canada), and 0.88% methylcellulose to obtain $4\text{-}5\times10^5$ cells/ml in the final concentration, and 1 ml of the mixed solution was dispensed to plastic petri dishes. The mixture was cultured in a 5% CO_2 incubator with adequate humidity at 37°C for 7-14 days. Chromosome specimens were prepared from the colonies obtained.

Preparation of Chromosome Specimens from Single Colonies

Chromosome specimens were prepared on day 9-10 of culture for granulocytic colonies and on day 12-13 for erythroid colonies. Our own technique[12] modified from the methods of Rajendra et al[13] and Dube et al[14] was used. A volume of 0.1 ml Colcemid (0.1 μg/ml) was added to each of the culture dishes and cultured for a further 1-2 hours. Single colonies were collected with a micropipette under an inverted microscope. The cells were mixed well with 0.075 M KCl (10 μl) on a slide coated with 0.1% (W/V) poly-L-lysine (Sigma, P1524). The slide was immediately turned over and subjected to hypotonic treatment at 37°C in a wet chamber for 25 minutes. The slide was then turned upright, allowed to stand for 15 minutes, and then fixed. After adding a drop of 30% fixing solution (methanol : acetic acid, 3:1), the slide was allowed to stand for a further five minutes. After adding three drops of 20% ethanol and being allowed to stand for 10 minutes, the slide was immersed in 100% fixing solution for 10 minutes, immediately dried over an open flame and stained by the coventional Giemsa method.

Chromosome Study by Culture of PHA-stimulated Peripheral Leukocytes

Chromosome specimens were prepared by the conventional peripheral leukocyte (T lymphocyte) culture method[1] patterned after Moorhead et al.[15]

RESULTS

Of the 39 cases, 4 cases were excluded for bacterial pollution, 2 cases for absence of analyzable metaphase, and 1 case for absence of growth of GM-CFC and BFU-E. Therefore, the subjects studied were a total of 32 individuals of whom 21 were high dose survivors (10 males and 11 females) and 11 controls (5 males and 6 females). The number of chromosome specimens prepared from colonies of hemopoietic stem cells in these 32 cases varied with the

Connaught 研究所, Toronto, Canada) 2U/ml 及び 0.88% メチルセルロースとなるように調製混合し, 1ml ずつ分注した. これらを 37°C, 5% CO_2 で, 十分湿潤にした培養器内で7～14日間培養し, 得られたコロニーから染色体標本を作製した.

単一コロニーからの染色体標本の作製

標本の作製は顆粒球系コロニーでは培養9－10日目, 赤血球系コロニーでは12－13日目で行った. 方法は Rajendra ら,[13] Dube ら[14] の方法を改変した我々独自の技法[12] で行った. すなわち, あらかじめ各皿に 0.1ml コルセミド液 (0.1μg/ml) を加え, 更に1～2時間培養した. その後, 倒立顕微鏡下にマイクロピペットで個々のコロニーを採取した. 細胞は0.1% (W/V) poly-L-lysine (Sigma 社, P1524) 処理スライド上で 0.075M KCl (10μl) とよく混和した後, そのスライドを直ちに反転し, 37°C の湿潤箱中で25分間低張処理を行った. その後スライドを元に戻し15分間静置後, 固定処理を行った. 30%固定液 (メタノール：酢酸, 3：1) を1滴加え5分間静置した. その後20%エタノールを3滴加えて10分間静置後, スライドを 100% 固定液中へ10分間浸した. 取り出して後, 直ちに引火乾燥を行った. 染色は通常のギムザ法によった.

PHA 添加末梢血白血球培養の染色体検査

Moorhead ら[15] の方法に準じた通常の末梢血白血球 (Tリンパ球) 培養法[1] によって染色体標本を作成した.

結　果

39例のうち, 4例は培地感染のため, 2例は分析可能な中期核板がないため, 1例は GM-CFC 及び BFU-E が成長していないため除外した. したがって, 今回検討された被検者数は総計32名である. その内訳は高線量被爆者群が21名 (男10, 女11), 対照群が11名 (男5, 女6) であった. 32例の造血幹細胞コロニーからの染色体標本の作製枚数は, 採血量,

volume of blood drawn, the number of mononuclear cells used for implantation, and the rate of colony formation. The range of variation was 18-140 (mean 59) for GM-CFC and 0-161 (mean 50) for BFU-E. Of these, analyzable chromosome specimens (colonies) were examined, the results of which are presented hereunder.

In the 21 cases of the high dose group, the number of colonies available for chromosome analysis was a total of 131 for GM-CFC and 75 for BFU-E. Distinct chromosome aberrations were observed in 15 colonies (11.5%) of the former and in 9 colonies (12.0%) of the latter (Table 1).

植え込みに用いた単核細胞数並びにコロニー形成率の違いにより様々であった．その範囲はGM-CFCで18～140（平均59），BFU-Eで0～161（平均50）であった．このうち，解析可能であった染色体標本（すなわちコロニー）の検討結果について以下に示す．

高線量被爆者群21例で，染色体分析が可能であったコロニーの数はGM-CFCで延べ131個，BFU-Eで延べ75個であった．そのうち明らかな染色体異常が認められたコロニーの数は，前者で15個（11.5%），後者で9個（12.0%）であった（表1）．

TABLE 1 CHROMOSOME ANALYSIS OF HEMOPOIETIC COLONIES IN HIGH DOSE GROUP: FREQUENCY OF CHROMOSOME ABERRATIONS

表1　高線量被爆者群における造血コロニーの染色体分析：異常核型の出現頻度

Case	Sex	Age (yrs)	T65D in rad	Frequency of chromosome aberrations			
				PHA-stimulated leukocytes (%)	GM-CFC	BFU-E	Total frequency in stem cells
1	F	49	1107	38	1/5	0/1	1/6
2	F	57	787	44	0/0	3/5	3/5
3	F	39	710	20	1/10	0/4	1/14
4	F	48	673	40	1/3	1/1	2/4
5	M	52	526	28	1/12	2/6	3/18
6	M	44	521	26	0/3	0/0	0/3
7	F	54	477	38	2/8	0/9	2/17
8	F	49	423	10	0/1	0/1	0/2
9	F	56	416	23	2/4	0/2	2/6
10	M	40	390	23	0/2	0/9	0/11
11	M	45	329	39	1/1	0/0	1/1
12	M	51	322	16	1/6	0/0	1/6
13	M	53	284	22	0/8	0/5	0/13
14	F	52	281	14	0/17	0/2	0/19
15	M	55	264	15	1/3	0/0	1/3
16	F	47	264	10	0/4	1/5	1/9
17	F	52	255	15	1/7	1/6	2/13
18	F	53	255	19	2/20	1/4	3/24
19	M	52	228	12	0/0	0/12	0/12
20	M	51	220	11	0/0	0/1	0/1
21	M	54	163	13	1/17	0/2	1/19
				Total	15/131	9/75	24/206
					11.5%	12.0%	11.7%

In the 11 controls, no distinct chromosome aberrations were found in the chromosome analysis of 61 GM-CFC or 41 BFU-E colonies (Table 2). A highly significant relationship between the two groups was observed by an exact test for GM-CFC (P=0.003) and BFU-E (P=0.017).

対照群11例のGM-CFCにおいて，延べ61個のコロニーについて染色体分析を行い，またBFU-Eでは延べ41個のコロニーについて染色体分析を行ったが，いずれのコロニーからも明らかな染色体異常は認められなかった（表2）．GM-CFC（P＝0.003）及びBFU-E（P＝0.017）の精密なテストでは，両群間に極めて有意な関係が観察された．

TABLE 2 CHROMOSOME ANALYSIS OF HEMOPOIETIC COLONIES IN CONTROL GROUP: FREQUENCY OF CHROMOSOME ABERRATIONS

表2 対照群における造血コロニーの染色体分析：異常核型の出現頻度

Case	Sex	Age (yrs)	Frequency of chromosome aberrations		
			GM-CFC	BFU-E	Total frequency in stem cells
22	M	54	0/6	0/2	0/8
23	M	53	0/24	0/3	0/27
24	M	53	0/0	0/1	0/1
25	F	57	0/5	0/4	0/9
26	M	39	0/0	0/7	0/7
27	F	51	0/1	0/5	0/6
28	F	48	0/14	0/12	0/26
29	F	56	0/5	0/0	0/5
30	M	50	0/2	0/5	0/7
31	F	50	0/3	0/0	0/3
32	F	53	0/1	0/0	0/1
		Total	0/61 0%	0/41 0%	0/102 0%

Most of the abnormal karyotypes observed in hemopoietic stem cell colonies were confirmed in at least two or more metaphases per colony. All the karyotypes analyzed in colonies with abnormal karyotype showed the same karyotypic abnormalities as in individual colonies, except for numerical abnormalities probably due to random loss. The results of analysis of two colonies wherein many karyotypes were obtained are shown in Table 3 and Figure 1. A total of 24 karyotypic abnormalities in GM-CFC and BFU-E were obtained. These karyotypic abnormalities were mostly translocation, but deletion and marker chromosome were also found (Table 4). Whether the karyotype showing hypodiploid chromosome number was clonal or artifact could not be confirmed except in one colony with two analyzable metaphases (Figure 2a and b).

造血幹細胞コロニーで認めた異常核型の大部分は，少なくとも1コロニーあたり2個以上の中期核板について確認を行った．任意欠失によると思われる数の異常を除いては，異常な核型をもつコロニーにおける核型で分析したものは，すべて同じ核型異常を示した．多くの核型を得た2個のコロニーの分析結果を表3及び図1に示す．GM-CFC及びBFU-Eで合わせて24個の核型異常が得られた．その核型異常の多くは転座型であり，その他に欠失，マーカー染色体なども認められた（表4）．低倍数体染色体数を示す核型がクローン性か人工的欠落かについては，2個の分析可能な中期核板をもつ1個のコロニーを除き確認できなかった（図2a及びb）．

TABLE 3 CHROMOSOMAL ANALYSIS OF TWO COLONIES WITH MANY ANALYZABLE METAPHASES
表3 分析可能な多くの中期核板を有する二つのコロニーの染色分析

1. A granulocyte-macrophage colony with eight analyzable metaphases derived from GM-CFC of Case 1.

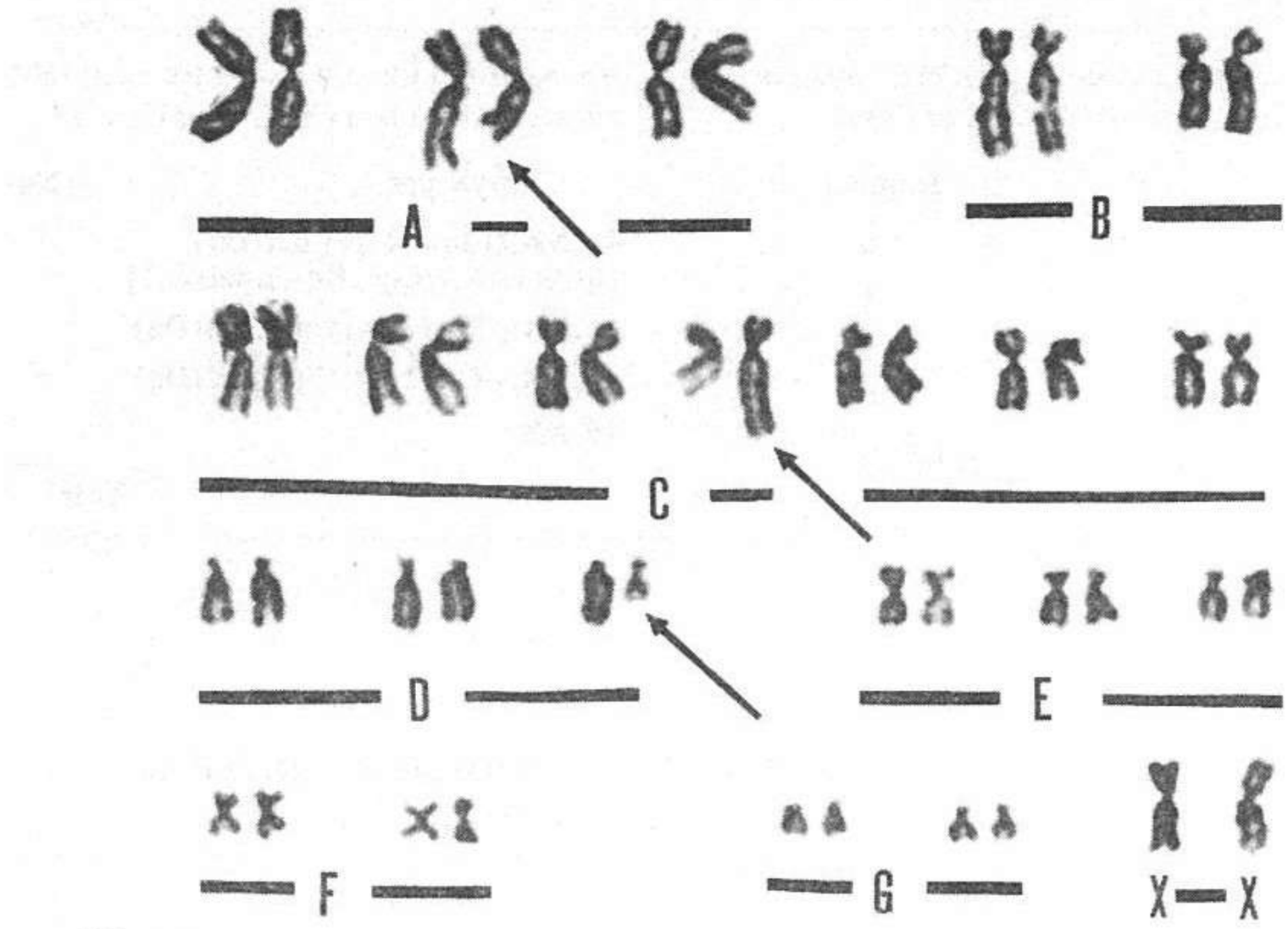
Karyotype	Number
46,XX,t(2q+;3q−)	6
45,XX,−C,t(2q+;3q−)	1
44,XX,−C,−E,t(2q+;3q−)	1
46,XX	0
Total	8

2. An erythroid burst with twelve analyzable metaphases derived from BFU-E of Case 17.

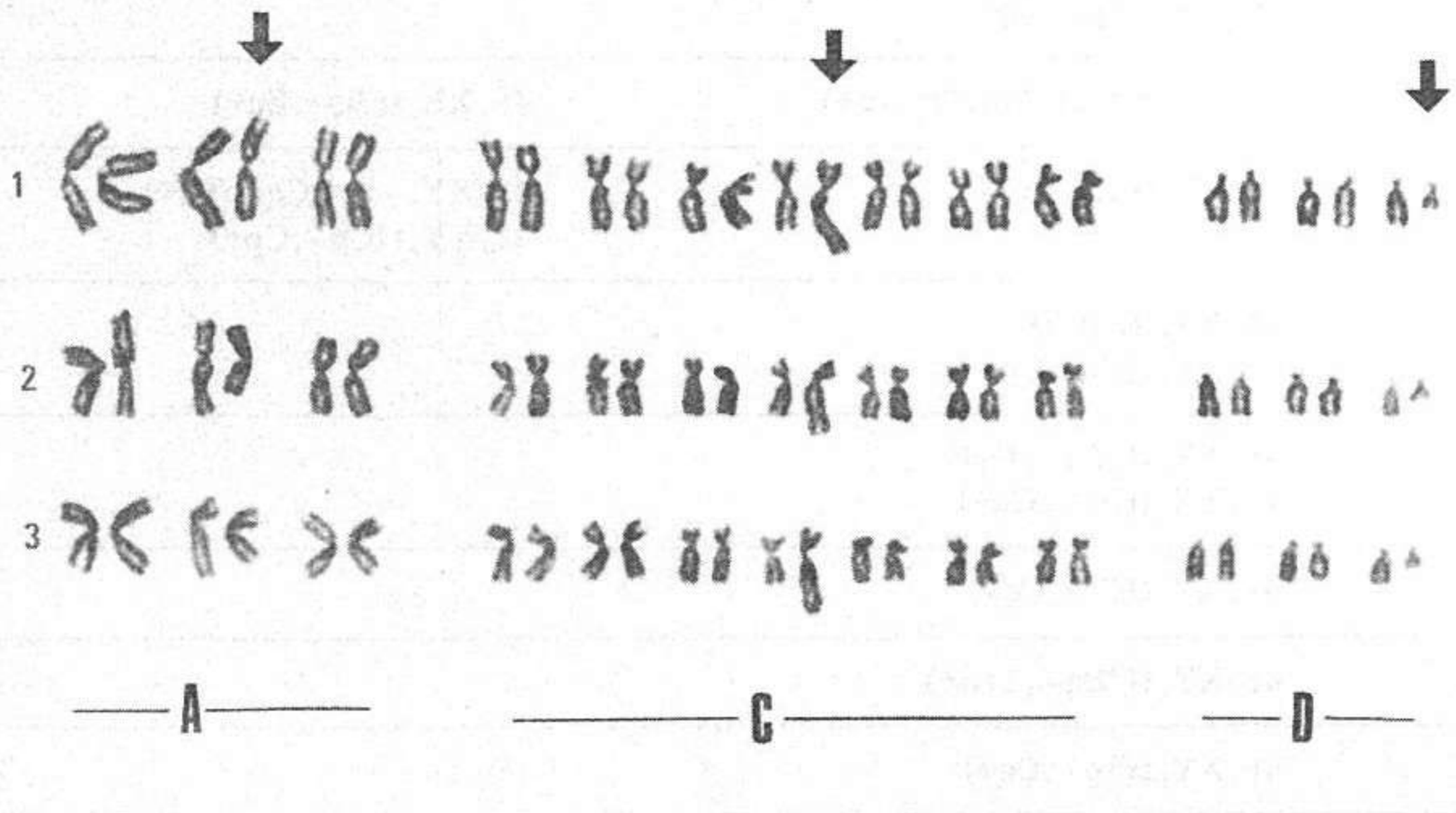
Karyotype	Number
46,XX,t(2q−;Cq+),del(Dq) [or 46,XX,t(Cq+;Dq−),del(2q)]	9
45,XX,−E,t(2q−;Cq+),del(Dq)	2
45,XX,−C,t(2q−;Cq+),del(Dq)	1
46,XX	0
Total	12

TABLE 4 ABNORMAL KARYOTYPES SEEN IN HEMOPOIETIC COLONIES
表4 造血コロニーでみられた異常核型

Case	GM-CFC	BFU-E
1	46, XX, t(2q+;3q−)	
2		46, XX, t(3q+;Cq−) 45, XX, −C, t(Cq−;Cq+) 45, XX, −C, −C, t(Cp+;Cq−),+marker
3	46, XX, t(3p−;Cq+)	
4	44, XX, −2, −C, ?inv(2p−q+)	46, XX, t(3q−;Eq+)
5	46, XY, t(3q−;Bq−)	45, XY, −B, t(Cq−;Eq+) 46, XY, t(Cp−;Cp+)
7	46, XX, del(Cq) 46, XX, t(Cp+;Cp−)	
9	46, XX, t(16q−;Fq+) 46, XX, t(Bq−;Gq+)	
11	46, XY, t(Cq+;Cq+)	
12	46, XY, t(?Bq−;17q+)	
15	46, XY, t(3p−;Cq+)	
16		46, XX, del(Bp)
17	46, XX, t(1p+;2p−)	46, XX, t(2q−;Cq+), del(Dq) [or 46, XX, t(Cq+;Dq−), del(2q)]
18	46, XX, t(Cq−;17q+) 46, XX, t(3q−;Cq+)	46, XX, t(3p−;Cq+)
21	45, XY, −F, t(Bq−;Cq+), t(Bp+;Fp−)	



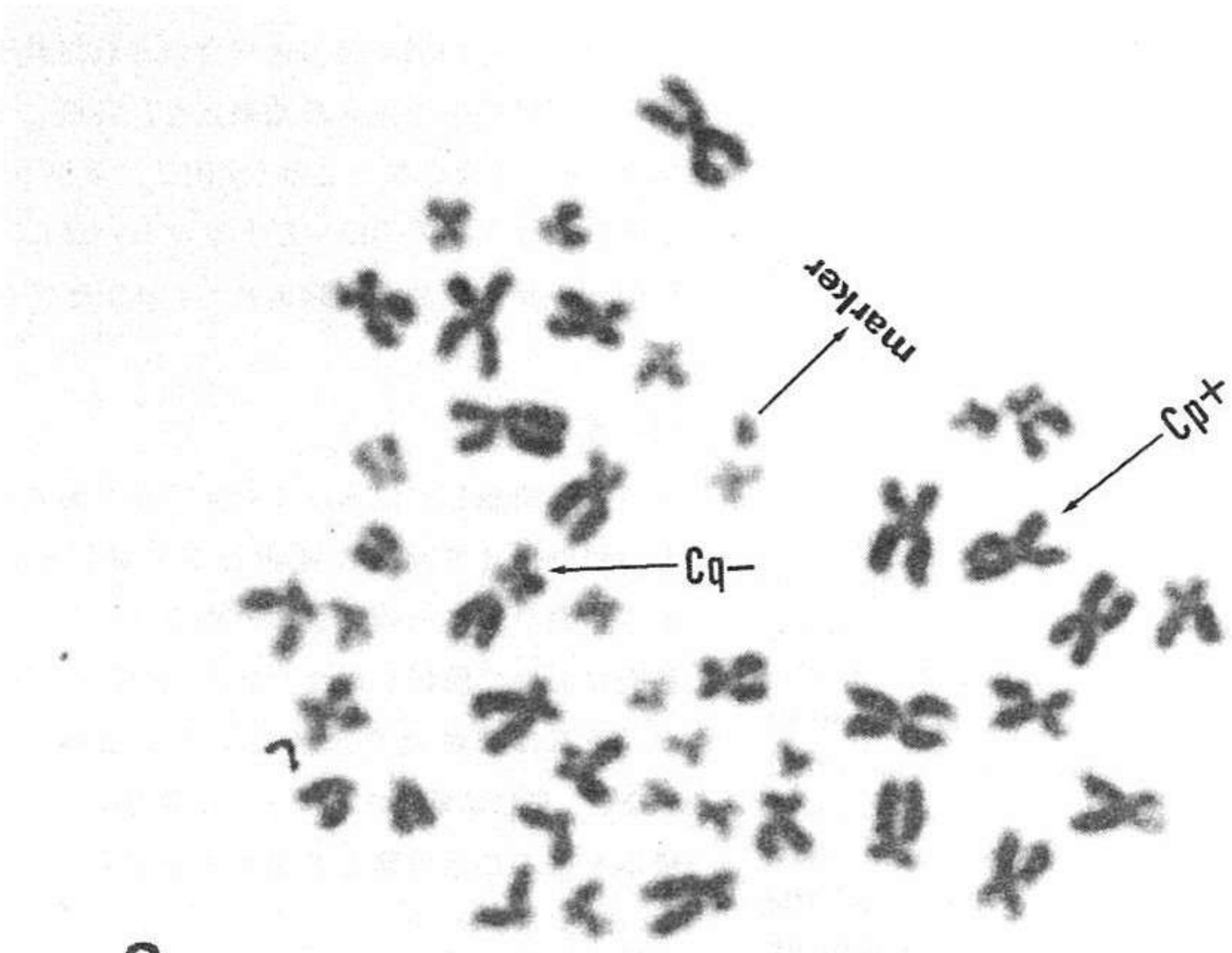
a



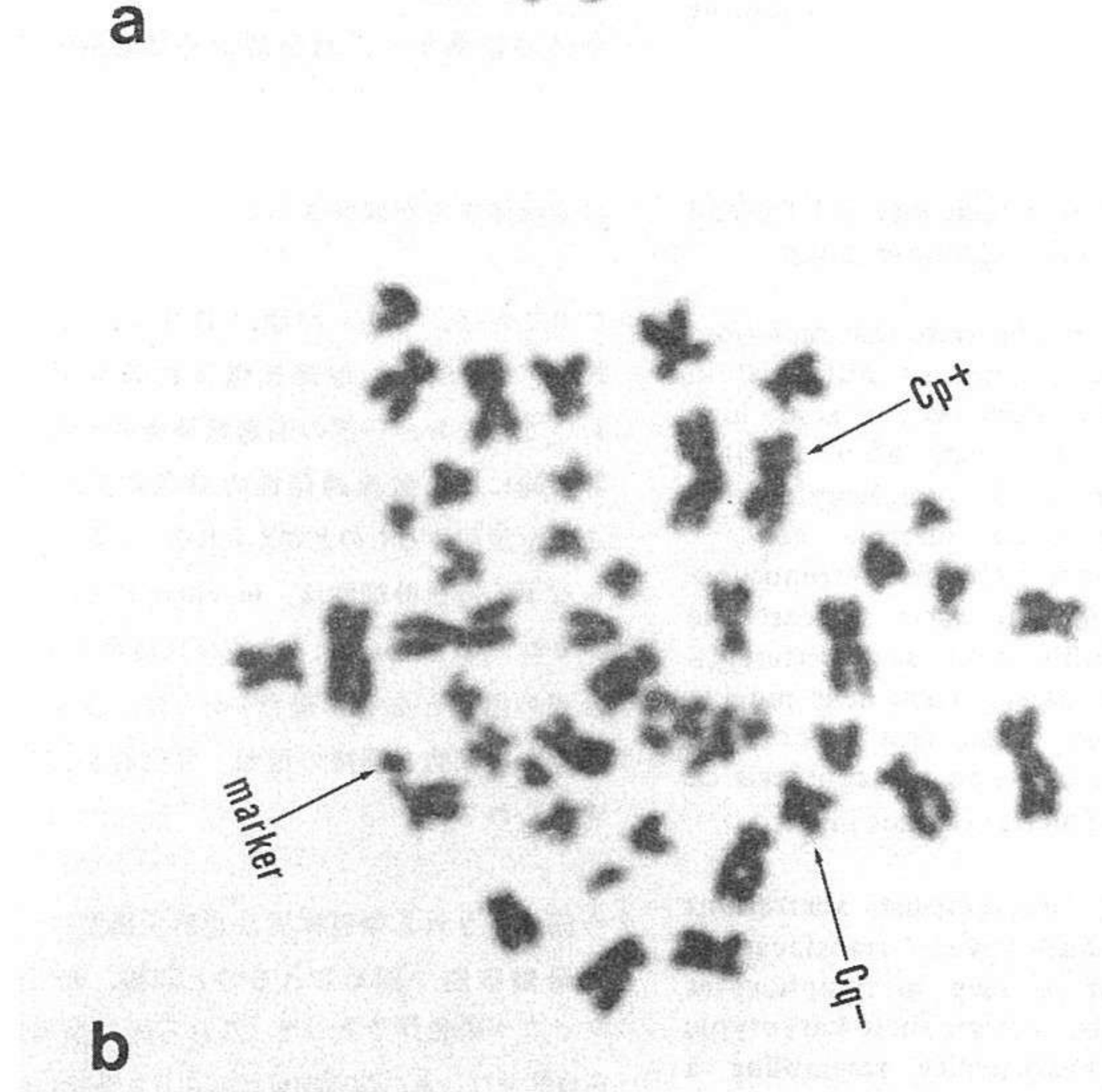
b

Figure 1. One complete karyotype (a) and three partial karyotypes (b) obtained from an erythroid burst of Case 17 (female). These show the identical chromosome aberration, t(2q−;Cq+), del(Dq) [or t(Cq+;Dq−),del(2q)].

図1　症例17(女)の赤血球系破裂で得られた一つの核型(a)及び三つの部分核型(b)．これはt(2q−；Cq+)，del(Dq)又はt(Cq+；Dq−)，del(2q)の染色体異常と同じものであることを示す．



a



b

Figure 2. Two different metaphases seen in an erythroid burst of Case 2 (female). Both (a) and (b) represent 45, XX, −C, −C, t (Cp+; Cq−), +marker.

図２　症例２（女）の赤血球系破裂でみられる二つの異なる中期核板．（a），（b）いずれも45，XX，−C，−C，t（Cp＋；Cq−），＋微小染色体，を示す．

The abnormal chromosome karyotypes in hemopoietic stem cells obtained in this study were compared for each case with the results of chromosome analysis of T lymphocytes obtained through the culture of PHA-stimulated peripheral leukocytes that had been conducted periodically. As a result, karyotypic abnormalities found in a pair of BFU-E and peripheral T lymphocyte closely resembled each other in two cases (Cases 2 and 5, Figures 3 and 4).

DISCUSSION

Since the commencement of this study, chromosome analyses were conducted on hemopoietic colonies derived from circulating hemopoietic stem cells for a total of 32 individuals of the high dose group and their controls. The number of colonies analyzable in each case was small owing to the small quantity of blood drawn, the small number of hemopoietic stem cells in the peripheral blood, and moreover to the difficulty in obtaining analyzable chromosome specimens from single colonies.

While no chromosome aberrations were found in the control group, they were observed in GM-CFC and BFU-E at a high rate (11.5% and 12.0%, respectively) in the high dose group.

This is considered to demonstrate that radiation-induced chromosome aberrations still exist in circulating hemopoietic stem cells of some high dose survivors even 40 years after A-bomb exposure as evident in T lymphocytes,[1] B lymphocytes,[16] and bone marrow cells.[2–4] These hemopoietic stem cells with chromosome aberrations are thought to have at least the same ability of proliferation and differentiation as normal hemopoietic stem cells judging from the morphology, size, and color tone (reddish brown as an index to the synthesis of hemoglobin) of hemopoietic colonies in vitro.

The karyotypes of chromosome aberrations analyzed in this study were translocation, deletion, and marker as seen in lymphocytes and bone marrow cells. Among these karyotypic abnormalities, one abnormality resembling a typical clonal chromosome aberration as seen in leukemia and its related diseases[17] was observed (Case 7, 46,XX,del(Cq)). Case 7 had also revealed chromosome aberration 46,XX,del(7q) twice (1976 and 1981) by the direct bone marrow method that had been conducted periodically.[18]

今回造血幹細胞から得られた染色体異常核型と，経年的に行っているPHA添加末梢血白血球培養から得たTリンパ球の染色体分析結果とを，各例につき比較検討した．その結果，2例（症例2，症例5）においてそれぞれ1組のBFU-EとTリンパ球に，極めて類似する核型異常が観察された（図3及び4）．

考　察

本研究を開始して以来，これまでに高線量被爆者群と対照群併せて32名の循環血中造血幹細胞に由来する造血コロニーの染色体分析を行うことができた．各例において解析し得たコロニー数はわずかであったが，それは採血量や末梢血中の造血幹細胞の数の少なさ，更には単一コロニーから染色体分析が可能な標本を得ることの難しさなどに基づくものであった．

今回の結果から，対照群からは染色体異常が見いだせなかったのに対し，高線量被爆者群からはGM-CFC及びBFU-Eからそれぞれ高率（11.5%，12.0%）に染色体異常が観察された．

このことは，Tリンパ球，[1] Bリンパ球[16]及び骨髄細胞[2-4]と同様，原爆被爆後約40年を経た現在においてもなお，一部の高線量被爆者の循環血中造血幹細胞には放射線誘発性の染色体異常が存在することを証明するものと考えられる．これら染色体異常を有する造血幹細胞は，in vitroにおけるその造血コロニーの形態，大きさ並びに色調（ヘモグロビン合成の指標となる赤褐色）からは，少なくとも健常の造血幹細胞と同様の増殖・分化能を有していると考えられる．

今回解析された染色体異常を示す核型は，リンパ球や骨髄細胞で認められるのと同様，転座型，欠失，マーカー染色体であった．これらの核型異常のうち，白血病並びにその関連疾患にみられる特異的なクローン性染色体異常[17]と類似するものを1個（症例7，46, XX, del(Cq)）認めた．更に症例7では，経年的に行っている骨髄直接法によって2回にわたり（1976年，1981年）染色体異常，46, XX, del(7q)を示した．[18]

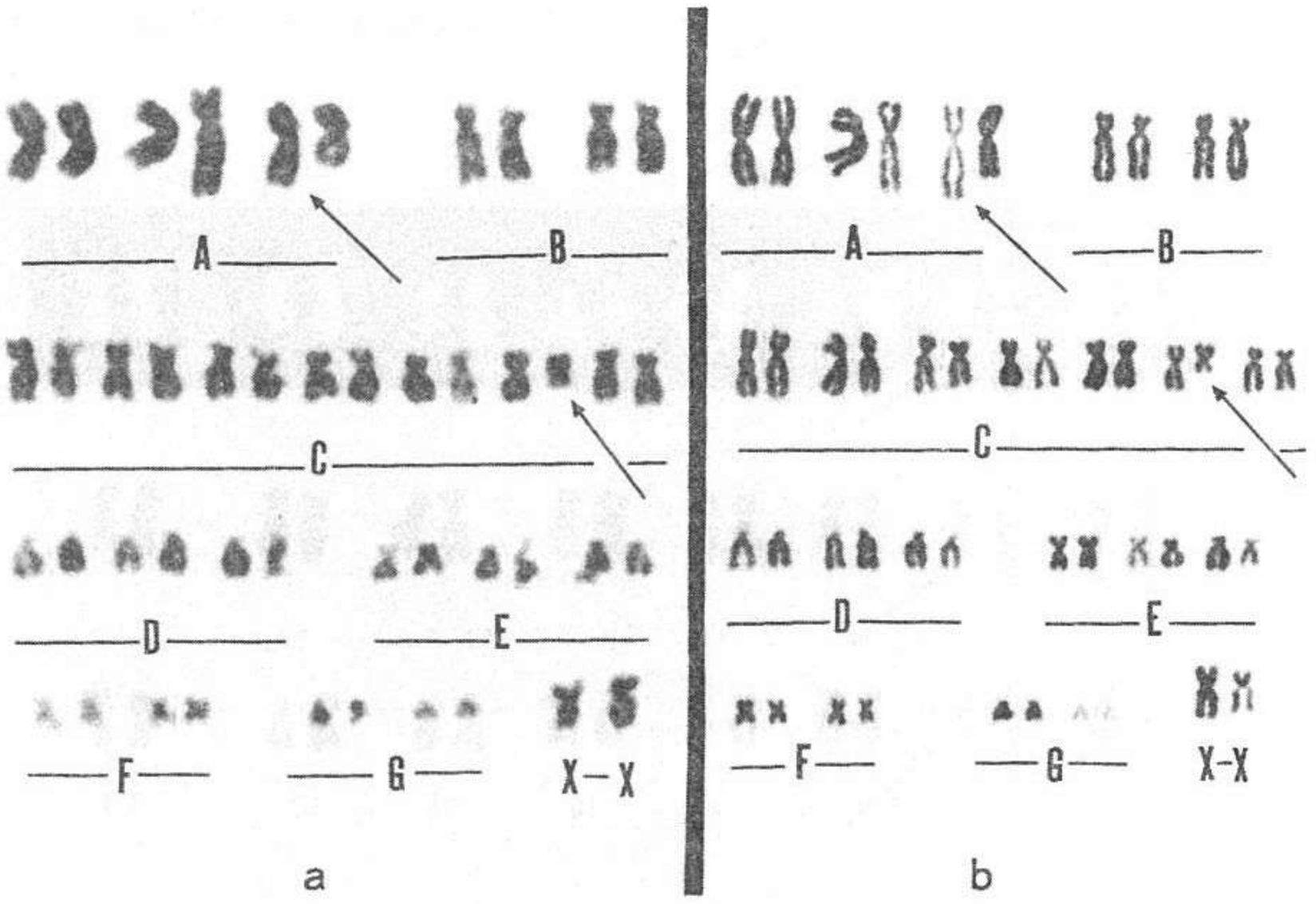


Figure 3. Resembling abnormal karyotypes 46,XX, t(3q+;Cq−) seen in BFU-E (a) and T lymphocyte (b) of Case 2 (female).

図3　症例2（女）のBFU-E(a)とTリンパ球(b)でみられた類似の異常核型，46，XX，t(3q＋；Cq－)．

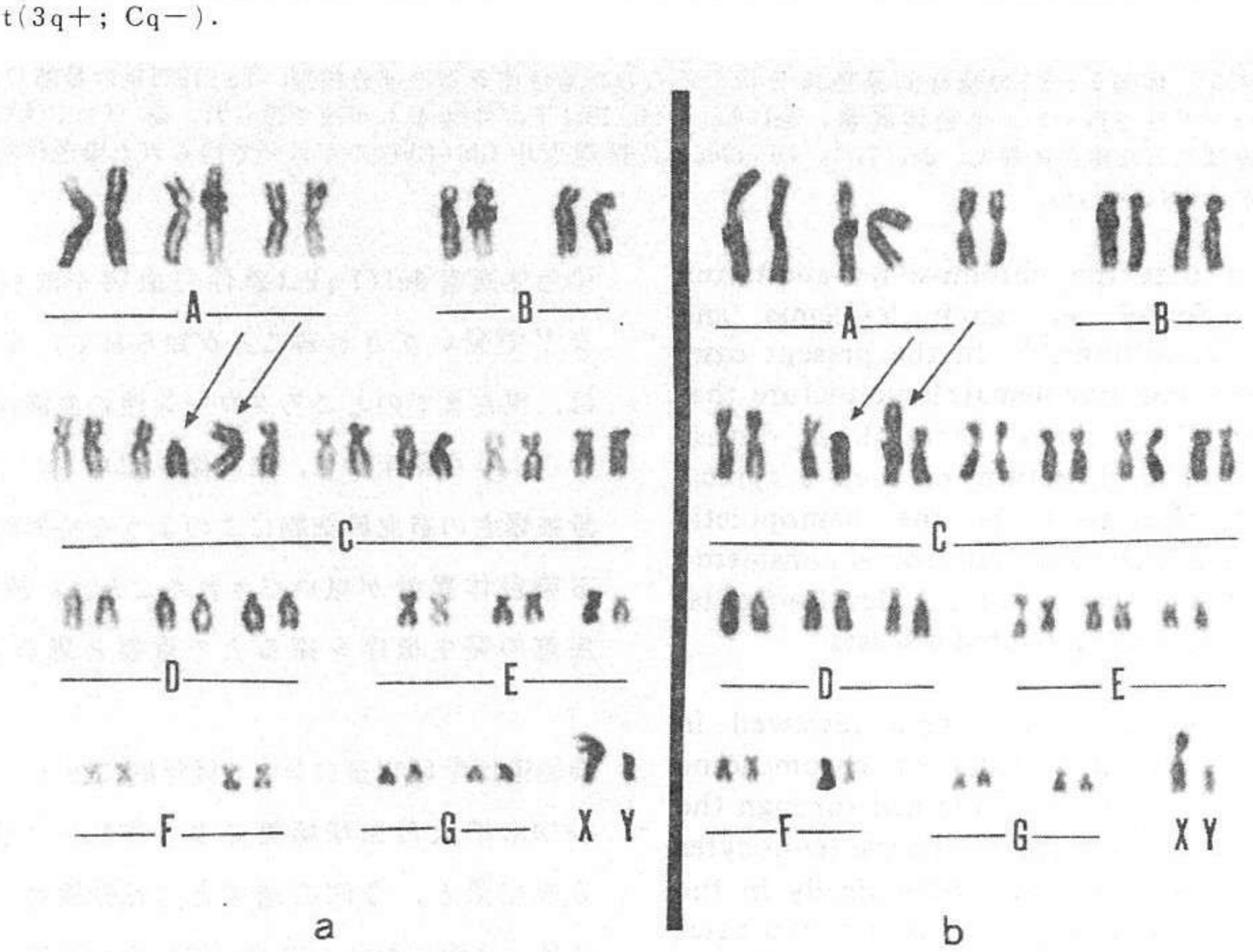


Figure 4. Resembling abnormal karyotypes 46,XY, t(Cp−;Cp+) seen in BFU-E (a) and T lymphocyte (b) of Case 5 (female).

図4　症例5（女）のBFU-E(a)とTリンパ球(b)でみられた類似の異常核型，46，XY，t(Cp－；Cp＋)．

In the present study, the karyotypic abnormality of del(Cq) which closely resembles del(7q) was found in the circulating GM-CFC of the same case (Figure 5).[19]

今回，del(7q)と極めて類似するdel(Cq)の核型異常を同例の循環血中GM-CFCでも観察した(図5).[19]

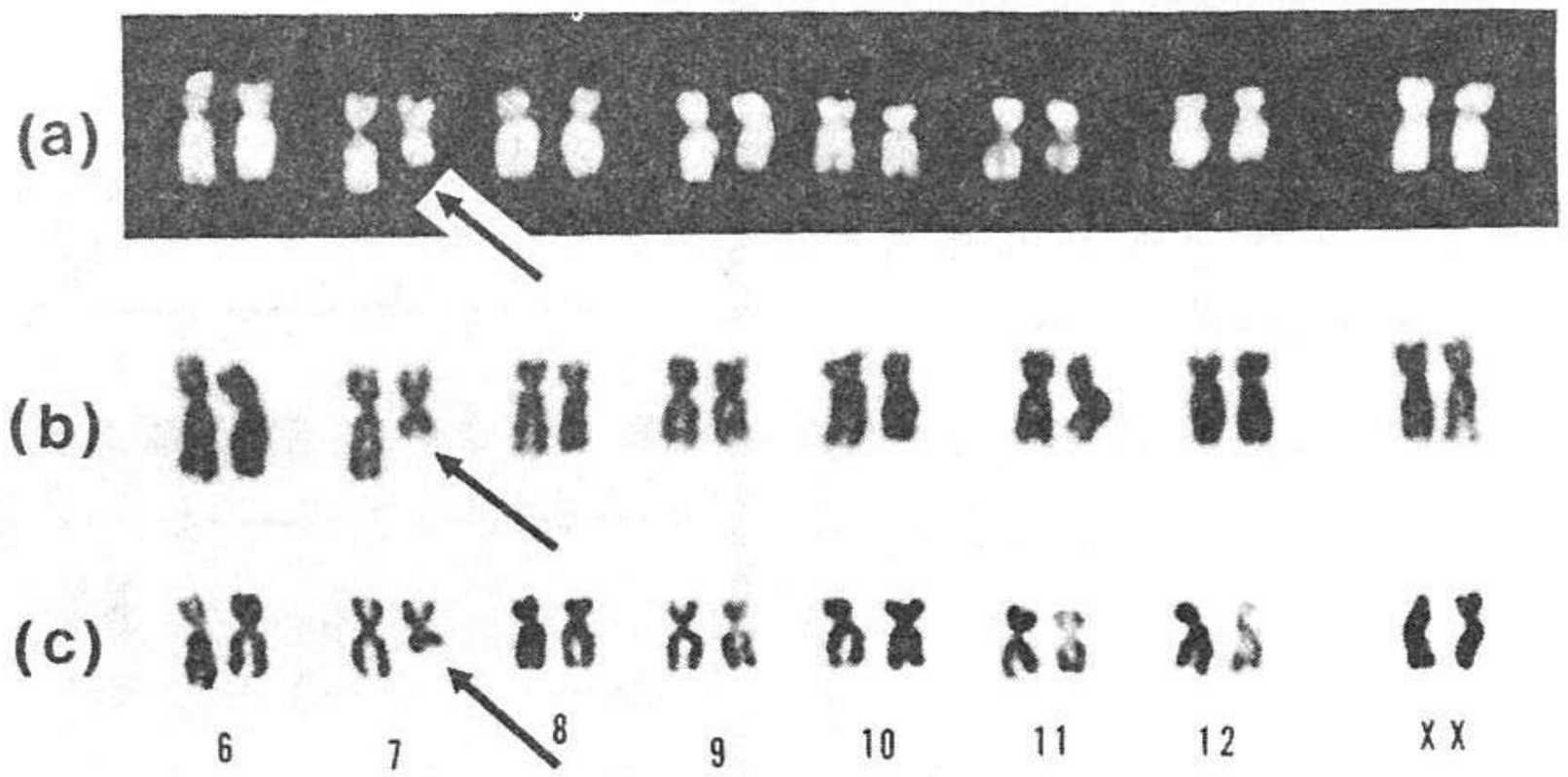


Figure 5. Partial karyotypes of chromosome aberration seen in Case 7 (female) by longitudinal chromosome analyses; (a) del(7q) observed in bone marrow by Q-banding in 1976, (b) del(7q) observed in bone marrow by Giemsa staining and comfirmed by Q-banding in 1981, (c) del(Cq) observed in circulating GM-CFC by Giemsa staining in 1982.

図5　症例7(女)の経時的染色体分析でみられた染色体異常の部分核型．(a)1976年の骨髄Q-バンド法で得られた染色体異常，del(7q)．(b)1981年の骨髄ギムザ法で得られ，Q-バンド法で確認された染色体異常，del(7q)．(c)1982年に循環血中GM-CFCのギムザで得られた染色体異常，del(Cq)．

It is known that the chromosome aberration del(7q) is found in acute leukemia and preleukemic conditions.[20] In the present case, no clinical findings nor hematologic picture that would suggest any clonal hematologic disease was found. The detection of such a typical chromosome aberration in the hemopoietic stem cells of a high dose survivor is considered important in the search for the developmental mechanism of radiation-related diseases.

染色体異常del(7q)は急性白血病や前白血病状態などで見いだされることが知られている.[20] 本例では，現在までのところクローン性の血液疾患を思わせるような臨床所見，血液像を認めていない．高線量被爆者の造血幹細胞にこのような特異的と思われる染色体異常が見いだされたことは，放射線関連疾患の発生機序を探る上で重要と思われる．

The results of this study were reviewed in comparison with the results of chromosome analysis of T lymphocytes obtained through the culture of PHA-stimulated peripheral leukocytes which has been carried out periodically in the Cytogenetics Laboratory. As a result, two cases showed an abnormal karyotype in a BFU-E which closely resembled that in T lymphocytes previously detected in those cases. Although it could not be clearly demonstrated because banding technique was not employed, it shows

細胞遺伝学研究室において経年的に行っているPHA添加末梢血白血球培養によるTリンパ球の染色体分析結果と，今回の結果とを比較検討した．その結果，2例において以前に認められたTリンパ球の異常核型と極めて類似する核型を，それぞれ1個ずつのBFU-Eにも見いだした．このことは，分染法でないために確定するには至らないが，造血幹細胞に

that radiation effects directly affected hemopoietic stem cells, and also suggests the possibility that relatively undifferentiated hemopoietic stem cells common to myeloid cells and lymphoid cells may exist in human adults. This is considered interesting together with the fact that acute leukemia which in some cases is considered to be a hemopoietic neoplasm of pluripotential stem cells[21,22] and chronic myeloid leukemia in which lymphoid cells also are considered as abnormal clones,[23,24] frequently occurred in A-bomb survivors,[25] and that hemopoietic dysplasia (or myelodysplastic syndrome) which is now considered as clonal hemopathy also occurred in A-bomb survivors.[26,27]

直接的に放射線の影響が及んだことを示しており，またヒト成人において骨髄系細胞とリンパ系細胞に共通したより未分化な造血幹細胞が存在する可能性を示唆する．これらは，一部の例で多能性造血幹細胞の造血腫瘍とされている急性白血病[21,22]や，リンパ系細胞も異常クローンとされている慢性骨髄性白血病[23,24]が原爆被爆者に多発したこと[25]や，現在clonal hemopathy と考えられている hemopoietic dysplasia（あるいは myelodysplastic syndrome）が原爆被爆生存者にも発症していること[26,27]と合わせて興味ある事実と考えられる．

It is felt that such hemopoietic stem cells with radiation-induced chromosome aberrations are involved in the recovery from hemopoietic failure immediately following A-bomb exposure and in the maintenance of hemopoiesis to this day. Moreover, it is probable that clonal cells with such abnormal chromosomes will continue to remain in the body repeating self-regeneration or in a state of pause.

このような放射線誘発性の染色体異常を有する造血幹細胞もまた，原爆被爆直後の骨髄障害からの回復や，現在に至るまでの骨髄造血能の保持に少なからず関与していることが想定される．更に，これらの異常染色体を有するクローン細胞は今後も自己再生を繰り返しながら，あるいは休止状態のまま体内に存続するものと考えられる．

The behavior of such clones with chromosome aberrations in relation to the associated chromosomes and their loci may be an interesting subject for future study.

今後このような染色体異常クローンがいかなる動向を示すかは，関与する染色体とその部位との関連からも極めて興味ある課題と言える．

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