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Micronuclei and Chromosome Aberrations Found in Bone Marrow Cells and Lymphocytes from Thorotrast Patients and Atomic Bomb Survivors*)

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ABSTRACT

As two cytogenetic parameters of radiation exposure, the frequency of micronucleus in erythroblasts, lymphocytes and red cells (Howell-Jolly body) as well as chromosome aberrations in bone marrow cells and in lymphocytes were studied in 24 thorotrast patients and in 32 atomic bomb (A-bomb) survivors who were exposed within one kilometer from the Hiroshima hypocenter. The incidence of both micronucleus and chromosome aberrations in these two exposed groups were significantly higher than that in non-exposed controls. So that these two parameters are useful guide for evaluating the residual effects of radiation, especially on hematopoietic cells. Because of its simple procedures, micronucleus test is also helpful as screening for prediction of chromosome aberrations. The characteristics of lymphocyte chromosome aberrations differed considerably between thorotrast patients and A-bomb survivors; the incidence of unstable type aberrations and intracellular complexity of chromosome aberrations were much higher in the former group. The incidence of micronucleus in erythroblasts and lymphocytes was also higher in thorotrast patients. Such differences are attributable to the differences in the radiation quality (α-ray or γ-ray+neutron) and in the mode of exposure (persistent or single) of these two groups.

INTRODUCTION

Because of high radiosensitivity, hematopoietic tissues are known injured with malformation of cells not only immediately but also long years after exposure to radiation. This has been documented in bone marrow cells of nuclear reactor workers 3.5 years after accidental exposure[3] or of atomic bomb (A-bomb) survivors 5 and even as late as 35 years after exposure[10,15]. The presence of such malformed cells in hematopoietic tissues may be interpreted to be an important index for assessing the dose of past radiation exposure or the degree of its persistent residual effect.

Thorotrast patients are the victims of radioactive thorium (ThO_2) injection for angiography during the World War II. As thorium deposited in phagocytic cells of reticuloendothelial tissue without extracorporeal excretion is a radioactive substance having considerably long half-life, intracorporeal generation of a very small amount of radiation is constant and long lasting. In this study, among malformed cells seen in bone marrow and peripheral blood from thorotrast patients, especially micronuclei

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were morphologically examined to determine whether they may be effectively used as a helpful index in the assessment of the effect of internal exposure.

Micronucleus test is in recent years attempted as one of the methods for screening of mutagenic agents using mouse marrow erythoblasts and erythrocytes[5,18]. Experiments with mouse cells or human lymphocytes have shown that the frequency of micronucles is correlated with the does of mutagenic agents and that the production of micronucleus is related to the loss of chromosomal segments due to chromosomal break or nondisjunction and also correlated with frequency of chromosomal aberration[4,22]. The same findings have been reported in people exposed to petroleum vapor[7]. The appearance of micronucleus in irradiation experiments has also been reported by some investigators with similar results to those obtained with chemical substances[6,16].

This paper also describes the results of chromosome analysis of peripheral lymphocytes recently undertaken in one thorotrast patient. To determine the characteristic effect specific to internal exposure in thorotrast patients micronucleus and chromosome aberration in A-bomb survivors exposed to a single but significant massive dose over the entire body are also reported for the sake of comparison.

SUBJECTS AND METHODS

1) *Micronucleus test*

Thorotrast patients examined were 24 males (ages 54-62) with a past history of thorotrast injection. The smear slides of bone marrow and peripheral blood were obtained from Aichi Cancer Center and National Kurume Hospital. A-bomb survivors examined were 32 subjects (18 males and 14 females, ages 27-84) exposed to A-bomb radiation within 1 km from the hypocenter in Hiroshima, of whom 21 were exposed within the radius of less than 0.5 km and 11 within the radius of 0.5-1.0 km. All of them were hospitalized for examination within the past 10 years in the Department of Internal Medicine of our Institute. Examination on admission disclosed no hematological abnormalities in all these thorotrast patients and A-bomb survivors. The bone marrow and peripheral blood smears were stained with Giemsa. The bone marrow smears were used to find the incidence of micronuclei appearing in 2,000 erythroblasts including proerythroblasts, basophilic and polychromatic erythroblasts. The incidence of Howell-Jolly bodies (H-J bodies) appearing in bone marrow erythrocytes was also examined from these smears. The peripheral blood smears were used to investigate the incidence of H-J bodies in 10^5 erythrocytes and micronucleus in 500 lymphocytes. Fifteen of 24 thorotrast patients (6 males and 9 females) and 15 of 32 A-bomb survivors (7 males and 8 females) described above were used for this analysis. In this paper, micronucleus and H-J body were discriminated, defining those seen in nuclear cells such as erythroblasts and lymphocytes as micronuclei and those seen in erythrocytes as H-J bodies. Ten healthy subjects without a history of exposure to radiation (5 males and 5 females, ages 18-73) were used as controls. Smear slides of bone marrow cells were prepared from these controls. Smear slides of peripheral blood were obtained from other 11 healthy subjects (6 males and 5 females, ages 19-45). The values obtained were statistically analyzed by maximum likelihood tests.

2) *Chromosome analysis*

The thorotrast patient used for chromosome analysis in this study was a 59-year-old man who had had in 1939 cerebral arteriography with thorotrast infusion for the treatment of a war wound. The amount of thorotrast infused was unknown. In May, 1977 he was admitted in Tsuyama Central Hospital because of severe pyrexia. The patient, however, died in October of the same year. Thorotrast deposition in the liver or spleen was not noted. Liver function test and hematologic analysis revealed no abnormalities. The bone marrow also appeared normal. The duration of exposure to thorotrast radioactivity lasted for 38 years.

Ten ml of peripheral blood was cultured for 50 hours with 7.9 ml of RPMI 1640 medium, 2.0 ml of fetal calf serum, and 0.1 ml of phytohemagglutinin (PHA), and two hours before cell harvest it was treated with colcemide at a concentration of 0.02 μg/ml. Hypotonic treatment and fixing was made and specimens were prepared by the air-drying method. The specimens for chromosome analysis were made by conventional staining and G-banding[10]. All the satisfactory metaphases were photographed

and all cells with chromosome aberrations on the photograph were used for karyotyping. For attempting to observe a large number of cells from this patient, some chromosome specimens were automatically analysed by computer[9].

RESULTS

1) *Micronuclei in bone marrow and peripheral blood*

Micronuclei were mostly detected in polychromatic erythroblasts. In Giema staining, both micronuclei and H-J bodies are well defined in small roundish outline and thus easily and distinctly discriminated from artifact. Figure 1 shows the incidence of micronuclei in bone marrow erythroblasts from the thorotrast patients and the A-bomb survivors. The values were significantly higher than those in the non-exposed controls. The controls had 0.75 micronucleus in 1,000 erythroblasts on an average. In contrast, A-bomb survivors and thorotrast patients had 2.63 and 3.27 micronuclei in 1,000 erythroblasts, respectively. Despite the absence of a significant difference between the thorotrast patients and A-bomb survivors, the incidence of micronuclei was higher than normal (meshed area in Fig. 1) in 22 of 24 thorotrast patients but in only 22 of 32 A-bomb survivors. On dividing the survivors into those (21 persons) exposed within the radius of 0.5 km and those (11 persons) exposed within the radius of 0.5-1.0 km, the values were higher than normal in 16 of the former 21 and in 6 of the latter 11. The symbols minus (−) and plus (+, ++, +++) above a closed circle in Fig. 1 denotes the rough incidence of H-J bodies detected in bone marrow erythrocytes (please see Fig. 1 in detalis). The proportion of erythroblastic micronuclei was positively correlated with that of erythrocytic H-J bodies.

Figure 2 shows the incidence of H-J bodies in peripheral erythrocytes. In contrast to their presence in none of 11 controls, they were detected in 8 of 15 thorotrast patients and 4 of 15 A-bomb survivors. The appearance of H-J bodies was obviously higher in thorotrast patients than A-bomb survivors.

The incidence of micronuclei per 100 peripheral lymphocytes was shown in Figure 3. They were detected in 2 cases of 11 controls, in only 4 cases of 15 A-bomb survivors and in 11 cases of 15 thorotrast patients. Though almost

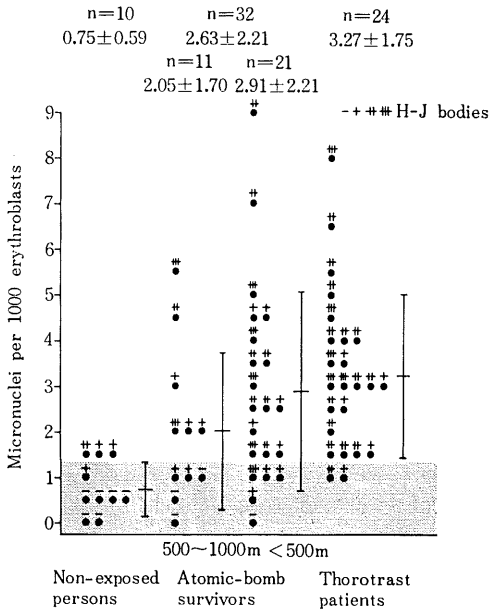

Fig. 1. Incidence of micronuclei and Howell-Jolly (H-J) bodies in bone marrow cells from thorotrast patients and A-bomb survivors. Four degrees (−, +, ++, +++) show the incidence of H-J bodies observed in bone marrow erythrocytes; −: not found, +: less than 5, ++: 6-16, +++: more than 16 during the analysis of 2,000 erythroblasts in each case. Meshed area shows normal range (mean+1SD) calculated from 10 control subjects.

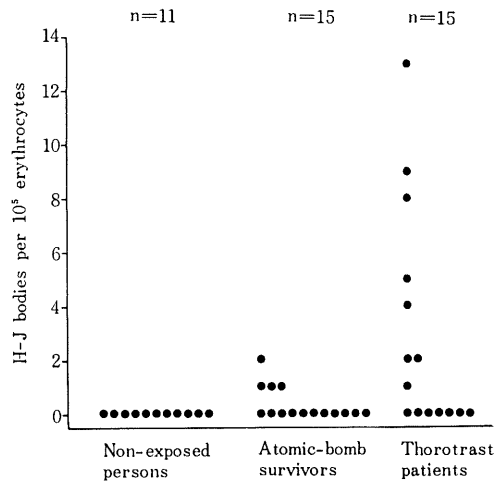

Fig. 2. Incidence of H-J bodies in peripheral erythrocytes from thorotrast patients and A-bomb survivors.

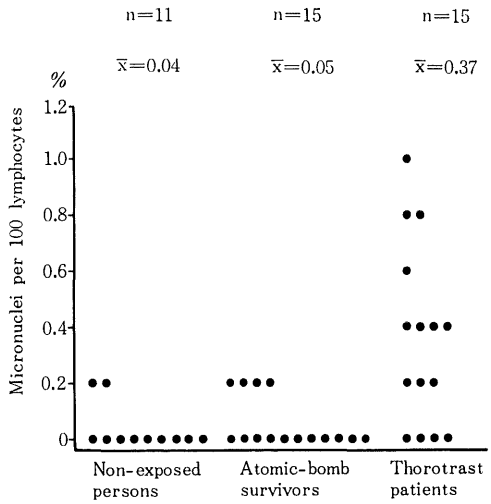

Fig. 3. Incidence of micronuclei in peripheral lymphocytes from thorotrast patients and A-bomb survivors.

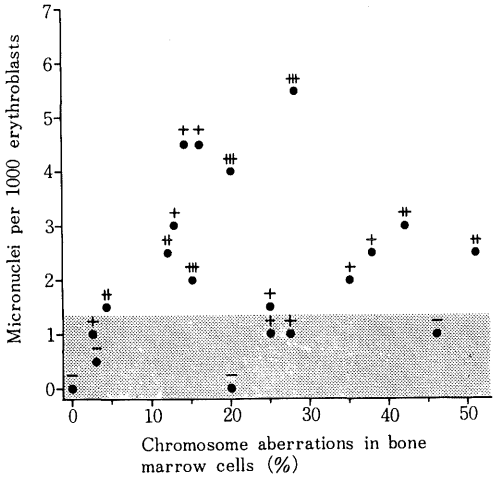

Fig. 4. Relationships between micronucleus frequencies and chromosome aberration frequencies in bone marrow cells from A-bomb survivors. Meshed area shows normal range calculated from 10 control subjects.

equally low in the A-bomb survivors (0.05%) and controls (0.04%), they were apparently high in the thorotrast patients (0.37%).

Micronuclei were also present, though very infrequently, in neutrophils, eosinoplils of thorotrast patients as well as A-bomb survivors. In some of thorotrast patients, the presence of macrophages that appeared phagocytising thorotrast granules was also noted in bone marrow smears.

The incidence of micronuclei and H-J bodies were then investigated in relation to the frequency of chromosomal aberration, estimated dose of exposure and age at examination. Figure 4 shows in bone marrow the relation between the frequency of chromosomal aberration previously obtained[9] and enythroblastic micronuclei in 22 A-bomb survivors. Although these 2 factors were not simply correlated, the survivors with a low aberration frequency seemed to show a low frequency of micronuclei. Of A-bomb survivors, the estimated exposure dose (T65D) was known in only 7 cases in our sample. As far as these 7 survivors were concerned, the incidence of micronuclei was high in those of high radiation dose of exposure. On studying the relation between the age at examination and the incidence of micronuclei, micronuclei were more frequent in old generations but there were large interindividual differences even in the same age bracket (Fig. 5). In the thorotrast patients, the incidence of micronuclei in erythroblasts and lymphocytes was not correlated with the thorotrast dose (10–20 ml) and the duration of exposure from administration to examination (28–40 years).

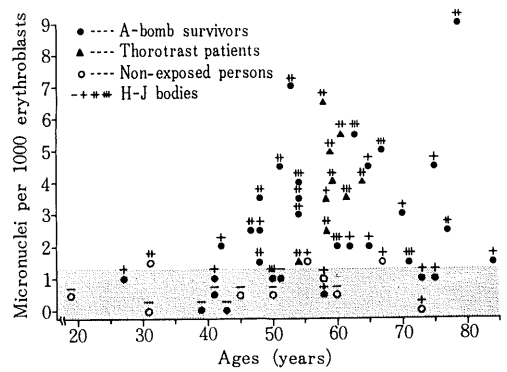

Fig. 5. Relationships between micronucleus frequencies in marrow cells and ages at the time of examination. Meshed area shows normal range calculated from 10 control subjects.

2) *Chromosome aberrations in peripheral lymphocytes*

Deliberate observation was attempted using a large number (1,189) of lymphocyte from our thorotrast patient. The results are shown in Table 1, and also compared with the results

Table 1. Chromosome aberration frequencies in a thorotrast patient and A-bomb survivors

Observed cells	Structural chromosome aberrations (%)							Numerical chromosome aberrations (%)
	Σ	Cs cells	Cu cells	(X_1Cu,	X_2Cu)	$\frac{\text{Dic+Ring}}{1,000 \text{ cells}}$	Cu/Cs	hyperdiploid
A-bomb survivors 3,136	651 (20.8)	635 (20.2)	16 (0.5)	10 (0.3)	6 (0.2)	2.87	0.03	44 (1.4)
Thorotrast patient 1,189	198 (16.7)	167 (14.0)	44 (3.7)	28 (2.4)	16 (1.3)	22.7	0.26	10 (0.8)

Cs: stable type, Cu: unstable type

Table 2. Chromosome aberration frequencies and chromosome break frequencies by exposure doses in a thorotrast patient and A-bomb survivors

	No. fo cases	Dose (mean)	Observed cells	Cells with structural chr. ab.	Breaks/Abnormal cells
Atomic bomb survivors					
Group Ⅰ	n = 4	≦100 rad (56 rad)	320	28 (8.8%)	1.94
〃 Ⅱ	n = 4	100～250 rad (150 rad)	366	64 (17.5%)	2.34
〃 Ⅲ	n = 6	250～700 rad (505 rad)	439	91 (20.7%)	2.36
〃 Ⅳ	n = 6	≧700 rad (853 rad)	621	185 (29.8%)	2.61
Thorotrast patient			1,189	198 (16.7%)	3.02

Note: Exposure dose in a thorotrast patient is unknown.

previously obtained from 39 A-bomb survivors exposed within the radius of less than 1 km. Aberrations in these survivors amounted to 6.4-42.3% (mean: 20.8%). In our thorotrast patient, aberration frequency was 16.7% and the aberrations in observed cells consisted of 81.5% of stable type (Cs) and 18.5% of unstable type (Cu). In A-bomb survivors, on the other hand, 98.0% were the Cs and only 2.0% the Cu. The Cu/Cs ratio was 0.26 in the thorotrast patient and 0.03 in A-bomb survivors. The frequencies of dicentric chromosomes and ring chrosomes (Dic+Ring) in 1,000 cells was 22.7 in our thorotrast patient and 2.87 in A-bomb survivors. With regard to numerical chromosome aberrations, hyperdiploids without structural chromosome aberration appeared at the frequency of 0.8 in our thorotrast patient and 1.4 in A-bomb survivors, thus slightly higher in the latter. Figure 6 shows the unstable type aberration in the thorotrast patient, in which A indicates a cell having dicentric chromosome with fragment and minute. Such a cell which is called an X_1Cu cell by Buckton[1] is a cell that has never been divided since the occurrence of chromosome aberration by exposure to radiation. B in the figure indicates a cell that has ring chromosome. Such a cell without fragment and minute is a cell that has undergone cell division at least once since the occurrence of chromosome aberration and is usually called an X_2Cu cell.

Complexity of chromosome aberration within an individual cell was investigated. The estimated dose (T65D) of radiation exposure is known in 20 of 39 A-bomb survivors. These 20 survivors were divided arbitrarily by the estimated dose into 4 groups; below 100 rad, 100-250 rad, 251-700 rad and over 700 rad (Table 2). Chromosome aberrations occurred in 8.8%, 17.5%, 20.7% and 29.8% of respective groups. The number of chromosome breaks per cell

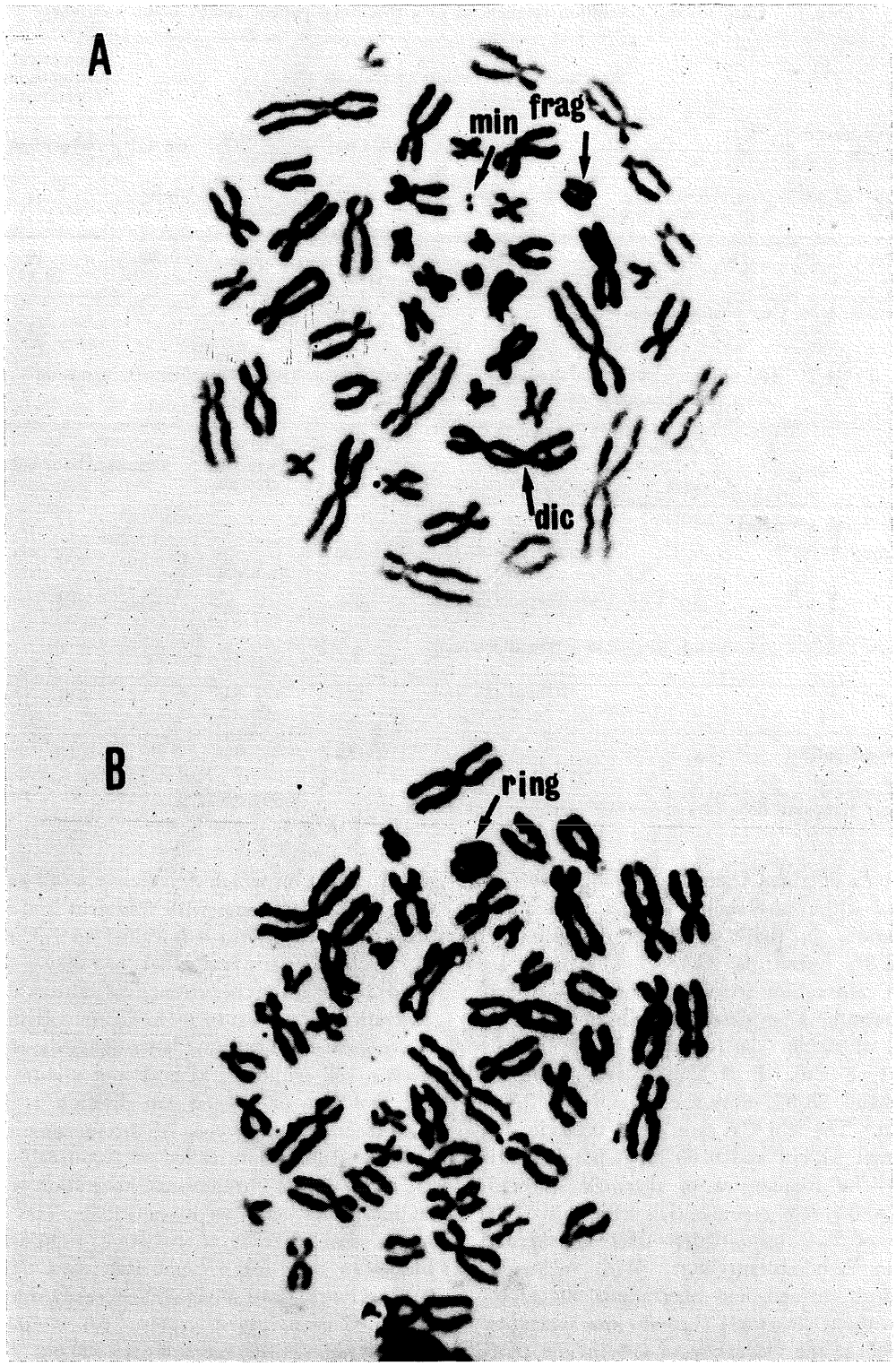

Fig. 6. Dicentric and ring chromosomes observed in lymphocytes from the thorotrast patient. dic: dicentric chromosome, ring: ring chromosome, frag: fragment, min: minute chromosome

was 1.94, 2.34, 2.36 and 2.61 respectively. On the other hand, chromosome aberration in our thorotrast patient amounted to 16.7% which almost corresponded to that in Group II (100–250 rad) of A-bomb survivors. However, the number of chromosome breaks per cell in our thorotrast patient (3.02) was remarkably higher than in Group IV (above 700 rad) of A-bomb survivors. The distribution of chromosome breaks per cell is shown in Fig. 7. In all the exposed groups, most frequently seen were the cells with 2 chromosome breaks, and the cells with more than 3 breaks increased with an increase in the exposure dose. The cells with 2 breaks were less frequent and those with more than 3 breaks were more frequent in our thorotrast patient than in A-bomb survivors. In other words, the thorotrast patient demonstrated a more complex aberration pattern within individual cells. Such aberration features seen in our thorotrast patient were also observed in unstable and stable type cells (Fig. 8). As shown on the left side of the figure, the X_1Cu cells in the thorotrast patient contained complicated aberrations, as compared with the survivors. These X_1Cu cells, as earlier de-

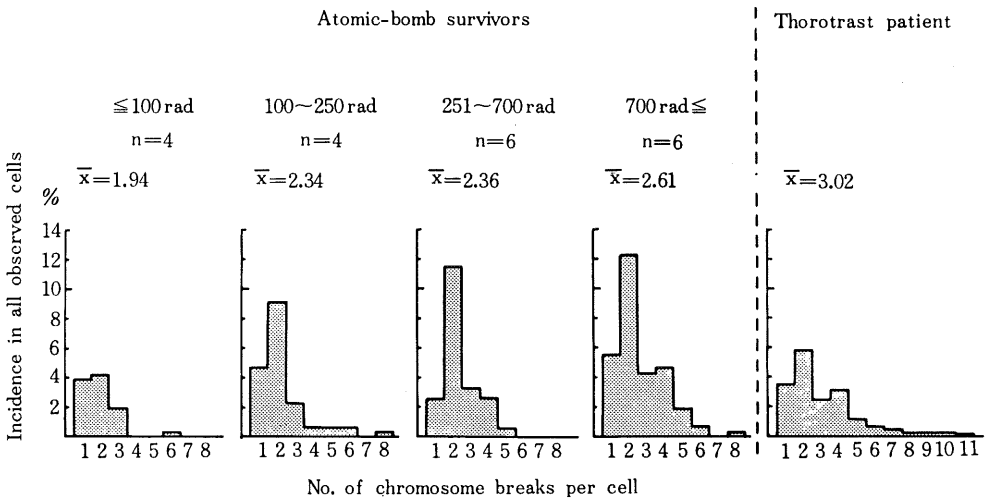

Fig. 7. Distribution of the number of chromosome breaks by exposure doses in a thorotrast patient and A-bomb survivors.

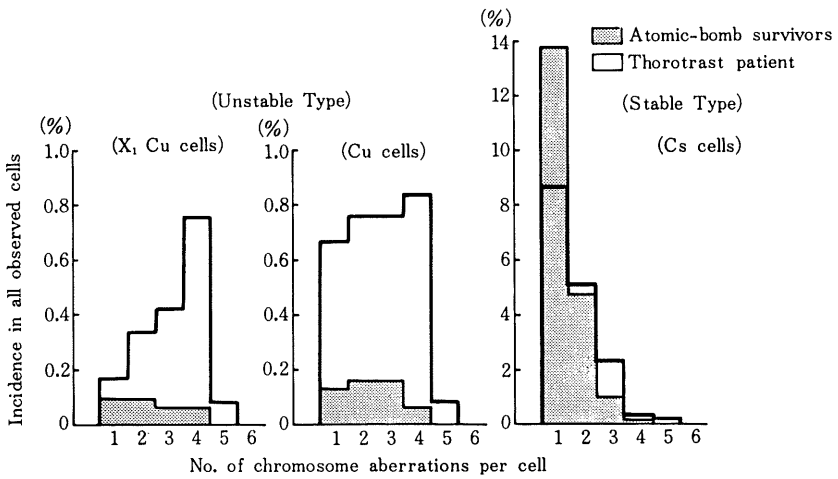

Fig. 8. Distribution of the number of chromosome aberrations in a thorotrast patient and A-bomb survivors.

Table 3. Distribution of breakpoints within chromosomal groups in a thorotrast patient and A-bomb survivors

	Chromosome number												
	1	2	3	4～5	6～X～12	13～15	16	17	18	19～20	21～22	Y	
Thorotrast patient													
Obs.	29	69	30	101	144	62	2	4	18	14	14	4	$\chi^2=92.44$
Exp.	44.7	42.3	34.2	62.4	199.0	53.6	15.4	14.6	13.6	23.1	16.3	10.9	d.f. =11
χ^2	5.51	16.9	0.52	23.9	15.2	1.32	11.7	7.7	1.42	3.58	0.32	4.37	$p<0.001$
	⊖*	⊕***		⊕***	⊖***		⊖***	⊖**				⊖*	
Atomic bomb survivors													
Obs.	98	86	88	195	459	189	53	49	59	55	80	3	$\chi^2=81.26$
Exp.	128.8	121.7	98.6	179.6	555	154.3	44.4	42.0	39.3	66.6	49.4	7.61	d.f. =11
χ^2	7.37	10.5	1.14	1.32	16.6	7.8	1.67	1.17	9.88	2.02	19.0	2.79	$p<0.001$
	⊖*	⊖**			⊖***	⊕**			⊕**		⊕***		

Expected breaks based on the individual chromosome length obtained by Caspersson et al. (1971).
*** $p<0.001$, ** $p<0.01$, * $p<0.05$

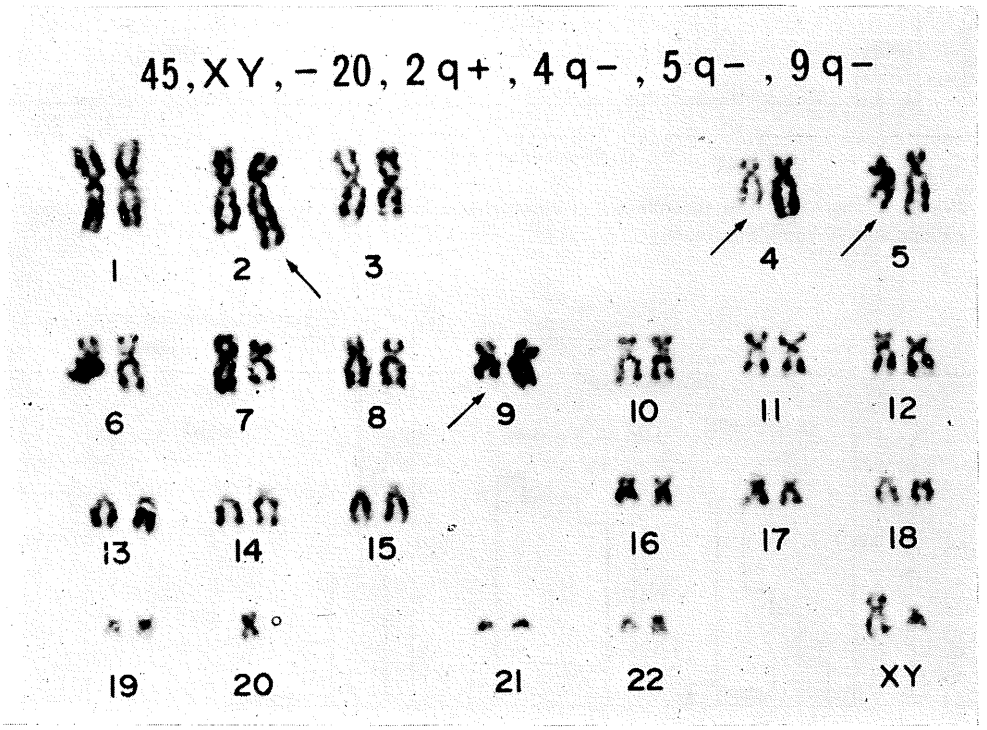

Fig. 9. Aberrant cells with breakpoint on chromosome #2 (2q+) observed in the thorotrast patient. This cell also had aberrations in chromosomes #4, 5 and 9. This cell shows a complex stable type aberration involving four chromosomes designated by arrows.

scribed, have never undergone division after exposure to radiation. Therefore, such a distribution pattern may be interpreted to reflect the difference in the effect of radiation immediately after exposure between α-ray and γ-ray+neutrons. The significant difference was absent between our thorotrast patient and A-bomb survivors in all of the Cu cells, as shown on the middle of the figure. The stable type cells shown on the right side of the figure contained complex aberrations within individual cells in this thorotrast patient, compared to A-bomb survivors. This indicates that the thorotrast patient had more than 2 chromosome aberrations more frequently than A-bomb survivors.

Lymphocytes in this thorotrast patient appeared to contain clones with the extended long arm of chromosome #2 (2q+), though at a low frequency (0.3%). In 39 A-bomb survivors, clone formation was suspected in 3, in whom the frequency was low (2.4%-3.8%).

To investigate whether chromosome aberration by irradiation was frequent to specific chromosomes, the distribution of breaks into chromosomal groups was investigated (Table 3). On calculating the expected number of breaks from the length of chromosome in our thorotrast patient, the number of breaks was significantly large in Group B chromosome and chromosome #2 and low in Group C chromosomes and in chromosomes #1, 16 and 17 and Y. In A-bomb survivors, it was significantly large in Group D chromosomes, chromosome #18 and Group G chromosomes and low in Group C chromosomes[20]. Thus, the pattern of break distribution differed between the thorotrast patient and A-bomb survivors. Furthermore, cells with aberrant chromosome #2 amounted to 29.8% of the entire abnormal cells in the thorotrast patient. Apart from 2q+, there were also other structural aberrations such as translocation, dicentric chromosome, inversion and shortening of the long arm (2q−) but the position of break point differed depending on cells. An example is shown in Fig. 9. This cell also had aberrations in chromosomes #4, 5 and 9. A similar phenomenon was observed in one of 39 A-bomb survivors. In this case, X chromosomes were frequently aberrant (21.9 % of the entire abnormal cells).

DISCUSSION

Micronucilei in bone marrow cells or peripheral blood after exposure to radiation have been studied by some investigators. Radiotherapy patients or radiologists are known to have significantly high incidence of micronuclei in lymphocytes[16]. However, the persistent presence of a high level of micronuclei in the bone merrow 3.5 years after accidental exposure to radiation or 5 years after A-bomb radiation has not so far been estimated[3,15]. In the absence of valid data, it is uncertain whether micronuclei after long years from radiation exposure may be used as a reliable index for assessment of the dose of radiation at the time of exposure.

The present observation showed a higher frequency of erythroblastic micronuclei in both thorotrast patients and A-bomb survivors, especially in the former, than the controls. The appearance of peripheral lymphocytic micronuclei was significantly frequent only in thorotrast patients. In A-bomb survivors, the incidence of erythroblastic micronuclei was found more or less correlated with the frequency of chromosomal aberration in bone marrow cells, estimated radiation dose and distance from the hypocenter. All these findings indicate that micronuclei in erythroblasts and lymphocytes can be used as a useful guide for evaluating the effect of radiation in people exposed to radiation. Moreover, micronucleus test, unlike chromosome analysis, is an advantageous method of screening without a need for technical difficulty, time-consuming manipulations. Therefore, routine examination of micronucleus in people exposed to radiation would provide a clue to detection of chromosome aberration.

Frequent appearance of H-J bodies in erythrocytes of thorotrast patients has already been reported[12]. Fibrosis, atrophy and resultant malfunction of the spleen due to internal radiation from thorotrast granules deposited in phagocytic cells may be related to frequent appearance of H-J bodies.

The characteristics of lymphocyte chromosome aberrations differed considerably between one of the thorotrast patients and A-bomb survivors in the incidence of unstable type aberrations and intracellular complexity of chromosome aberrations. The frequency of

micronuclei in erythroblasts and lymphocytes also differed between the internal and external exposure patients. These differences are attributable to the difference in the radiation quality between α-ray and γ-ray+neutron and in the mode of exposure; in the thorotrast patient, unstable type cells were constantly produced as a results of continuous internal exposure, while in A-bomb survivors, unstable type cells were constantly lost after a single, massive exposure to the A-bomb radiation. Furthermore, in contrast to topical intracorporeal exposure in thorotrast patients in whom only the lymphocytes passing through the vicinity of radioactive thorotrast granules are exposed, exposure in A-bomb survivors is almost entirely extracorporeal type. This difference may also be responsible for the different distribution patterns of aberrant chromosomes within an individual cell.

Chromosome aberrations in thorotrast patients has been reported mainly in Occidental countries and Japan[1,2,8,11,17,21]. In these reports, lymphocyte chromosome aberrations were characterized by complexity of aberration within an individual cell, aberration range between 1% and 20% depending on cases and high percentage of unstable type amounting to or exceeding 50% of aberrations. The case presented in this paper had 16.7% aberrations and hence may belong to the group of severe internal radiation exposure among the thorotrast patients so far reported. In this case, unstable type cells amounted to only 18.5% of all abnormal cells, which is slightly lower than reported elsewhere. One of the reasons may be that unstable type aberrations with acentric fragments and minutes might have been included in stable type, as a result of the loss of acentric fragments and minutes during cell division in the course of long culture process. Cells were cultured for 50 hours in this analysis. However, if the culture time is associated with loss of unstable type cells, a correct method should be planned for precise detection of unstable type aberration in patients exposed to radiation.

In our thorotrast patient, 22.7 dicentric and ring chromosomes were counted in 1,000 lymphocytes. In consideration of far less dicentric and ring chromosomes (0.78 in 1.000 cells) as reported in nonexposed subjects[13], the considerably high rate of these chromosomes in this patient indicates that the accumulated doses was remarkably higher by constant exposure than in the nonexposed subjects. Since the frequency of chromosome aberrations is regarded not correlated with the dose of thorotrast infusion, the amount of deposition and the accumulated doses[11], estimation of the exposure dose from the frequency of chromosomal aberration has been attempted without success. The presence of 2.87% dicentric and ring chromosomes in A-bomb survivors indicates that despite gradual clearance over the 3 decade after exposure, they persisted at 3.3 times the level in the nonexposed subjects.

Clone production is said to be frequent in thorotrast patients with chromosome aberration of bone marrow cells[8]. This is also observed in A-bomb survivors[20]. The presence of clones in peripheral lymphocytes must be a considerably less occasion. Buckton noted the presence of clones in 2 of his 36 thorotrast patients[1]. Although clones were found in 3 of 39 A-bomb survivors in this study, only 3 cells (the minimum of the criteria) with the same abnormal karyotype were identified in them. Furthermore, by observation of a large number of lymphocytes, again only 3 cells with the same abnormal karyotype were identified in our thorotrast patient. Clone formation defines as that if more than 3 cells of the same abnormal karyotype are identified for 48-hour culture, cells of the same origin were already in the course of proliferation in vivo before culture. More discretion may be needed, however, for accurate judgement of clone formation.

The thorotrast patient reported in this study disclosed frequent aberrations of chromosome #2. One of 39 A-bomb survivors disclosed frequent aberrations of X-chromosomes. This may suggest that chromosomes liable to injury by radiation are present in some people.

Such comparison study as this between thorotrast patients and A-bomb survivors, who were exposed to different qualities of radiation, is important to clarify the radiation effects on human chromosomes.

Epidemiological studies showed a significant incidence of malignant neoplasm such as hepatoma, and leukemia in thorotrast patients[14]. Thorotrast infused may partially stay deposited in the site of infusion but is mostly accumelated

in the liver, spleen and bone marrow via the blood stream and occasionally causes chromosome aberration in bone marrow cells[8]. Chromosome aberration also occurs in leukemic cells. Therefore, examination of chromosomes in hematopoietic cells (bone marrow cells and lymphocytes) is also important for health control, when considering the late effect in thorotrast patients.

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