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Cytogenetic Studies in 12 Patients with Itai-Itai Disease*

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Summary. Among 12 Itai-Itai disease patients examined, 8 patients showed a remarkably high frequency of chromatid aberrations, whereas the other 4 patients showed a much lower frequency of such aberrations, although a significant number of stable type aberrations was observed also in the latter patients. The frequencies of aneuploid cells of all 12 patients were significantly higher than those of the controls. The abnormalities were found in 50-hour and 72-hour cultures, from which it can be concluded that the aberrations occurred in the blood stem cell of the patients. In addition to these structural and numerical aberrations, satellite associations of the D and G group chromosomes were often observed.

Introduction

Itai-Itai disease has been found to occur frequently around the central basin area of the Jinzu River in Toyama Prefecture, Japan, and attacks mostly elderly women. Pain first occurs in the lumbar area, the extremities, and then spreads to involve the entire body. As a result of this, the patients gradually begin to suffer from walking disturbances and after a long time it becomes very difficult for the patient to move. At this stage, bones in the body will fracture from even the slightest pressure or force and so the patient groans with pain, "Itai-Itai" (Ouch-Ouch). Examination of the blood reveals marked anemia, decrease of inorganic phosphorous and an elevated alkaline phosphatase in the serum. Other important findings in Itai-Itai disease are constant proteinuria, glycosuria and elevated urinary excretion of calcium and the heavy metal cadmium. The patients usually have lived in the area of the cadmium pollution for more than 30 years, having used water, rice and fish of the area. There exists a strong etiological correlation between the cadmium pollution and the incidence of the disease. Therefore, Itai-Itai disease has been thought to be caused by chronic cadmium poisoning (Ishizaki, 1971). However, the pathogenesis of the disorder is obscure, and it is disputed by many scientists whether this element is related directly to the cause of Itai-Itai disease. Recently, several approaches have been pursued in an effort to clarify the pathogenesis of the disease. In a previous paper (Shiraishi and Yosida, 1972) we reported remarkable chromosome aberrations (chromosome breakages and rearrangements) in Itai-Itai disease patients. Moreover, we observed very similar damage in cultured human leucocytes which were treated with cadmium sulfide *in vitro* (Shiraishi and Yosida, 1972). However, the relationship

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of these chromosomal aberrations to the pathogenesis of Itai-Itai disease is unknown. Also it has not been established whether the abnormalities are present *in vivo* or whether they occur only *in vitro*. It, therefore, appeared reasonable to investigate systematically the chromosomes in a group of patients in order to determine if any abnormalities could be found. To ascertain the origin of the chromosomal abnormalities in the patients, follow-up studies on 12 Itai-Itai disease patients were carried out.

Materials and Methods

Peripheral blood samples obtained from 12 patients with Itai-Itai disease and 9 control subjects were examined in the present study. The age and sex of each individual and the date that the samples were examined are presented in Tables 1, 2 and 4. Table 1 presents the results of examination in 1972, and Tables 2 and 4, in 1973. Of the 12 patients, serial chromosome examinations at 2 and/or 3 different times were performed in 8 patients (I-1—8). These patients were followed up by examinations at 3 months intervals.

The methods used in this study were essentially the same as those previously described (Shiraishi and Yosida, 1972). 1 ml heparinized blood, freshly drawn from each individual, was added to 10 ml of RPMI 1640 media (Nissui Seiyaku Co. Ltd., Tokyo, Japan) containing 20% calf serum and 0.1 ml phytohemagglutinin (Burroughs Wellcome). Cultures were incubated for 50 and 72 hrs at 37°C, and colcemide at a concentration of 0.02 μg/ml was added to the cultures 3 hrs before harvest in order to obtain metaphase chromosomes. The 72-hour culture contains a mixed population of the first and the second generations of dividing cells (Bender and Gooch, 1962). Buckton and Pike (1964) have pointed out that in cultured lymphocytes the frequencies of unstable type abnormalities are influenced by time of incubation in culture, and that 48-hour incubation is an optimum time for chromosome observation, since the majority of cells are in the first division in culture. In January 27, April 27 and July 27, 1972, cells were examined at 72 hrs of cultivation, and in the 1973, the 50-hour culture material was used. Slides for the observation of chromosome were made by an air-drying technique and stained with Giemsa.

In each case, 155—700 metaphase cells (randomly chosen) were examined by scoring the frequency of chromosome aberrations. In order to delineate the chromosome condition as accurately as possible, each sample was examined by the following procedure. In all instances the counts were made only on intact metaphase plates to avoid the risk of including broken cells. The cells with chromosome aberrations were classified into those with chromatid aberrations (chromatid and isochromatid breaks, chromatid exchanges), chromosome type aberrations (unstable: dicentric, acentric fragment; stable type:), polyploid, aneuploid and endomitotic cells. All the cells scored as aberrants, namely, isochromatid breaks, unstable and stable type abnormalities were photographed for detailed karyotypic analysis. Special note was taken to identify the isochromatid breaks from the fragments. Isochromatid breaks can be clearly identified from the acentric fragments by analysis of their karyotype. In the present classification, isochromatid breaks were classified as chromatid type aberrations.

χ^2 tests were used for the statistical analyses of the difference of chromosome aberrations between Itai-Itai disease patients and controls.

Results

The results of the chromosome analysis in 9 control subjects and 12 Itai-Itai disease patients are presented in Tables 1—6. Table 1 presents the results of the 72-hour cultures in January, April, and July of 1972. Tables 2—5 present the results of the 50-hour cultures in April, 1972 and in February, 1973. Table 6 presents an analysis of aneuploid cells observed in the patients. As seen in these tables, the peripheral blood cultures from Itai-Itai disease patients revealed a

marked increase of chromosome aberrations, when compared to control subjects. Almost all the types of chromatid and chromosome type aberrations have been found in the patients (Figs. 1—9).

Chromosome Examination in 1972 (72-hour Culture)

Controls. The results are given in Tables 1—3. The frequencies of total abnormal cells ranged from 1.5 to 3.8%, the mean value being 2.6%, and polyploid cells from 0.5 to 2.2%, the mean value being 1.2%. The abnormal cells consisted of chromatid and isochromatid breaks, and other abnormalities were not observed. The frequency of aneuploid cells was about 1.3%. These values would be considered to be within the normal range. The predominant chromosome number was 46 in all control cases and these showed normal karyotypes.

Itai-Itai Disease Patients. The results are given in Table 1. The frequency of all chromosome aberrations ranged from 8.9 to 51.2% (mean value: 26.7%), and was significantly higher than that in the controls ($P < 0.001$). Polyploid and aneuploid cells were also observed at a frequency of 0.6 to 3.3% (mean: 2.4%), and 1.5 to 5.6% (mean: 3.6%), respectively, and, again, this frequency was higher than that of the controls ($P < 0.001$). It is to be noted that hyperdiploidy, which is less likely to be technique-induced than hypodiploidy, occurred at a comparatively high frequency (controls: 0.35%; patients: 3.2%). With regard to structural aberrations, 8 (I-1—8) out of the 12 patients exhibited high frequencies of chromatid and unstable type aberrations, although they varied from case to case. Ring chromosomes were seen less frequently than dicentrics and fragments. An increased number of chromatid exchanges was noted in 7 patients except for case I-8. Some of the figures found in the patients are shown in Fig. 1a—f. A few of the figures were so structurally aberrant that it was not possible to determine which chromosomes were involved. In general, however, the majority was from A, B, or C. The deletions of the long arm in the G group chromosomes (GLD, Table 1) were seen in I-1,5,7. The other 4 (I-9—12) patients exhibited a comparatively low frequency of chromatid and unstable type aberrations, whereas significant numbers of the stable type aberrations (1.5—4.1%) were observed in these patients, and the frequencies of total chromosome aberrations were significantly higher than in controls ($P < 0.001$). Thus, the chromosome aberrations in the patients were quite different from those in normal subjects, both qualitatively and quantitatively. However, considering that the frequency of the aberrations varied from case by case, and a 72-hour culture is a mixed cell population, with both first and second division products, it becomes necessary to determine whether these aberrations are present *in vivo*, or result from damage of the blood stem cells. Hence, the 5 patients (I-1,3,4,6,7) who exhibited a high frequency of aberrations in the examination of 1972, were followed up with 50-hour cultures, in which all of the cells were in their first mitosis in culture.

Chromosome Examination in 1973 (50-hour Culture)

Controls. 9 subjects, whose cells were examined in 1972, were included in the present chromosome study. The frequency of total structural aberrations ranged from 1.5 to 4% (mean value: 2.5%); the numerical aberrations from 1.5 to 3.2%

Table 1. Types and frequencies of chromosomal aberrations observed in

Case No.	Age and sex	Date examined	No. of cells observed	Total abnormal cells (%)	Polyploid cells (%)	Chromatid aberrations	
						CB	Iso-CB
Control							
C-1	58-F	1972.1.27	600	20 (3.3)	4 (0.7)	4	0
C-2	62-F		700	26 (3.7)	13 (1.8)	6	0
C-3	67-F		650	10 (1.5)	3 (0.5)	6	1
C-4	70-F		580	20 (3.5)	6 (1.1)	6	2
C-5	70-F	1972.4.27	550	9 (1.6)	3 (0.6)	5	0
C-6	78-F		500	14 (2.8)	10 (2.0)	12	1
C-7	65-M		450	17 (3.8)	10 (2.2)	10	1
C-8	68-M	1972.7.27	550	9 (1.6)	4 (0.7)	5	0
C-9	75-M		500	8 (1.6)	5 (1.0)	12	0
		Total	5080	133	58	66	5
Itai-Itai disease							
I-1	52-F	1972.1.27	205	105 (51.2)	3 (1.5)	116	20
		1972.4.27	215	62 (28.8)	5 (2.3)	76	20
I-2	66-F	1972.1.27	188	90 (47.9)	4 (2.1)	140	21
		1972.4.27	205	55 (26.8)	5 (2.4)	64	20
I-3	69-F	1972.1.27	200	93 (46.5)	6 (3.0)	130	34
		1972.4.27	210	49 (23.3)	5 (2.4)	58	19
		1972.7.27	200	45 (22.5)	5 (2.5)	51	17
I-4	69-F	1972.1.27	190	24 (12.6)	4 (2.1)	23	6
		1972.4.27	200	39 (19.5)	3 (1.5)	37	13
I-5	70-F	1972.1.27	239	105 (43.9)	10 (2.9)	135	39
		1972.4.27	240	139 (30.8)	19 (3.3)	73	27
I-6	70-F	1972.1.27	245	110 (44.9)	5 (2.0)	92	15
		1972.4.27	220	64 (29.5)	4 (2.3)	67	24
		1972.7.27	200	43 (21.5)	6 (3.0)	27	15
I-7	73-F	1972.1.27	189	119 (38.1)	3 (1.6)	80	6
		1972.4.27	260	159 (29.2)	6 (2.3)	82	15
I-8	70-F	1972.4.27	210	23 (11.0)	4 (1.9)	10	5
		1972.7.27	250	34 (13.6)	5 (2.0)	13	6
I-9	72-F	1972.4.27	200	19 (9.5)	4 (2.0)	17	2
I-10	72-F	1972.4.27	155	18 (12.0)	2 (1.3)	5	2
I-11	71-F	1972.4.27	170	17 (10.0)	2 (1.2)	9	3
I-12	72-F	1972.4.27	180	16 (8.9)	1 (0.6)	6	1
		Total	4571	1428***	111***	1311***	330***

CB: Chromatid break, Iso-CB: Isochromatid break, Exch: Chromatid exchange, Dic: Dicentric chromosome, A.f.: Acentric fragment, R.chr.: Ring chromosome, TR: Stable cells with translocations, GLD: G(21) long arm deletion, GSL: G(21) short arm large.

***: $P < 0.001$, **: $P < 0.005$, *: $P < 0.25$.

Itai-Itai disease patients and the normal control subjects (72-hour culture)

Chromatid aberrations	Chromosome aberrations						Aneuploid cells	
	unstable type			stable type			hypodiploid	hyperdiploid
Exch	Dic.	A.f.	R.chr.	TR	GLD	GSL	$2n = 45$	$2n = 47$
0	0	0	0	0	0	0	7	5
0	0	0	0	0	0	0	4	3
0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	5	1
0	0	0	0	0	0	0	1	2
0	0	0	0	0	0	0	—	—
0	0	0	0	0	0	0	—	—
0	0	0	0	0	0	0	—	—
0	0	0	0	0	0	0	—	—
0	0	0	0	0	0	0	29	
21	8	9	0	2	1	0	1	6
12	2	7	3	5	2	2	0	12
16	1	5	1	2	0	0	0	7
6	4	3	0	4	0	0	3	9
12	1	0	0	0	0	0	0	2
0	2	0	0	3	2	0	2	8
4	3	3	0	2	3	0	2	10
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	6
20	2	6	0	0	0	10	0	7
8	4	7	0	0	0	15	0	0
6	3	3	0	0	0	0	1	2
2	2	3	0	0	0	0	1	15
6	3	4	0	2	0	0	0	6
7	2	4	0	0	0	8	1	2
4	6	6	0	0	0	10	0	3
0	4	2	0	4	0	0	1	8
0	2	7	0	5	2	0	1	10
1	0	0	0	3	0	0	0	7
0	0	0	0	5	0	0	0	12
0	0	0	0	8	0	0	0	5
0	0	0	0	5	0	0	0	8
126***	49***	69***	4*	50***	10**	45***	158***	

Table 2. Structural abnormalities (control subjects) 1973 (50-hour culture)

Case No.	Date examined	Total No. of cells observed	Chromatid aberrations			Chromosome aberrations						Total structural aberrations (%)
			CB	Iso-CB	Exch	unstable type			stable type			
						Dic.	A.f.	R.chr.	TR	GLD	GSL	
1	1973.2	200	3	2	0	0	0	0	0	0	0	5 (2.5)
2	1973.2	200	2	0	0	0	1	0	0	0	0	3 (1.5)
3	1973.2	300	5	1	0	0	0	0	0	0	0	6 (2.0)
4	1973.2	300	6	0	0	0	0	0	0	0	0	6 (2.0)
5	1973.2	200	4	0	0	0	0	0	0	0	0	4 (2.0)
6	1973.2	250	6	3	0	0	1	0	0	0	0	10 (4.0)
7	1973.2	300	6	3	0	0	0	0	0	0	0	9 (3.0)
8	1973.2	200	5	0	0	0	0	0	0	0	0	5 (2.5)
9	1973.2	300	3	2	0	0	1	0	0	0	0	6 (2.0)
	Total	2200	40	11	0	0	3	0	0	0	0	54 (2.5)

Table 3. Chromosome count (control subjects) 1973.4

Case No.	Total No. of cells observed	< 45	45	46	47	47 >	Aneuploid cells	No. of polyploid cells	No. of endomitoses	Total numerical aberrations (%)
1	200	0	2	197	0	0	2	1	0	3 (1.5)
2	200	1	1	196	0	1	3	1	0	4 (2.0)
3	300	1	2	295	1	1	5	0	0	5 (1.7)
4	300	2	4	294	0	0	6	0	0	6 (2.0)
5	200	1	4	197	0	0	5	0	0	5 (2.5)
6	250	2	1	242	1	2	6	2	0	8 (3.2)
7	300	3	2	295	4	0	9	0	0	9 (3.0)
8	200	1	3	193	0	1	5	1	0	6 (3.0)
9	300	1	2	295	3	0	6	2	0	8 (2.7)
Total	2200						47	7	0	54 (2.5)

Table 4. Structural abnormalities (Itai-Itai disease patients) 1973.4 (50-hour culture)

Case No.	Date examined	Total No. of cells observed	Chromatid aberrations			Chromosome aberrations						Total cells with structural aberrations (%)
						unstable type			stable type			
			CB	Iso-CB	Exch	Dic.	A.f.	R.chr.	TR	GLD	GSL	
1	1973.2	200	75	26	10	2	3	0	7	0	0	68 (34)
3	1973.2	300	50	13	5	2	6	0	5	0	0	57 (19)
4	1973.2	200	25	20	2	0	0	0	4	0	0	39 (19.5)
6	1973.2	300	60	25	6	1	1	0	6	5	0	63 (21)
7	1973.2	200	25	14	1	0	2	0	3	1	18	43 (21.5)
2	1972.4.27	200	35	10	2	2	6	0	4	2	3	54 (27)
5	1972.4.27	200	26	10	2	1	3	0	3	5	36	52 (26)
	Total	1600	296***	118***	28***	8**	21***	0	32***	13***	57***	376*** (23.5)

Table 5. Chromosome counts (Itai-Itai disease patients) 1973.4

Case No.	Total No. of cells observed	<45	45	46	47	47>	Aneuploid cells	No. of polyploid cells	No. of endomitoses	Total numerical aberrations (%)
1	200	2	12	172	10	0	24	3	1	28 (14)
3	300	4	21	251	18	0	43	4	2	49 (16)
4	200	0	19	168	11	0	30	1	3	34 (17)
6	300	0	10	271	16	0	26	2	2	30 (10)
7	200	0	8	167	22	0	30	1	2	33 (16.5)
2	200	0	16	175	5	0	21	2	0	23 (11.5)
5	200	0	7	184	6	0	13	1	2	16 (8)
Total	1600						187***	14**	12***	213*** (13.3)

Table 6. Analysis of numerical aberrations in Itai-Itai disease patient

Cell type	C(+1)	C(—1)	E(+1)	E(—1)	G(+1)	G(—1)
No. of cells	184	83	19	9	30	12
(%)	(54.60)	(24.63)	(5.64)	(2.67)	(8.90)	(3.56)

C(+1): cells with extra C group chromosome. $2n = 47$.
C(—1): cells with monosomic C group chromosome. $2n = 45$.
E(+1): cells with extra E group chromosome (E18). $2n = 47$.
E(—1): cells with monosomic E group chromosome (E18). $2n = 45$.
G(+1): cells with extra G group chromosome. $2n = 47$.
G(—1): cells with monosomic G group chromosome. $2n = 45$.

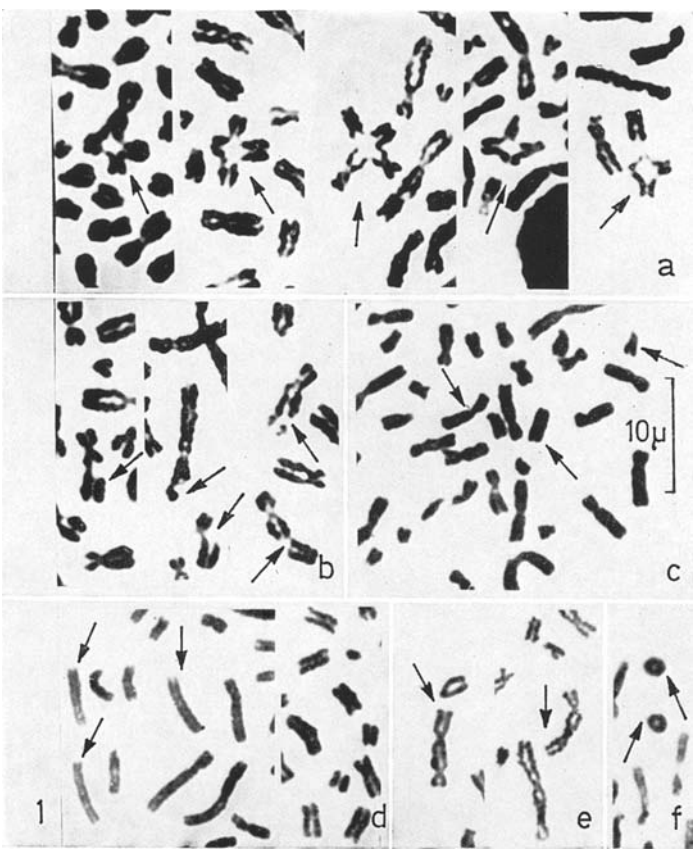

Fig. 1a—f. Chromosomal aberrations observed in Itai-Itai disease patients. Arrows indicate each type of aberrations. (a) Chromatid exchange. (b) Chromatid gap and break. (c) Isochromatid break. Fragment of a long arm (arrow, middle right) and a short arm (arrow, upper right) of a C chromosome. Compare with the normal one of the homologue (arrow, middle left). (d) Acentric fragments. (e) Dicentric chromosomes. (f) Ring chromosomes

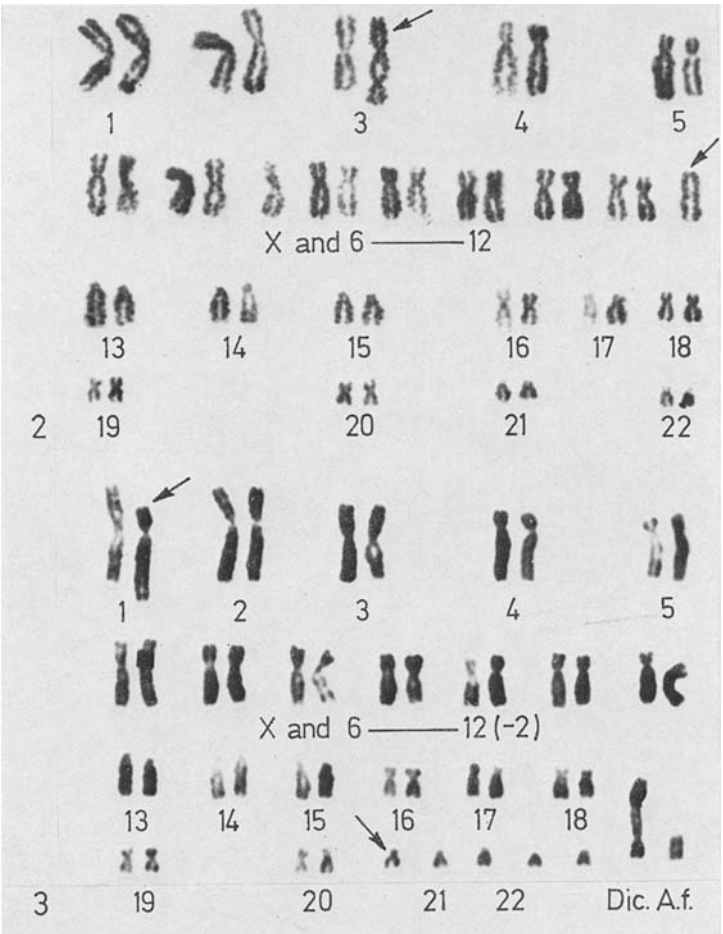

Fig. 2. Karyotype with dicentric chromosome and acentric fragment (arrow)

Fig. 3. Karyotype with stable type aberration (No. 1), dicentric and fragment, and an extra G chromosome

(mean value: 2.5%). These values were within the normal range. The predominant chromosome number was 46 in all cases and showed normal karyotypes.

Itai-Itai Disease Patients. Results obtained in 50-hour cultures are shown in Tables 4 and 5. Patients I-2 and -5 were examined on 27th April, 1972 (Table 4). They died of stomach cancer after the examination. The frequency of total cells with structural aberrations ranged from 19 to 34% (mean value: 23.5%) and total numerical aberrations from 8 to 16.5% (mean value: 13.3%); these were higher than those of controls ($P < 0.001$). Among the structural aberrations, chromatid, unstable type and stable type aberrations were observed in all 7 patients. Fig. 2 shows karyotypes with dicentric and acentric fragment (I-1). Fig. 3 shows a stable type (No. 1), dicentric and a fragment, and an extra G chromosome

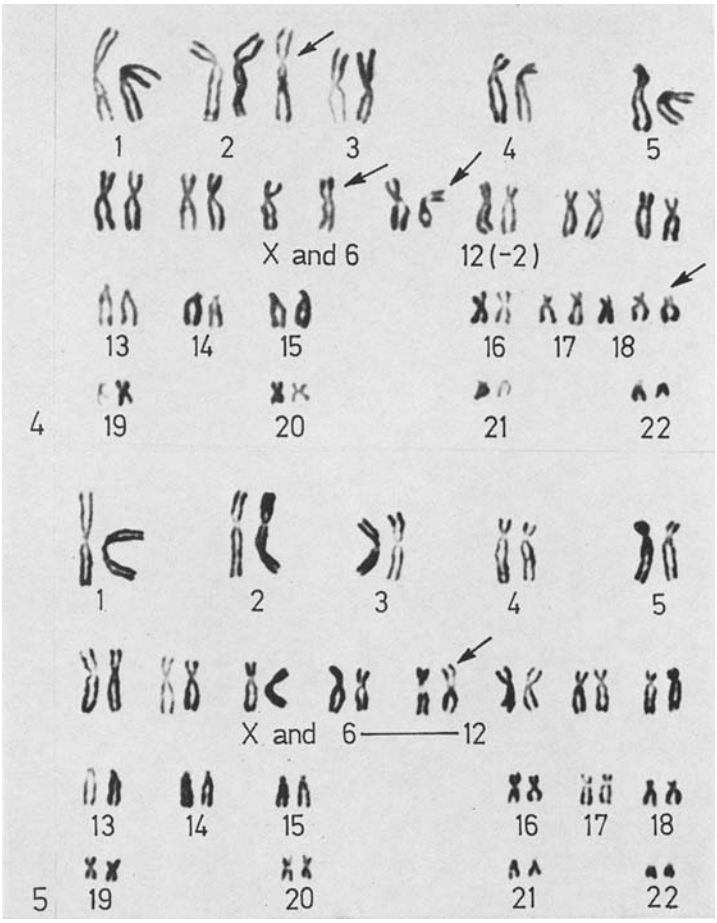

Fig. 4. Karyotype having extra an abnormal chromosome 2, loss of 2 C chromosomes, and addition of an extra E chromosome (18?). Note the abnormality of the chromosome pair 9 (pericentric inversion)

Fig. 5. Karyotype with pericentric inversion No. 9

in one metaphase cell. Fig. 4 shows a karyotype with an extra abnormal chromosome (No. 2), loss of 2 C chromosomes, abnormal morphology in pair No. 9, and an extra E chromosome (I-6). It is noted that the abnormal chromosome in pair No. 9 (Figs. 4 and 5) is the same as that reported by Wahrman *et al.* (1972) as a pericentric inversion of chromosome No. 9. Fig. 7 also shows a karyotype with 2 stable type aberrations, aberrant chromosome No. 2 and isochromosome of one of the C chromosomes. Noticeable is the fact that one or two of the C group chromosomes often participated in the formation of a majority of the structural aberrations. Fig. 8 shows the karyotype of a cell with large short arms of the G group chromosome (I-5). Fig. 6 shows a balanced translocation between B and D chromosomes. Since these aberrations were seen in the 50-hours cultures, in which

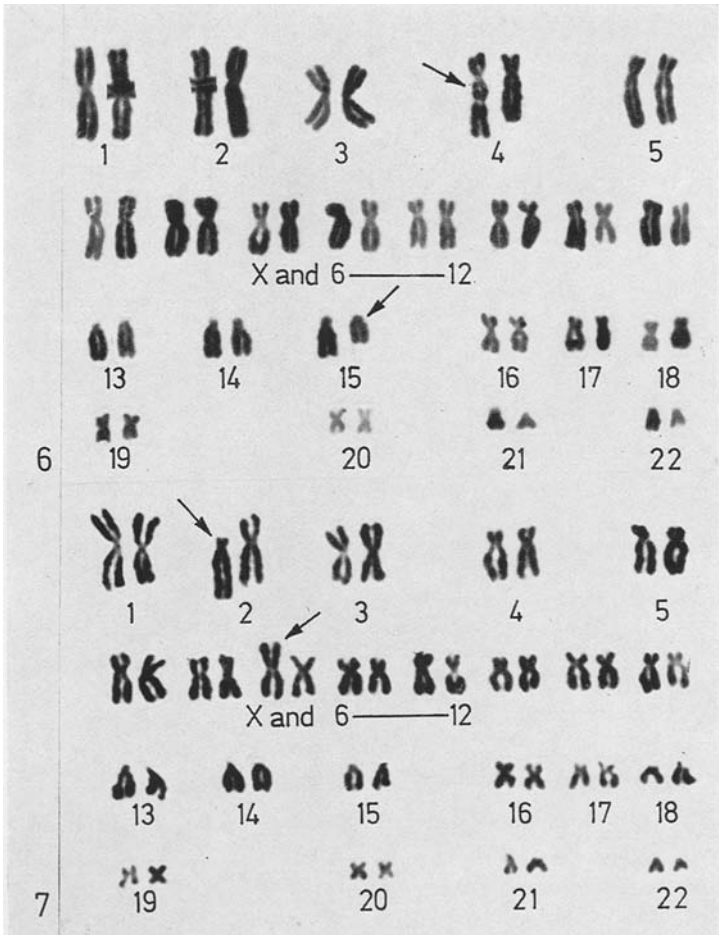

Fig. 6. Karyotype with the reciprocal translocation (B/D)

Fig. 7. Karyotype with the stable type aberration (No. 2) and isochromosome of a long arm of one chromosome in the C group

all of the cells were in their first mitosis, they were assumed to be derived aberrations that appeared *in vivo* or in blood stem cells. The levels of chromosome aberrations were compared on the basis of the frequency of chromatid, unstable type, and stable type aberrations. The observed number of chromatid aberrations was somewhat higher in the 72-hour cultures than in the 50-hour ones. The number of unstable type aberrations was also higher in the 72-hour cultures than in the 50-hour ones in cases I-1,3,6,7. A similar result was also obtained by the direct comparison between 50- and 72-hour cultures in cases I-2,5 in 1972 (Tables 1 and 4). Monocentric abnormal chromosomes resulting from translocations (TR) were obtained in all the cases at a fairly constant frequency of 1.5 to 3.5%, although the occurrence in the 72-hour cultures varied from case to case. With

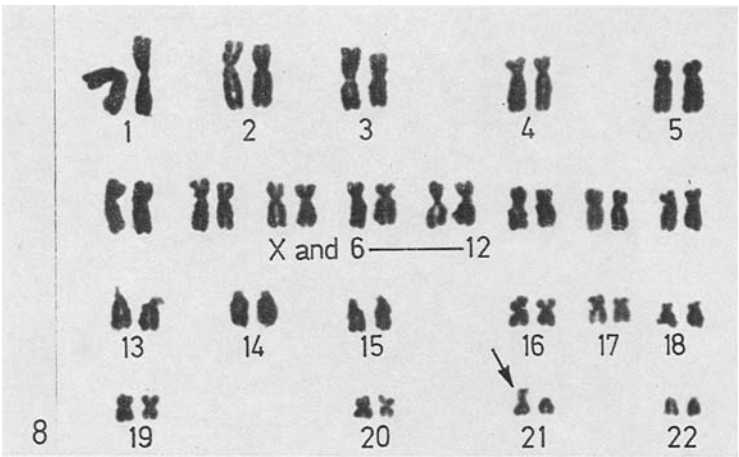

Fig. 8. Karyotype with an elongated short arm in the G chromosome

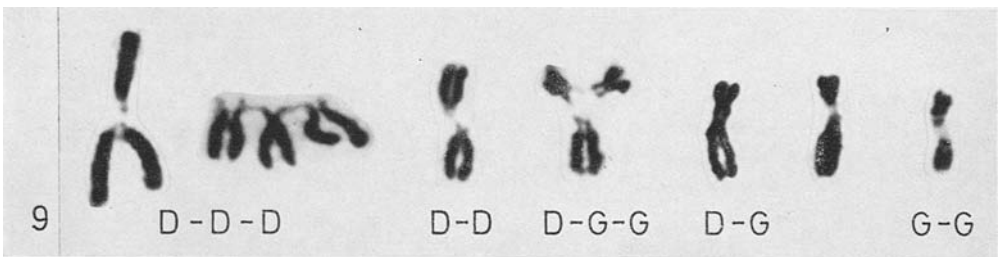

Fig. 9. Satellite associations (D-D-D, D-D, D-G, G-G)

regard to this point, the presence of increased frequencies of chromatid aberrations in one metaphase may often make identification of stable types difficult in the 72-hour cultures. There was no tendency for any particular culturing time to yield a higher frequency of aberrations. The results are in contrast to those previously obtained for Thorotrast-injected cases (Ishihara and Kumatori, 1965) and the cases of Buckton and Pike (1964) in which there was a rapid decrease of unstable abnormalities with lapse of time in culture. Accordingly, with regard to the increase of unstable type aberrations in the 72-hour culture, it may be considered as follows. If the chromatid aberrations do not result in the mechanical difficulties in the separation of chromatid at mitosis, they will pass into the daughter cells, and reappear as "derived" chromosome type aberrations at the following mitosis. Another possibility may be the existence of an external agent that could induce these chromosomal aberrations.

Aneuploidy (hyperdiploid and hypodiploid) was present at a comparatively high frequency and occurred at about four times or more the frequency in the patients than in the controls. Among the 345 aneuploid cells (hyperdiploid: 233, hypodiploid: 112), 337 cells were analysed, and the results are presented in Table 6. Almost all hyperdiploid cells in the patients had 47 chromosomes, including

extra C, E, or G group chromosomes. Many of the hypodiploid cells had 45 chromosomes as monosomic types of C, E and G group chromosomes. It is to be noted that most of the hyperdiploid cells (184/233) were characterized by an extra C chromosome, the incidence being significantly higher than that of the trisomies of any other chromosomes ($P < 0.001$). The presence of cells with 47 chromosomes with an extra C chromosome is an uncommon occurrence in normal peripheral blood cultures in our laboratory. The occurrence of a missing C chromosome in the hypodiploid cells (83/112) was also significantly more frequent than that of any other chromosomes ($P < 0.001$). Therefore, it should be necessary to determine whether the specific chromosome is related to the disease.

In addition to these structural and numerical aberrations, satellite associations of the D and G group chromosomes were often observed in the cells of the patients, although the cause of the phenomenon is unknown (Fig. 9). Satellite associations of D-D-D, D-D, D-G and G-G are shown in this figure.

Discussion

Among 12 patients with Itai-Itai disease examined by us, there was a remarkably high frequency of chromosome aberrations in their blood cells, as compared with the results in control subjects. Comparing the cytogenetic findings in the 12 patients in detail, it was found that 8 patients showed a remarkable high frequency of chromatid aberrations, whereas the other 4 patients showed a much lower frequency of such aberrations, although significant numbers of the stable type aberrations were observed in the latter patients. The results also showed that the frequency of aneuploidy among the 12 patients was significantly higher than in the controls. Analysis of the aneuploidy in cells with 47 chromosomes showed three types of trisomy, i.e., extra C, E or G chromosomes. In cells with 45 chromosomes, the same C, E and G chromosomes were found to be monosomic. The incidence of cells with extra C or missing C chromosomes is significantly higher than that of any other chromosomes. Therefore, it is very important to determine whether this aneuploid condition and structural aberrations are derived from the stem cells or not, and whether a specific chromosome is related to the disease.

Since the 72-hour culture consisted of a mixture of first and second division cells, it was necessary to establish whether or not chromosome aberrations existed in cells in a 50-hour culture. Among the 7 patients who showed a very high frequency of chromosome aberrations in the 72-hour culture, we also observed the same chromatid and stable aberrations, dicentric and fragments, an extra or missing C chromosome in the 50-hour culture. From this, we can conclude that there is a strong possibility that the aberrations occurred in the stem cells. Also, if is true that the aberrations originated within the stem cell, then we also must consider the possibility of some agent having caused this condition. Since it is thought that Itai-Itai disease arises from chronic cadmium poisoning, the relation between cadmium and the chromosome aberrations of somatic cells is a focal point. Thus, the chromosome breaks induced by cadmium sulfide *in vitro* in our preliminary experiment is a very interesting finding. However, whether or not chromosomal aberrations could be induced *in vivo* by exposure to cadmium at a

much lower concentration over a much longer period of time remains uncertain. Therefore, we must consider the possibility that the chromosome aberrations in patients with Itai-Itai disease are not due to chronic cadmium poisoning, but that the chromosomes of these patients have an unusual high susceptibility to cadmium.

The chromosome aberrations which are observable in the Itai-Itai disease patients are comparable to those of chromosome breakage syndromes. There is a belief that where these kind of chromosome aberrations occur, cancer, leukemia and related diseases occur at a high frequency. Since death through cancer is occurred among the Itai-Itai disease patients, further exploration of this relationship is necessary. A study is now in progress concerning the identification by banding pattern of these structural aberrations and the trisomy or monosomy chromosomes.

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