57. *Chromosomal Abnormalities in Cultured Leucocyte Cells from Itai Itai Disease Patients*[*)]

By Yukimasa SHIRAISHI[**)] and Tosihide H. YOSIDA[***)]

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In the previous study (Shiraishi *et al.* 1972) we found that the cadmium sulfide (CdS) produced a marked increase in the frequencies of chromosomal aberrations such as chromatid and isochromatid breaks, translocations and dicentrics, when the compound was added to cultures of human peripheral leucocytes. This compound is known as the cause of Itai Itai disease, which is a kind of diseases by environmental pollution. However, it is discussed by many scientists whether this compound is related directly to the cause of Itai Itai disease. To determine this problem, we examined the chromosome of the disease patients. The results of the observations are preliminarily described in this paper.

Materials and methods. Human peripheral leucocytes were obtained from seven patients diagnosed as a typical Itai Itai disease. As control the leucocytes of six normal females were examined. The leucocytes were cultured according to the routine procedures of the whole blood culture. One ml heparinized blood taken from each individual was added to 10 ml of media 1640 (Nissui Seiyaku Co. Ltd., Tokyo, Japan) containing 20 per cent calf serum and 0.1 ml phytohemagglutinin (Burrough Wellcome). All cultures were incubated for 72 hours at 37°C, and colcemide at a concentration of 0.02 μg/ml was added to the cultures 3 hours before harvest in order to obtain metaphase chromosomes. Slides were prepared by the routine air-drying methods and stained with Giemsa. In each case, fifty well-spread metaphase plates were examined for the chromosomal analysis.

Finding and remarks. In cultured leucocytes from the patients with Itai Itai disease, various chromosomal aberrations, such as translocations, chromatid and isochromatid breaks, dicentric chromosomes and acentric fragments, were observed in a very high frequency. In the Fig. 1, some of the aberrations are illustrated.

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**) Department of Anatomy, School of Medicine, Kanazawa University, Kanazawa, 920.

***) Department of Cytogenetics, National Institute of Genetics, Misima, 411.

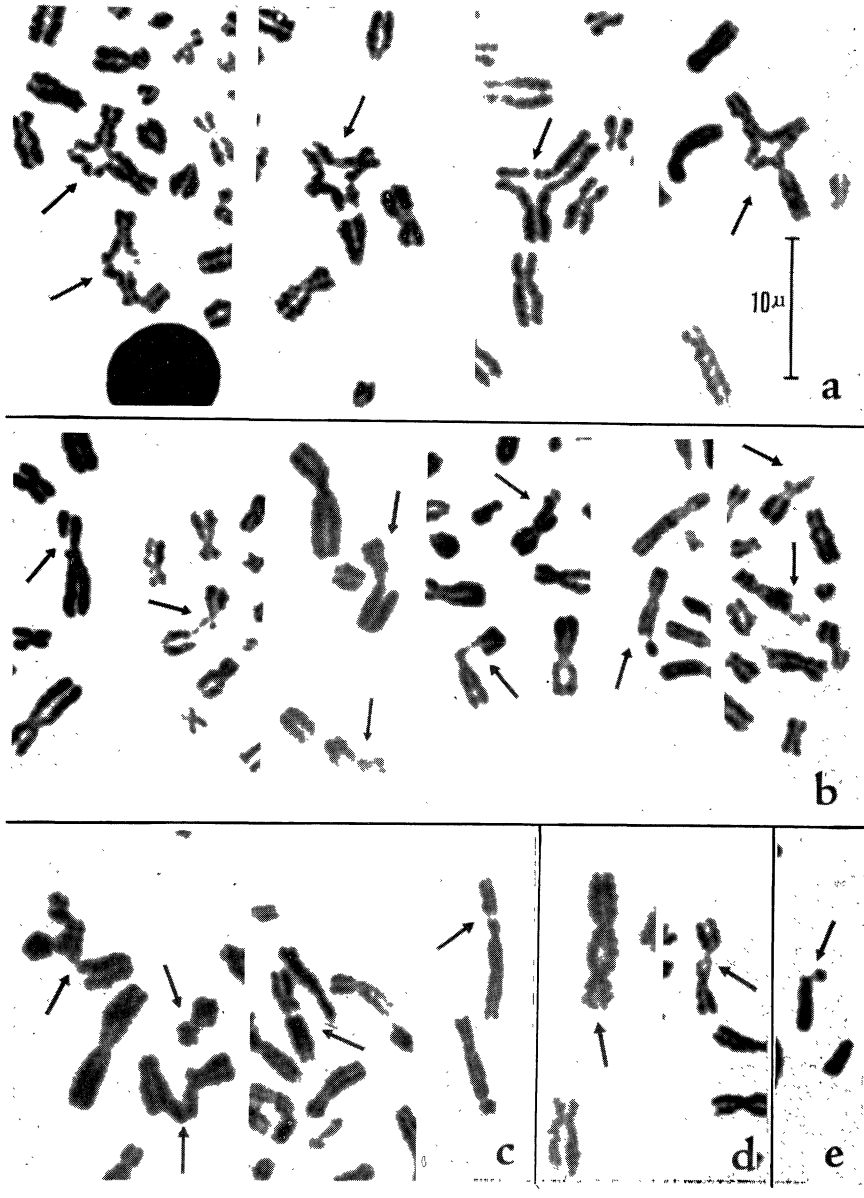

Fig. 1. Chromosomal aberrations observed in Itai Itai disease patients.
a: Chromatid translocations (quadriradial and triradial formations).
b: Single chromatid breaks, c: Isochromatid breaks or gaps, d: Dicentric or dicentric like chromosomes. e: Acentric fragment. Arrows indicate each type of chromosome aberrations.

Among the aberrations, quadriradial and triradial formations (Fig. 1a) developed by chromatid translocations, which were very rare in normal subjects, were observed frequently. The chromatid

Table I. Types and frequencies of chromosomal aberrations observed in Itai Itai disease patients and the normal control subjects

Case No.	Age and sex	Total No. of cells observed	No. of cells with single chromatid breaks	No. of cells with isochromatid breaks or gaps	No. of cells with chromatid translocations	No. of cells with dicentric or dicentric like chromosomes	No. of cells with acentric fragments	Total No. (%) of cells with chromosome abnormalities
	Controls							
C-1	58-F	50	1	0	0	0	0	1(2)
C-2	62-F	50	0	0	0	0	0	0(0)
C-3	67-F	50	0	0	0	0	0	0(0)
C-4	70-F	50	1	0	0	0	0	1(2)
C-5	70-F	50	0	0	0	0	0	0(0)
C-6	78-F	50	0	0	0	0	0	0(0)
	Itai Itai disease							
I-1	52-F	50	15	2	8	2	3	30(60)
I-2	66-F	50	18	3	6	0	1	28(56)
I-3	69-F	50	14	3	6	0	0	23(46)
I-4	69-F	50	6	1	0	0	0	7(14)
I-5	70-F	50	19	3	7	1	2	32(64)
I-6	70-F	50	12	2	3	2	1	30(60)
I-7	73-F	50	15	2	6	1	3	27(54)

breaks are also observed rather frequently in the leucocytes (Fig. 1b). Isochromatid breaks or gaps are shown in Fig. 1c. Dicentric or dicentric like chromosomes and acentric fragments are illustrated in Figs. 1d and 1e. Out of the above aberrations, ring chromosomes are observed in a few cells.

The frequency of cells with the chromosome abnormalities was assessed in 50 cells in each of the subjects (Table I). The data show that the frequency of the chromosome abnormalities in all Itai Itai disease patients was significantly higher than that of the control subjects. Especially, the chromatid breaks were observed as common type of chromosome abnormalities in all patients. In addition, cells showing translocations were found more frequently than dicentrics and acentric fragments. Of the 7 patients studied, one (case No. I-4) was found to show more or less low frequency of chromosome abnormality; the frequency of cells with chromatid and isochromatid breaks or gaps were 14 per cent, while the other six patients (case No. I-1, I-2, I-3, I-5, I-6 and I-7) showed that the frequencies of cells

with chromosome abnormalities were at approximately 50 to 60 per cent.

A very similar damage was observed in the cultured normal leucocytes which were treated with cadmium sulfide *in vitro* (Shiraishi *et al.* 1972). In this experiment the chromosome damage appeared after a short time treatment (4 and 8 hours). High occurrence of chromosomal damages in the Itai Itai disease patients suggests an additional long-term effect of the cadmium, because they have used the water containing cadmium for a long period of time. The chromosome abnormalities observed in the patients do not seem to be an effect of medical treatment, because they have not received any specific therapeutic treatments which cause a induction of chromosomal abnormalities. In the present time, it is not determined whether the chromosomal abnormalities result from the permanent damage of the blood stem cells, or damage in the peripheral blood cells which were affected by the chemical included in the patient body. Detailed examinations are in progress now on many other Itai Itai disease patients and their pedigrees.

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Reference

Shiraishi, Y., H. Kurahashi, and T. H. Yosida: Proc. Japan Acad., **48**, 133–137 (1972).