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Chromosome Studies in Acute and Chronic Myeloid Leukaemia

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The development of cytogenetic techniques suitable for the study of human chromosomes (17,26) has yielded additional information about acute and chronic leukaemia. Now a closer approach to the problems of the nature and evolution of leukaemia has become possible.

We obtained chromosome preparations from short-term cultures of peripheral leukocytes according to a slight modification of the technique described by MOORHEAD et al. (26) using colchicine and in some instances vinca-leukoblastine and the air-drying technique (37).

Table 1 depicts the findings in 3 cases of acute para-myeloblastic leukaemia. Similar to the results previously published (4, 18, 19, 30, 44), we found that these patients had morphologically normal chromosome sets, whereas the chromosome counts varied. As suggested by FORD et al. (14) for chromosome preparations from normal leukocytes, we also regard at least part of the changes in chromosome number of leukaemic cells as artefacts of technique. Perhaps blast cells are more susceptible to osmotic and mechanical lesions during the preparation. From vincaleukoblastine-treated cultures metaphases of a quality were obtained similar to those which were

treated with colchicine.

On the contrary in chronic myelogenous leukaemia the identification of an abnormal chromosome (Ph^1-chromosome) in the great majority of the cases studied (1-3, 13, 20, 29-32, 35, 38, 39, 44) has supported the assumption that for the first time a constant structural chromosome change for one type of leukaemia could be detected. Few authors, however, reported on a minute chromosome similar to the Philadelphia one which was found to be also visible in healthy persons (45) as well as in acute paramyeloblastic leukaemia (24). In the peripheral blood of 3 treated and 2 untreated patients with chronic myelogenous leukaemia we found the Ph^1-chromosome to be positive in 9 to 56 per cent, the values of the untreated ones were 38 and 44 per cent respectively. For closer studies the application of direct marrow techniques has been recently postulated (38, 39).

Two cases of chronic myelogenous leukaemia disclosed unusual findings which seem worthwile to report in detail.

The findings in the first case refer to a woman, 43 years of age, who developed evidence of chronic myeloid leukaemia in February 1961. During April and May 1961 and from February to March 1963 she was given the total dose of 500 mgm. myleran. Since she developed a blast crisis in April 1963 she recieved 1.2 gm. purinethol in a daily dosage of 50 mgm. until the first leukocyte culture was started. The white cell count was then 4,200 with 35 per cent blasts, 2 per cent promyelocytes, and 21 per cent myelocytes in the differential count. Chromosome counts (table 2) revealed considerable numerical aberrations (42) which cannot be due to artefacts only. 19 cells or 11 per cent showed the feature of endoreduplication. It has been described in man in one case of acute leukaemia (10), in one case of chronic myeloid leukaemia (16), and very seldom also in

Nr.	pretreatment		<44	44	45	46	47	48	92	total
203	2,5 mg Vincristine	VLB	4	3	3	9	–	–	1	20
37	11,5 mg Vincristine	COL	–	3	8	18	–	–	–	29
42	ø	COL	7	3	8	39	4	1	–	62
90	1,075g Purinethol	COL		1	3	29	2	–	–	35

COL = Colchicine
VLB = Vincaleukoblastine

Table 1: Chromosome counts in 3 acute paramyeloblastic leukaemias

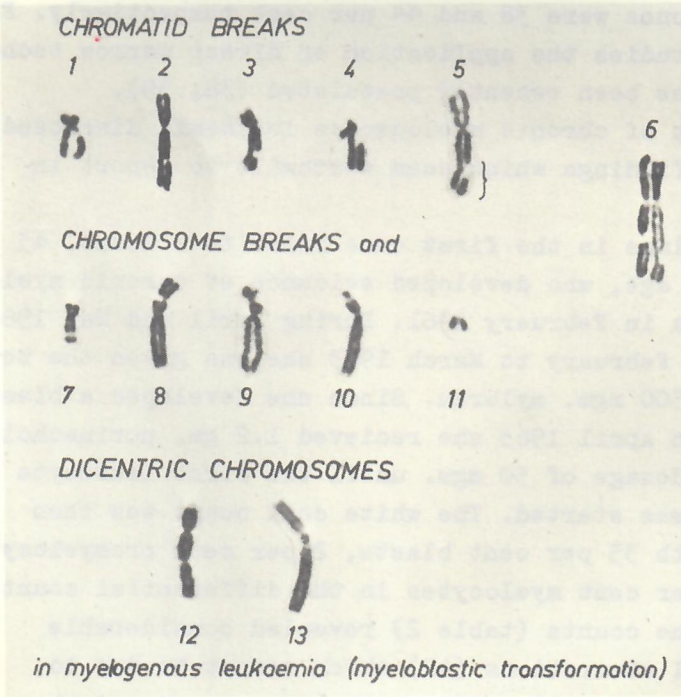

Figure 1: Examples of structural chromosome abnormalities (see text)

normal cells (21). It is regarded as a masked endomitosis (36). In the resting stage an extra division of the chromosomes takes place and diplochromosomes will be present during prophase and metaphase (22, 23). The centromeres hold together four chromatids instead of two. The endoreduplication was a constant finding in three cultures, while in the controls and in all the rest of the analysed cultures of normal patients we could never detect this phenomenon, only with the exception of overdosage experiments (8) with tritium-labelled thymidine. This is also in accordance with the results of chromosome analyses in X-ray treated cultures (5, 6, 33). Additionally in this case we found in 48 per cent of the scored cells chromatid and chromosome breaks Some examples are depicted by the next slide. (figure 1) We classified them as follows:

1) chromatid breaks proximal from the centromere
2) intermediate
3) distal
4) with dislocation of a chromatid
5) with translocation, as revealed in the idiogram and a broad achromatic gap
6) a chromatid and chromosome break in a diplochromosome
7) chromosome breaks distal from the centromere
8) intermediate in the short arms
9) with dislocation
10) with a broad achromatic gap
11) an acentric fragment probably resulting from a chromosome break.

Furthermore dicentric chromosomes were detected (12) and 13) one or more questionable new dicentric chromosome with a narrow space between the two centromeres were found in five cells.

The detectable morphological chromosome aberrations are summarised in the next slide (table 2). It could be

proved that the majority of the chromatid and chromosome breakages occurred in the long arms and distal from the centromere. A complex chromosome aberration proved to be a breakage reunion "bivalent". Further chromosome aberrations are revealed by idiogram analysis. The first one shows a chromosome break in number 3 and probably in number 1 near the centromere. The second slide reveals a questionable translocation between number 2 and 3, with a chromatid break and a wide achromatic gap. A fragment is attached to number 18 and a number 15 has perhaps lost part of its long arms. The next slide shows a probable translocation between number 1 and 2, and a chromosome break in the short arms of number 4 with dislocation. And in the last idiogram the numbers 2, 5 and two of the group number 7-12 are missing, while 5 abnormal chromosomes are present: a questionable dicentric one with a narrow space between the two centromeres, another dicentric, a chromosome with a probable break in the centromere, and two chromosomes with abnormal arm proportions.

In another male patient, now 38 years of age, the diagnosis of chronic myeloid leukaemia was established in 1957. In 1954 he had had an X-ray irradiation of 1350 r of the lumbosacral spinal column because he suffered from osteochondrosis. After the onset of the leukaemia he recieved in the same institute from 1958 to 1960, the total dose of 3025 r applied to the spleen and the bone marrow. From June 1961 to January 1963 he was given cytostatic agents (42 gm. mannit-myleran, 4.87 gm. thiadiazole, 95 mgm. vincaleukoblastine and 0.598 gm. myleran). During the four months preceeding the start of the first leukocyte culture he did not recieve any therapy except 8 mgm. methylprednisolone per day. The haematological data were then as follows: haemoglobin 10.1 gm. per cent, mean corpuscular haemoglobin 35, red cell count 2.98 mill., white cell

<44	44	45	46	47	48	50	54	56	58	92	>92	endo-reduplication	total
6	7	10	106	11	6	1	1	1	1	4	1	19	174

	chromatid breaks		chromosome breaks	
	short arm	long arm	short arms	long arms
proximal	2	5	1	
intermediate	2 1(Nr.1)	6	2	4
distal	1 1(Nr.1)	9	3	1 Nr.(16-18) 12
with a broad achromatic gap		4	–	
with dislocation		1	1	1
with translocation	1			
acentric fragments	–		16	
single fragments	6			
chromatid- and chromosome break in a diplochromosome	1			

Ph¹-chromosome	dicentrics	abnormal length of satellites (Nr.13-15)	complex chromosome aberration
16	8	3	1

metaphases total 174
with chromosome aberrations 83 = 48 per cent

Table 2: E.G., 43, (61, 81, 86)
Chromosome counts and structural aberrations in chronic myelogenous leukaemia (myeloblastic transformation)

count 12,300 and platelets 10,000. In the differential count all stages of myelopoiesis were present, no blast crisis was detectable.

Similar to the case just reported, the chromosome counts also revealed numerical aberrations and the evidence of endoreduplication, in 8 per cent of the scored cells (table 3). Structural chromosome aberrations were detected in 45 per cent of the cells. Distal chromatid and chromosome breaks also outnumbered the other forms of breakage. The long arms of the chromosomes exhibited the majority of breaks. A ring chromosome was also present in one of the cells. From the complex chromosome aberrations a breakage reunion "bivalent" was seen, three others showed the features of a three-armed star. In the first example, shown in the next slide, this abnormality could not clearly be distinguished either as a complex chromosome abnormality with a centromere holding together 6 chromatid arms, or the attachment of two centromeres, or as an occasional position of two chromosomes, one out of the group number 3 and the other as a trisomic one of the group number 13-15. The second example depicts a chromosome number 15 with attached chromatid fragments, two supernumary chromosomes, a distal chromosome break in number 2, and a missing number 6. In the next example, a probable number 12 chromosome of which the centromere can be clearly seen has become attached to unidentifiable chromosome fragments, an additional chromosome is also present. These complex structural aberrations may be caused by the previous irradiation.

The chromosome data of the two patients indicate that a variety of numerical and morphological aberrations may also occur in treated myelogenous leukaemia. Manifold breakagefusion-events lead to various structural changes, however, on the one hand the influence of irradiation

<44	44	45	46	47	48	52	60	66	78	84	86	92	endo-reduplication	total
5	3	9	60	6	2	1	1	1	1	1	1	3	8	102

	chromatid breaks		chromosome breaks	
	short arm	long arm	short arms	long arms
proximal		2		3
intermediate	1	3	2	2
distal		4		10
with a broad achromatic gap	2		–	
acentric fragments	–		11	
single fragments	7			
ring chromosome			1	

Ph'-chromosome	dicentrics	complex chromosome aberrations
17	5	4

metaphases total 102
with chromosome aberrations 45 = 45 per cent

Table 3: H.H., 38, (67, 89)

Chromosome counts and structural aberrations in chronic myelogenous leukaemia.

(9, 11, 25, 34, 40, 43), cytostatic agents, and other events (27) and on the other hand the influence of culture conditions and preparation techniques must be taken into consideration. We have to be aware of the fact that with the applied cytogenetic techniques only gross chromosome damages can be made visible. It is now possible, however, to estimate the relative deoxyribonucleic acid synthesis in each chromosome. The next slide shows a labelled metaphase and in the next one the chromosomes of the same metaphase are classified according to the Denver nomenclature. (12) The statistical analysis of the incorporation of tritiated thymidine in chromosomes (7, 15, 27, 41) of normal and leukaemic leukocytes will perhaps yield further information.

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