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Chromosome abnormalities in chronic active hepatitis[1]

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SYNOPSIS An investigation on human peripheral blood lymphocyte chromosomes in chronic active hepatitis was carried out. A higher percentage of chromatid and chromosome lesions was recorded in all patients studied as compared with control groups—normal individuals, healthy subjects who had suffered from acute viral hepatitis, patients with alcoholic liver disease, and patients with mechanical jaundice due to cancer. The possible origin of these abnormalities is discussed.

Chromosome abnormalities have been observed in peripheral blood lymphocytes (Mella and Lang, 1967) and in bone marrow cells (Matsaniotis, Kiossoglou, Maounis, and Anagnostakis, 1966, 1970) of patients with acute viral hepatitis. The hepatitis virus was assumed to be the chromosome-damaging agent since the cell-free plasma from patients with acute viral hepatitis was able to transfer the effect to normal lymphocytes cultured *in vitro* (El-Alfi, Smith, Biesele, 1965; Baroyan, Barinsky, and Shatkin, 1970). It is known that a significant proportion of patients with acute viral (serum and infectious) hepatitis had hepatitis-associated (HA) antigen in the serum (Okochi and Murakami, 1968; Gocke and Kavey, 1969; Elling, Nielsen, and Dietrichson, 1970). The HA antigen is considered by some authors to be closely related to, if not identical with, hepatitis virus (Bayer, Blumberg, and Werner, 1968; Blumberg, Sutnik, London and Millman, 1970). This antigen is also encountered in chronic active hepatitis, though less frequently (Wright, McCollum, and Klatskin, 1969; Hadziyannis, Merikas, and Afroudakis, 1970). If hepatitis virus is involved, at least in part, in the pathogenesis of chronic active hepatitis—as it has already been suggested (Prince, Leblanc, Krohn, Masseyeff, and Alpert, 1970; Maynard, Sadikali, Anthony, and Barker, 1970; Nielsen, Dietrichson, Elling, and Christoffensen, 1971)—chromosome abnormalities could be assumed to occur in the cells of patients with chronic active hepatitis.

In order to test this hypothesis we have studied the chromosomes of peripheral blood lymphocytes from 12 patients with chronic active hepatitis, all of them having a past history of viral hepatitis. The results of this investigation are presented here.

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Material and Methods

The patients were diagnosed as suffering from chronic active hepatitis on the grounds of the usual clinical and laboratory criteria (hepatosplenomegaly, elevation of serum SGOT transaminase and of gamma globulin levels, markedly raised bromsulphalein retention, antinuclear antibodies, and a clinical course of more than one year). In four patients (the only patients accepting it), needle liver biopsy was carried out and a histological picture typical of chronic active hepatitis was seen (parenchymal cell necrosis and chronic inflammatory cell infiltration most intensive in the perilobular region, erosion of the portal limiting plate, and periportal fibrosis). None of the patients studied suffered from any noticeable disease that might cause chromosome damage or were exposed to agents known to break the chromosomes.

As controls, four groups of subjects were used: five healthy persons with no past history of hepatitis; five persons who had had viral hepatitis at least five years before but who, by the time of this investigation, were clinically well, and the routine liver function tests did not reveal any biochemical change; three patients with alcoholic liver cirrhosis, with no history suggestive of acute viral hepatitis; four patients with mechanical jaundice due to cancer.

LEUCOCYTE CULTURE

The blood was collected on heparin and allowed to

stand at room temperature for about one hour. The supernatant leucocyte-rich plasma was centrifuged and the cells were suspended in standard IC-70 lymphocyte serum free medium (Brucher, Teodorescu, Stefănescu, and Titu, 1972) with phytohaemaglutinin M (Difco). The cells were harvested after 72 to 96 hours of incubation at 37°C, the last three hours in the presence of colcemid (CIBA), and routinely prepared for chromosome analysis.

Results

The results on individual cases are shown in Table I. It can be seen that all cases presented chromosome lesions in certain percentages of cells from cultured blood samples, as well as aneuploidy. The incidence of chromosome abnormalities in patients with chronic active hepatitis is prominently higher than in control groups (Table II). Both chromosome and chromatid type aberrations were recorded. These aberrations consisted mostly in gaps and breaks but a few rearrangements were also noticed. Mention must be made that no differences in percentages of the blast cells and in mitotic indices in 72-hr cultures were found between study and control groups.

As may be seen in Table II, the incidence of chromosome abnormalities in the five subjects who suffered from viral hepatitis but were clinically and biochemically well is not different from those

Case	*Age*	*Sex*	*Viral Hepatitis (yr before)*	*Normal Cells in 100 Cells Examined*	*Chromosome Abnormalities*[1]						
					Cell Distribution		*Chromatid Aberrations*		*Chromosome Aberrations*		
					Aneuploid Cells	*Polyploid Cells*	*Gaps and Achromatic Areas*	*Breaks*	*Quadri-radials*	*Fragments*	*Dicentrics*
1[2]	38	F	9	81	10	1	2	2	—	6	1
2[2]	51	F	12	79	10	—	6	—	—	4	1
3[2]	22	F	15	84	6	—	7	—	1	3	—
4[2]	25	F	4	83	6	1 (EDR)	6	—	—	5	—
5	64	F	24	77	9	—	2	9	—	6	—
6	26	F	3	82	2	—	8	3	—	6	—
7	48	M	2	91	2	—	2	1	—	5	—
8	67	M	19(?)	78	6	2	5	5	—	4	2
9	60	M	28	85	2	1	6	3	—	6	—
10	58	M	8	78	6	—	2	6	—	9	—
11	58	F	22	76	5	3 (1=EDR)	10	4	1	4	—
12	56	F	21	87	2	—	6	3	—	4	—

Table I *Chromosome abnormalities in the present series*

[1]Different abnormalities observed in the same cell were recorded separately in their respective columns.
[2]Liver biopsy.

Group	*Total Cells Examined*	*Normal Cells (%)*	*Percentage Chromosome Abnormalities*						
			Aneuploid	*Polyploidy*	*Chromatid Aberrations*			*Chromosome Aberrations*	
					Gaps	*Breaks*	*Quadri-radials*	*Fragments*	*Dicentrics*
Chronic active hepatitis	1200	81·8	5·5	0·7	5·2	3·0	0·17	5·2	0·3
Normal persons having history of viral hepatitis	500	94·0	2·4	0	1·4	2·0	0	0·2	0
Normal persons with no history of viral hepatitis	500	95·6	2·0	0·4	1·6	0·4	0	0	0
Patients with alcoholic liver disease	300	92·1	3·3	1·0	2·3	1·6	0	0·66	0
Patients with mechanical jaundice due to cancer	400	93·1	2·7	1·2	2·7	1·0	0	0·5	0

Table II *Percentage of chromosome abnormalities*

noticed in the five healthy individuals. Similarly, the frequency of chromosome abnormalities in patients with alcoholic liver disease and with mechanical jaundice was in the range recorded in normal controls (Table II).

Discussion

So far, chromosome abnormalities in patients with chronic active hepatitis have not reported. They may be induced by the hepatitis virus which might be chronically carried by lymphocytes as was lately shown to be the case for adenoviruses and some herpes viruses (Nász, Kulcsar, Dán, and Sallay, 1971).

Alternatively, the chromosome abnormalities could be caused by certain damaging substances released by the injured liver. This hypothesis seems, however, to be overruled, since the patients with alcoholic liver disease, and those with mechanical jaundice did not exhibit chromosome abnormalities, although their liver cells were also injured.

Since our study was carried out on long-lived small lymphocytes, the chromosome abnormalities found in these cells could develop during the acute viral disease and persist for many years. This hypothesis is also overruled because the lymphocytes of healthy persons who had suffered from viral hepatitis at least five years before did not display chromosome abnormalities more than those in the control group. It seems therefore that persistence of the chromosome aberrations since the acute viral disease is unlikely.

Further experiments well under way in our laboratories are being carried out to establish the actual nature of the chromosome-damaging agent for lymphocytes in patients with chronic active hepatitis. In any case, the presence of chromosome breakage in lymphocyte cultures from patients with chronic active hepatitis may be useful for diagnostic purposes.

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References

Baroyan, O. V., Barinsky, I. F., and Shatkin, A. A. (1970). Inhibitory effect of sera from patients with Botkin's viral hepatitis on the differentiation and mitotic activity of lymphocytes and integrity of their chromosomes in the course of serial passaging in vitro. *Acta virol.*, **14**, 324.

Bayer, M. E., Blumberg, B. S., and Werner, B. (1968). Particles associated with Australia antigen in the sera of patients with leukaemia, Down's syndrome and hepatitis. *Nature (Lond.)*, **218**, 1057-1059.

Blumberg, B. S., Sutnik, A. I., London, W. T., and Millman, I. (1970). Australia antigen and hepatitis. *New Engl. J. Med.*, **283**, 349-354.

Brucher, J., Teodorescu, M., Stefănescu, D. T., and Titu, H. (1972). Proliferative activity and antibody synthesis in culture of lymphoid cells grown in a serum free medium. *Arch. roum. Path. exp.*, **31**, 105-111.

El-Alfi, O. S., Smith, P. M., and Biesele, J. J. (1965). Chromosomal breaks in human leucocyte cultures induced by an agent in the plasma of infectious hepatitis patients. *Hereditas (Lund.)*, **52**, 285-294.

Elling, P., Nielsen, J. O., and Dietrichson, O. (1970). Australia antigen in patients with various liver diseases. *Acta path. microbiol. scand.*, Sect. B, **78**, 657-663.

Gocke, D. J., and Kavey, N. B. (1969). Hepatitis antigen: correlation with disease and infectivity of blood donors. *Lancet*, **1**, 1055-1058.

Hadziyannis, S. J., Merikas, G. E., and Afroudakis, A. P. (1970). Hepatitis-associated antigen in chronic liver disease. *Lancet*, **2**, 100.

Matsaniotis, N., Kiossoglou, K. A., Maounis, F., and Anagnostakis, D. E. (1966). Chromosomes in infectious hepatitis. *Lancet*, **2**, 1421.

Matsaniotis, N., Kiossoglou, K., Maounis, F., and Anagnostakis, D. (1970). Chromosomal aberrations in infective hepatitis. *J. clin. Path.*, **23**, 553-557.

Maynard, E. P., Sadikali, F., Anthony, P. P., and Barker, L. F. (1970). Hepatitis-associated antigen and cirrhosis in Uganda. *Lancet*, **2**, 1326-1328.

Mella, B., and Lang, D. J. (1967). Leukocyte mitotis: suppression in vitro associated with acute infectious hepatitis. *Science*, **155**, 80-81.

Nász, I., Kulcsar, G., Dán, P., and Sallay, K. (1971). A possible pathogenic role for virus-carrier lymphocytes. *J. infect. Dis.*, **124**, 214-216.

Nielsen, J. O., Dietrichson, O., Elling, P., and Christoffensen, P. (1971). Incidence and meaning of persistence of Australia antigen in patients with acute viral hepatitis: development of chronic hepatitis. *New Engl. J. Med.*, **285**, 1158-1160.

Okochi, K., and Murakami, S. (1968). Observations on Australia antigen in Japanese. *Vox Sang. (Basel)*, **15**, 374-385.

Prince, A. M., Leblanc, L., Krohn, K., Masseyeff, R., and Alpert, M. E. (1970). S.H. antigen and chronic liver disease. *Lancet*, **2**, 717-718.

Wright, R., McCollum, R. W., and Klatskin, G. (1969). Australia antigen in acute and chronic liver disease. *Lancet*, **2**, 117-120.