POSSIBILITY OF PASSAGE OF EPIDEMIC HEPATITIS VIRUS FROM THE BLOOD SERUM OF PATIENTS TO LEUKOCYTE CULTURES FROM HEALTHY DONORS

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The possibility of passage of the virus of epidemic hepatitis from patients' blood serum to leukocyte cultures from healthy donors was demonstrated. The action of this agent is not only to cause injury to the chromosomes of leukocytes of the infected cultures, but also to suppress their mitotic activity and their ability to undergo blast transformation.

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Experiments to isolate the agent of epidemic virus hepatitis by infecting tissue cultures and animals have not yet produced hopeful results. The strains obtained by investigators in the Soviet Union and elsewhere have been identified mainly as members of a latent group of already known viruses. Possibly the detection of herpes viruses or picornaviruses in material from patients with epidemic heptatitis is explained by the presence of a concomitant virus flora or by laboratory contaimination. On the other hand, the regular isolation of adenoviruses [1, 6] from patients with heptatitis calls for further intensive research. In comparative studies of the pathogenesis of virus heptatitis in man and animals [2, 3, 8] we have found the viruses of heptatitis of dogs, ducks, and mice for long periods and in high titers in the blood and in organs rich in reticulo-endothelial cells and lymphocytes: spleen, bone marrow, lymph glands, and liver. For this reason, cultures of human circulating leukocytes attracted our attention as a possible adequate system of lymphoid tissue for the study of the etiology of epidemic hepatitis in vitro. The cytogenetic study of blood leukocyte cultures from patients with virus hepatitis at various periods of the disease [4, 7], and also from donors carrying the virus [5] pointed to a high percentage of cells with gross chromosomal injuries. Similar anomalies were found in a series of passages to cultures of blood leukocytes from healthy donors infected with patients' sera [9]. In addition, the phenomenon of inhibition of blast transformation of lymphocytes has been observed in primary cultures of blood leukocytes from patients with infectious hepatitis [10].

Assuming that the phenomena described above can be attributed to the presence of the etiological agent of virus hepatitis, in this investigation we studied the possibility of its cultivation in blood leukocyte cultures from healthy donors, stimulated by phytohemagglutinin (PHA), by analyzing the degree of inhibition of differentiation of lymphotcytes into blast cells, their mitotic activity, and the integrity of the chromosomal apparatus.

EXPERIMENTAL METHOD

The infecting material, presumably containing the etiological agent, in the experiments of series I conisted of the blood serum from two paitents with virus hepatitis. The material was taken on the 3rd and 5th days after the appearance of jaundice. Gross structural injuries to the chromosomes were found in cultures of blood leukocytes from these patients by cytogenetic investigations at the times indicated above in 26 and 28% of lymphocytes examined. The serum was added in a volume of 0.1 ml to cultures of blood leukocytes from healthy donors. These cultures, containing $2 \cdot 10^6$ cells/2 ml medium, were grown on medium No. 199 with the addition of 10% autologous serum and PHA (Wellcome) up to a concentration of 0.01 mg/ml. The leukocyte cultures (infected and control) were used 72 h after infection for cytogenetic analysis by the method of Moorhead and co-workers [11], and for determination of the mitotic activity and percentage of blast transformation by counting 1000 mononuclear cells in each preparation. Some cultures were used as

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TABLE 1. Results of Experiments of Series I on Passage of Agent from Blood Serum of Patients with Virus Hepatitis to Blood Leukocyte Cultures from Healthy Donors

Dilution	Passage	Tested leukocyte cultures from blood of donors	Results of investigation after passage of blood serum of patient S to cultures of blood leukocytes from donors		Results of investigation after passage of blood serum of patient G to cultures of blood leukocytes from donors	
			Index			
			mitotic activity (in %)	cell with chromosomal injuries (in %)	mitotic activity (in %)	cells with chromosomal injuries (in %)
$1/2 \cdot 10^{-1}$	1st	Uninfected culture from donor K	27.5	4/2	27.5	4/2
		Culture + material for passage	19.5	16/10	15.3	10/8
$1/4 \cdot 10^{-2}$	2nd	Uninfected culture from donor V	29.0	2/0	29/0	2/0
		Culture + material for passage	16.3	22/12	12/0	16/14
$1/8 \cdot 10^{-3}$	3rd	Uninfected culture from donor L	28.5	2/2	28.5	2/2
		Culture + material for passage	12.5	32/16	10.7	28/16

Note. Results of cytogenetic investigation of cultures of blood leukocytes from patients: S, aged 10 years, 3rd day of icteric period – 26/16; G., aged 14 years, 5th day of icteric period – 28/20.
Legend: Numerator represents total number of anomalies, denominator number of gross anomalies without gaps (in %).

material for infection in a volume of 0.1 ml in three consecutive passages. The original sera were diluted 20, 400, and 8000 times during these passages.

EXPERIMENTAL RESULTS

The results given in Table 1 demonstrate the well marked injurious action of the agent present in the sera of patients with virus hepatitis on the chromosomal apparatus of the infected lymphocytes, leading to a successive decrease in mitotic activity of the cultures. In the course of the passages, despite repeated dilution of the original material, the injurious action was increased.

In the preceding series of experiments the test material was transmitted to cultures of blood leukocytes of the same donors. Blood serum from two other patients was used as infectious material for these cultures; the material was taken on the 5th day after the appearance of jaundice. The cultures of blood leukocytes of these patients, in contrast to those tested previously, were not subjected to cytogenetic study. The distinctive "cytogenetic background" of the infectious materials was thus absent in this series of experiments. In addition, serum from a healthy donor was also used as material for passage, and as an additional test to indicate inhibition of the vital activity of lymphocytes stimulated by the addition of PHA, the ability of the infected lymphocytes to undergo blast transformation was studied.

The results of these experiments are summarized in Tables 2 and 3. They reflect the successive decrease in the mitotic index of the cells and their ability to undergo blast transformation (by 50-67% at the 3rd passage) during passages of the material from patients with virus hepatitis. The number of lymphocytes with injured chromosomes, which was already increased after the first passage, increased still further by the 3rd passage to reach 3-6 times the control value (uninfected cultures and cultures inoculated with serum material from a healthy donor). It should be emphasized that, after triple passage of the sera used for infection, and also of the material from the 3rd passage of each series of experiments to human embryonic kidney cell cultures, no contaminating viral flora should be detected.

TABLE 2. Cytogenetic Investigation of Cultures of Donors' Leukocytes Infected with Material from Patients with Epidemic Virus Hepatitis (in passages)

Dilution of material	Passage	Culture of blood leukocytes from donor Sh						Culture of blood leukocytes from donor M					
		Material for infection						Material for infection					
		1-B		2-B		H		1-B		2-B		H	
		mitotic activity (in %)	cells with chromosomal injuries (in %)	mitotic activity (in %)	cells with chromosomal injuries (in %)	mitotic activity (in %)	cells with chromosomal injuries (in %)	mitotic activity (in %)	cells with chromosomal injuries (in %)	mitotic activity (in %)	cells with chromosomal injuries (in %)	mitotic activity (in %)	cells with chromosomal injuries (in %)
	Initial data for culture of donors' lymphocytes	28	12/4	28	12/4	28	12/4	29	4/2	29	4/2	29	4/2
$1/2 \cdot 10^{-1}$	1st	16.7	25/10	13.3	–	–	–	18	12/4	20	16/8	–	–
$1/4 \cdot 10^{-2}$	2nd	10.0	24/12	12.0	20/8	–	–	20.0	20/20	24.0	14/12	–	–
$1/8 \cdot 10^{-3}$	3rd	15.0	20/8	12.0	21/12	–	–	14.0	32/32	13.4	28/24	28.0	8/4

Legend: 1-B and 2-B denote sera of patients (3rd and 5th days of jaundice); H denotes healthy donor's serum; numerator – total number of anomalies (in %), denominator – gross anomalies without gaps (in %).

Percentage of metaphase plates with injured chromosomes in control group (healthy donors) was 8.

TABLE 3. Inhibition of Blast Transformation of Lymphocytes in Cultures of Donors' Blood Leukocytes Infected with Material from Patients with Epidemic Virus Hepatitis (in passages)

Dilution of material	Passage	Culture of blood leukocytes from donor Sh						Culture of blood leukocytes from donor M					
		material for infection											
		1-B		2-B		H		1-B		2-B		H	
		blast transformation (in %)											
		48 h	72 h	48 h	72 h	48 h	72 h	48 h	72 h	48 h	72 h	48 h	72 h
	Initial data for culture of donors' lymphocytes	90,4	96,9	90,4	96,9	90,4	96,9	65,8	89,7	65,8	89,7	85,8	89,7
$1/2 \cdot 10^{-1}$	1st	55,9	77,1	66,3	78,3	89,7	92,5	—	—	28,6	68,3	—	—
$1/4 \cdot 10^{-2}$	2nd	63,9	75,8	66,0	78,3	90,1	92,7	33,0	69,9	40,3	61,9	—	—
$1/8 \cdot 10^{-3}$	3rd	65,8	73,6	66,5	71,8	87,7	93,2	30,1	49,8	—	—	—	—

Legend: see Tables 1 and 2.

The preliminary results of these experiments, as well as other observations [9], thus demonstrate the possibility of in vitro passage of the agent contained in the blood serum of patients with virus hepatitis to cultures of blood leukocytes of healthy donors stimulated with PHA. In the present experiments, in addition to data obtained by other workers [9], the effect of this agent was not only to damage the chromosomes of lymphocytes of the infected cultures, but also to depress their mitotic activity and to inhibit their ability to undergo blast transformation. The hypothetical virus nature of the agent transmitted in the present experiments is confirmed by earlier cytogenetic studies of cultures of blood leukocytes from patients with epidemic heaptitis, convalescents, and donors carrying the virus, demonstration of the specific fluorescence of lymphocytes in immunofluorescence experiments [6], and also the detection of distinctive virus particles during the electron-microscopic study of cultures of blood leukocytes from patients with epidemic virus hepatitis.

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