Lymphocyte response to hepatitis B surface antigen

Findings in hepatitis and Indian childhood cirrhosis

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Chandra, R. K. (1975). *Archives of Disease in Childhood*, 50, 559. **Lymphocyte response to hepatitis B surface antigen: findings in hepatitis and Indian childhood cirrhosis.** The lymphocyte delayed hypersensitivity response to phytohaemagglutinin (PHA) and hepatitis B antigen (HBsAg) was evaluated by two *in vitro* tests—leucocyte migration inhibition and DNA synthesis. Patients convalescing from HBsAg-positive hepatitis showed the presence of a state of cell-mediated immune responsiveness to the antigen. In Indian childhood cirrhosis, there was a failure of response to HBsAg and a slight but significant depression of reaction to PHA. It is suggested that the lack of immune reactivity to HBsAg, perhaps determined genetically, may be a significant factor in the evolution of cirrhosis in Indian children.

Cirrhosis of the liver is a major killer of young children in India. It has a characteristic clinical (Achar, Raju, and Sriramachari, 1960) and histopathological (Liver Disease Subcommittee, 1955) picture. Epidemiological data (Achar *et al.*, 1960; Chawla *et al.*, 1973) and evolution of histomorphological changes (Aikat and Srivastava, 1956) suggest an important pathogenetic role of hepatitis-like illness. Hepatitis B surface antigen (HBsAg, Australia antigen, hepatitis-associated antigen) is detected in a significant proportion of affected children (Chandra, 1970) and may persist in the serum, other body fluids, and excretions for weeks and months, suggesting a failure of lymphocyte-mediated immunity to eliminate the virus. There is mild to moderate impairment of cellular immunity, as judged by cutaneous delayed hypersensitivity to common antigens and lymphocyte transformation response to phytohaemagglutinin (PHA) (Chandra, *et al.*, 1972) but this may well be a consequence of inanition brought about by a serious illness. Since these patients tolerate other infections reasonably well, it was considered desirable to look at the specific reactivity of lymphocytes to HBsAg.

Materials and methods

Subjects. Four groups of children were studied. 10 were patients with the diagnosis of Indian childhood cirrhosis based on clinical features and histological ex-

amination of percutaneous and needle biopsy of the liver. Their ages were from 11 to 36 months. All were boys and had shown HBsAg in the serum at some stage of their illness. 10 male patients with HBsAg positive hepatitis were studied 4–24 weeks after clinical recovery. By the time of the study, all had become negative for HBsAg. Their ages ranged from 15 to 36 months.

Four children with postnecrotic cirrhosis negative for HBsAg and antiHBs were also investigated. 10 healthy children with no history of jaundice or blood transfusion, matched for age and sex, served as controls. Their sera were negative for HBsAg and anti-HBs.

Hepatitis antigen and antibody. HBsAg and anti-HBs were detected initially by counterimmunoelectrophoresis and confirmed later by radioimmunoassay.

Lymphocyte stimulation response. Cell separation was achieved by Ficoll-Hypaque gradient centrifugation. Cells were washed twice and cultures established containing 2×10^6 lymphocytes/ml in medium 199 + 15% pooled AB serum. Lymphocyte cultures were set up in triplicate. To one set of cultures, PHA was added in doses of 3, 30, and 300 $\mu\text{g/ml}$ and the cells harvested at 96 and 120 hours. For analysis of results, values of cultures stimulated with 30 $\mu\text{g/ml}$ PHA and examined at 96 hours were used, since these culture conditions yielded maximum counts. To the second set of cultures, HBsAg purified by isopyknic fractionation and zonal centrifugation was added in a concentration of 200 $\mu\text{g/ml}$ and the cells examined at 120 hours, since these dose-time variables achieved maximum

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stimulation. The third set was kept as unstimulated control. DNA synthesis was measured by ^3H -thymidine incorporation using a scintillation counter. The morphology of 200 cells was studied for blast transformation.

Leucocyte migration inhibition. The method of Bendixen and Soborg (1969) was followed. Heparinized venous blood was allowed to sediment at 37°C , the leucocyte rich plasma was removed and centrifuged at $120 g$ for 20 minutes at 15°C . The cell pellet was resuspended in medium 199 containing 20% fetal calf serum and penicillin-streptomycin, and made up to a concentration of 10^7 mononuclear cells/ml. The cells were packed into a capillary tube, centrifuged, and the glass tube cut at the cell-liquid interface. The capillaries were laid in specially contrived chambers containing culture medium with or without HBsAg-containing serum and incubated at 37°C for 24 hours. Migration of cells into the medium was measured by planimetry and calculated by the formula $\text{Aa}/\text{Ao} \times 100$, where Aa is the area of migration in the presence of HBsAg and Ao the area of migration in the absence of HBsAg.

Results

Lymphocytes of children convalescing from HBsAg-positive hepatitis showed a significant ($P < 0.01$) increase in DNA synthesis when cultured in the presence of HBsAg (Table). This was corroborated by examination of cell morphology to detect lymphoblasts. Cells of healthy controls negative for HBsAg and anti-HBs, and cells of HBsAg-positive cirrhotic patients did not have increased ^3H -thymidine incorporation on stimulation with the antigen. With PHA, the lymphocyte transformation response was comparable in control and hepatitis groups, and slightly but significantly reduced ($P < 0.05$) in cirrhosis.

Leucocytes of convalescent patients with hepatitis had significant ($P < 0.01$) inhibition of migra-

tion in the presence of HBsAg (Table). The migration of cells obtained from cirrhotics was comparable to that of controls, confirming the lack of a state of hypersensitivity to HBsAg in each of the groups.

Discussion

The consequences of contact with material containing HBsAg are variable, from the asymptomatic to the fulminant (Dudley, Fox and Sherlock, 1971; Chandra, 1974a), and such contact may also lead to a chronic carrier state, cirrhosis, or hepatoma. It is likely that host factors, especially the immune response, are significant in determining the outcome. Besides nonspecific factors, antibodies and lymphocyte-mediated specific reactivity help in the elimination of pathogens. For viruses, cellular immunity seems more important.

We have employed two *in vitro* tests to evaluate the presence of a specific immune state of delayed hypersensitivity to HBsAg. The unequivocal inhibition of leucocyte migration and stimulation of lymphocyte DNA synthesis in patients with hepatitis indicated the existence of such a state. This was not observed in healthy controls who had no evidence of infection with HBsAg in the past. Using serum rich in HBsAg, Yeung Laiwah (1971) and his colleagues (Yeung Laiwah, Chaudhuri, and Anderson, 1973) found that blast transformation of lymphocytes occurred in patients with serum hepatitis. Recently *in vivo* and *in vitro* delayed hypersensitivity to purified HBsAg has been shown in chimpanzees (Ibrahim, Vyas, and Prince, 1974) and in man (Ibrahim, Vyas, and Perkins, 1975).

Failure to show a specific immune reactivity to HBsAg in Indian childhood cirrhosis is intriguing, since HBsAg had been shown in the serum of these

TABLE

Hepatitis B surface antigen (HBsAg) and antibody (anti-HBs), lymphocyte stimulation, and leucocyte migration. (Mean and range of values are given)

Group (and no.)	No. positive		Lymphocyte DNA synthesis (cpm)			Blast transformation (%)		Leucocyte migration in presence of HBsAg (%)
	HBsAg	anti-HBs	Saline control	PHA	HBsAg	PHA	HBsAg	
Healthy (10)	0	0	236 (190-258)	14 132 (9871-23 681)	102 (87-151)	82 (75-92)	7 (3-12)	121 (90-143)
Hepatitis (10)	10	3	121 (107-159)	10 361 (7671-17 639)	1 137 (857-1813)	80 (65-91)	58 (42-82)	58 (20-85)
Indian childhood cirrhosis (10)	10	1	258 (112-337)	5297 (4176-7103)	108 (80-136)	55 (43-73)	14 (3-42)	103 (75-130)
Postnecrotic cirrhosis (4)	0	0	196 (139-251)	7368 (5913-14 032)	143 (96-210)	71 (53-86)	11 (5-23)	87 (79-96)

patients at some stage of their illness. There was no significant increase in spontaneous release of migration inhibition factor (MIF) from lymphocytes of patients, so the lack of augmentation of the response with HBsAg is not a likely result of lymphocyte stimulation *in vivo*. The absence of increased DNA synthesis in unstimulated cultures lends support to this explanation.

The lymphocyte DNA synthesis in response to PHA was only mildly impaired in Indian childhood cirrhosis, which may be the result of changes in the metabolic milieu and inanition associated with a serious illness, and excludes a marked general depression of cellular immunity. It is likely that the lack of lymphocyte immune response to HBsAg in such patients permits persistence of antigenaemia and continued progressive liver cell damage. An analogous situation is seen in patients with generalized necrotic vaccinia after smallpox immunization, in whom there may be a failure to develop delayed hypersensitivity to the virus, dissociated from antibody synthesizing capacity and cellular response to other agents (Fulginiti, *et al.*, 1966).

Three patients, who were sibs of previously confirmed cases of Indian childhood cirrhosis, had been tested and found to be negative for HBsAg and anti-HBs before the onset of symptoms. This would make it unlikely that the lack of immune response was due to the phenomenon of tolerance seen in asymptomatic HBsAg carriers (Yeung Laiwah *et al.*, 1973), especially in those who acquire the infection early in postnatal life (R. K. Chandra, unpublished data).

The immunological abnormality in Indian childhood cirrhosis may well be determined genetically since the disease has a peculiar geographical distribution. There is a familial aggregation of cases (Achar *et al.*, 1960), abnormal dermatoglyphs (Chandra, 1969) and the segregation ratio on genealogical analysis suggests inheritance on an autosomal recessive basis (Chandra, 1974b). The concept of a particular genetic soil predisposing to persistent virus infection is new but logical. The capacity to mount a T (thymus-dependent) cell response to certain defined antigens is controlled genetically in some animals, the ability to respond being transmitted as a single mendelian autosomal characteristic. These genes have been named the Ir (immune response) genes, and are linked to the loci bearing the principal transplantation antigens or those governing reactivity in the mixed lymphocyte reaction.

It is suggested that Indian childhood cirrhosis may develop from infection with hepatitis B virus

in a genetically predisposed infant, and that failure to mount an adequate immune response results in persistent antigenaemia, progressive liver cell necrosis, and death. Most of the biochemical and immunological abnormalities described in the syndrome (Chandra 1968, 1970, 1974b; Chandra *et al.*, 1972) are epiphenomena or the consequence of liver injury rather than the cause of it.

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