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Clinically important immunological processes in acute and fulminant hepatitis, mainly due to hepatitis B virus

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SUMMARY Clinically useful criteria were found by studying immunological functions on admission in 15 African children with acute hepatitis (AH) (11 of whom were HB_sAg positive) and in 11 children with fulminant hepatic failure (FHF) (8 of whom were HB_sAg positive), and by comparing these results with normal controls. Nine of the FHF patients died. All the AH patients survived despite the development of transient liver failure in seven. There was significant diminution of components of the classical and alternative pathways of complement and total haemolytic complement in FHF compared with AH, and in both groups in comparison with controls. Cellular immunity tested by phytohaemagglutinin and HB_sAg transformation of lymphocytes and leucocyte migration inhibition with HB_sAg, were more impaired in FHF than AH. These indices were reduced in both groups of patients compared with controls, except for HB_sAg lymphocyte transformation which was significantly higher in AH than in controls. The most important index correlating with severity of clinical disease was C3. It was lowest in FHF, but within this group was highest in 2 patients who survived, and in AH the C3 on admission was significantly lower in patients who subsequently showed signs of transient liver failure than in those who did not. The prothrombin index was less sensitive in differentiating serious from mild illness. It is suggested that C3 levels can be helpful in monitoring patients with acute liver disease.

In infection due to hepatitis B virus, which is non-cytopathogenic, hepatic injury and therefore clinical disease, appear to be the result of a variable immune response of the infected individual.[1] In chronic carriers of hepatitis B virus, large amounts of HB_sAg are detected in blood and liver tissue without associated clinical disease.[2] If HB_sAg is associated with clinical disease some of the tests of immune function appear to be compromised in acute hepatitis (AH) and to an even greater extent in fulminant hepatic failure (FHF).[3] A few studies have shown reduced complement components in AH, FHF, and chronic liver disease.[4 5] These tests however, did not consistently predict outcome of the illness. A recent study of FHF in children also failed to detect any prognostic indices, other than prothrombin index.[6]

We report a study of biochemical and immunological parameters in patients with AH and FHF mainly due to HB_sAg, with a view to finding out if immune functions influence the clinical outcome, and also to identify prognostic factors which may make possible early institution of appropriate treatment.

Patients and methods

Patients. African children with AH and FHF were studied.

Acute hepatitis

AH was defined as acute illness with jaundice and biochemical evidence of acute liver disease, in the absence of clinical evidence of liver failure, on admission to hospital. Such cases were further subdivided into uncomplicated and complicated cases. Complicated cases were those in which at least one of the following developed subsequently: fetor hepaticus, flapping tremor, or encephalopathy; the last was graded on a I to IV scale.

These children received no specific treatment.

Fulminant hepatic failure

FHF was defined as jaundice and encephalopathy

Table 1. *Clinical biochemical and immunological details of patients with acute hepatitis and fulminant hepatic failure*

Case	Age (years)	Sex	HB_sAg	Encephalopathy grade	PI index (% normal)	Serum bilirubin (μmol/l)	AST (U/l)	C3 (mg/100 ml)	C4 (mg/100 ml)	THC (% normal)	AP (% normal)	Factor B (mg/100 ml)	Leucocyte migration inhibition (HB_2Ag 1/10) inhibition index	Lymphocyte transformation (HB_2Ag 1/100) d/min	Lymphocyte transformation (PHA) d/min × 10^3
Acute hepatitis															
1	6	M	+	–	93	80	210	84	46	0	0	10	0	161	15·5
2	11	F	–	–	101	214	37	66	20	0	90	36	57	76	3·8
3	9	M	+	–	76	228	1746	80	32	90	89	101	3	73	27·0
4	3½	M	+	–	86	78	438	82	28	100	126	22	6	1363	10·7
5	8	M	+	–	75	375	510	48	14	0	0	0	NA	NA	NA
6	6	M	+	–	75	1893	1893	66	20	66	0	5	−8	78	10·6
7	9	F	+	–	80	462	462	72	28	NA	NA	NA	NA	NA	NA
8	3	F	–	–	65	1529	1529	72	32	NA	NA	NA	NA	NA	NA
9	8	M	+	2	72	1932	1932	34	24	0	60	71	−17	1207	12·9
10	8	M	+	2	70	185	185	38	30	21	0	0	−1	1881	9·3
11	9	M	+	2	59	1308	1308	44	14	76	0	0	10	522	11·9
12	6	M	+	2	50	1326	1326	38	28	56	0	0	9	340	10·5
13	5	M	+	1	55	644	644	62	20	0	0	0	4	509	12·9
14	5	M	+	2	56	374	374	42	14	0	0	0	−9	358	9·2
15	11	F	+	2	52	890	NA	32	<1	134	94	75	2	323	24·0
Fulminant hepatic failure															
1	4	M	+	4	30	276	79	36	18	42	60	NA	1	80	7·9
2	9	M	+	4	20	174	780	38	12	0	0	0	1	113	1·4
3	11	M	–	2	20	171	642	12	1	0	0	0	−3	55	2·10
4	11	F	+	4	45	78	803	16	10	72	0	0	13	85	7·3
5	6	M	+	2	35	268	1626	16	8	0	0	0	−7	64	12·5
6	4	M	+	1	20	32	903	10	<1	45	59	0	0	61	12·0
7	6	M	+	2	20	300	582	16	1	0	0	0	−15	61	NA
8	6	F	+	3	20	217	2629	<1	<1	0	0	0	0	221	4·3
9	6	M	+	2	45	300	756	<1	4	0	0	0	0	65	14·4
10	12	M	–	3	20	170	NA	0	0	0	0	0	3	111	16·6
11	2	M	–	4	65	191	1485	48	4	0	0	0	NA	NA	NA

PI=prothrombin index; THC=total haemolytic complement; AP=alternative pathway; NA=not available.

on admission, with no history of previous liver disease, and the present illness being of less than 8 weeks' duration.

These children were given antibiotics, intravenous dextrose, protein-free diet, vitamin K, and high bowel washouts.

Steroids were not used in either group of patients.

Controls. Normal values in African children for the various tests used had already been established in this laboratory. Controls for migration inhibition and lymphocyte transformation due to HB_sAg were 15 normal African children, within the same age range as the patients, who formed part of an epidemiological study of prevalence of HB_sAg carrier rate. All 15 were negative for HB_sAg.

Methods

All tests were performed within 24 hours of admission. Biochemical tests were performed by standard methods.

The HB_sAg was detected by the haemagglutination-inhibition test. Complement components C3, C4, and factor B were estimated in plasma by radial immunodiffusion, and total haemolytic component and alternative pathway measured by radial assay.

Phytohaemagglutinin (PHA) and HB_sAg transformation of lymphocytes was performed by a routine method and assessed by incorporation of ^{14}C-thymidine into lymphocytes in inactivated human antibody serum, cultured for 72 hours if using PHA and for 168 hours if using HB_sAg. Results are expressed as disintegrations per minute (d/min).

HB_sAg was prepared from a pool of known HB_sAg plasma by ultracentrifugation for 3 hours at 150 000 *g*. The pellet was mixed with a small quantity of supernatant to achieve a high concentration of HB_sAg. This was filtered through a 0·42 μm millipore filter and used at a dilution of 1/10 for migration inhibition and at 1/100 for lymphocyte transformation. These dilutions had been shown to give optimum results in preliminary experiments.

Migration inhibition of leucocytes using HB_sAg was performed in agarose medium. Results are expressed as the inhibition index which was derived from the following formula: % migration inhibition (MI)=extent of migration with antigen/extent of migration without antigen × 100; inhibition index= 100—% MI.

Statistical analysis was carried out by the Mann-Whitney U test and Spearman's rank correlation coefficient.

Results (Table 1)

Patients.

Acute hepatitis (n=15)

The age range was 3 to 11 years and 11 were boys. Thirteen were HB_sAg positive. The condition of 7 of these 15 patients became complicated during the first week. The duration of fetor hepaticus, flapping tremor, or encephalopathy was between 3 and 7 days.

Fulminant hepatic failure (n=11)

The age range was 2 to 11 years and 9 were boys. Eight were HB_sAg positive and 2 patients had positive blood cultures for *Escherichia coli*. Two patients survived but the others died within 72 hours of admission. One patient was azotaemic. Liver histology at necropsy, which was available in 8 patients, showed acute massive liver necrosis in all.

Table 2 *Complement components and tests of cell-mediated immunity in acute hepatitis and fulminant hepatic failure compared with controls*

Patients	*C3 (mg/100 ml)*	*C4 (mg/100 ml)*	*Alternative pathway (%)*	*Factor B (mg/100 ml)*	*Total haemolytic component (%)*	*Lymphocyte transformation (d/min)*		*Migration inhibition HB_sAg 1/10 inhibition index*
						PHA	*HB_sAg 1/100*	
Controls	119±17†	54±14	100*	39± 9	68±7·5	20±7‡	148±151	17± 7
Acute hepatitis	57±18	23±10	35±48	25±35	42±47	13±6	474±542	6±19
Fulminant hepatic failure	17±16	5± 6	11±24	0	14.5±25	9±5	92±47	—1± 7
Significant P values								
Controls: AH	<0·002	<0·002	—	—	—	<0·02	<0·002	<0·02
Controls: FHF	<0·002	<0·002	—	—	<0·002	<0·002	—	<0·002
AH:FHF	<0·002	<0·002	<0·002	—	<0·002	—	<0·02	<0·002

*Value for pooled normal human serum: †mean±SD; ‡ ×10³.

Immunological tests (Table 2)

Complement. The C3 both in AH (mean 57±SD 18 mg/100 ml) and FHF (17±16 mg/100 ml) was significantly lower than in controls (119±17 mg/100 ml) ($P<0.002$) and the level in FHF was significantly lower than in AH ($P<0.002$). Furthermore, in the AH group, the levels in complicated cases (41±9 mg/100 ml) were significantly lower than in the uncomplicated (71±11 mg/100 ml) ($P<0.002$) ones. In the FHF group, the 2 patients with the highest levels of C3 survived.

C4, total haemolytic component, factor B, and alternative pathway were each significantly lower in both AH and FHF than in controls ($P<0.002$). The reductions were more profound in FHF but, unlike C3, there was no significant difference between complicated and uncomplicated AH and the 2 survivors of FHF did not have the highest levels.

Cell mediated immunity. Results were available for 12 of 15 AH patients and for 11 of 12 FHF patients. The PHA transformation of lymphocytes in AH (13±6 d/min) 10^3 was significantly lower than in controls (20±7 d/min) 10^3 ($P<0.02$) and was even more reduced in FHF (9±5 d/min) 10^3 ($P<0.002$). There was no statistically significant difference between AH and FHF although in the latter PHA transformation of lymphocytes was lower.

Lymphocyte transformation with HB_sAg (474± 542 d/min) was significantly greater than in controls in AH (148±151 d/min) ($P<0.002$), but not in FHF (92±47 d/min). There was a significant difference between AH and FHF ($P<0.02$).

Migration inhibition of leucocytes in both AH (6±19 d/min) and FHF (—1±7 d/min) was significantly less than in controls (17±7 d/min) ($P<0.02$ and <0.002 respectively) and in addition in FHF was significantly less than in AH ($P<0.002$).

Biochemical tests

The prothrombin index was considerably less in both AH (70±15%) and FHF (30±15%) compared with controls (100%) and furthermore was significantly less in FHF compared with AH ($P<0.002$). It was significantly less in complicated (59±8%) than in uncomplicated AH (81±11%) ($P<0.05$). One of the two survivors in FHF had the highest level of prothrombin index (65%) in that group.

The results of the remaining biochemical tests (aspartate transaminase and serum bilirubin level) were similar in AH (889±687 U/l; 235±15 μmol/l respectively) and FHF (1029±677 U/l; 198±93 μmol/l respectively).

Correlation. There was a significant correlation between C3 and prothrombin index ($P<0.05$) in FHF and AH but not among any of the other parameters studied.

Discussion

We have shown that in comparison with normal controls, children with AH and FHF have a significant diminution in quantity and function of complement proteins and this is accompanied by alterations in tests of cellular immunity. Such changes were nearly always more profound in FHF. The results indicate that there were abnormalities in both classical (C4) and alternative pathways of complement in both groups of patients. The most important index correlating severity of clinical disease with immunity was C3. This accords with the pivotal role of C3 in the complement cascade. C3 was lowest in fulminant disease but within this group was highest in children who survived. In AH, the C3 on admission was lower in patients who subsequently developed signs of transient liver failure than in those who did not. C3 may therefore predict survival in FHF and impending liver failure in AH. C3 measurements are simple assays and may be used easily and frequently to monitor patients with acute liver disease. In support of these findings is the observation that persistent infection and progression to chronicity after AH can be predicted by the demonstration of complement on hepatocytes during the acute phase.[7]

The treatment of FHF is unsatisfactory and there is a high mortality. Newer methods of treatment for this disease—for example haemodialysis using polyacrylonitrile membrane [8]—may be initiated and appropriately assessed according to C3 levels. Most children with AH do not require any specific treatment and therefore C3 measurements will not materially alter the management. However, in the few who develop signs of impending liver failure and who need more intensive observation and treatment, C3 measurements can be helpful. The large proportion of patients with AH who developed signs of liver failure in this series reflects the fact that only the moderately and severely ill were admitted to hospital.

The diminution of complement demonstrated in both diseases may in part be due to excessive consumption. The wide range of specific antibodies,[9] autoantibodies,[10] specific lymphocytotoxicity,[11] the presence of IgG on hepatocytes,[12] and circulating soluble immune complexes in hepatitis B virus disease,[13] indicate the extent of possible mechanisms capable of triggering activation of complement. We have preliminary evidence for the presence of C3

breakdown products in AH and FHF sera which supports this interpretation of the results. The greatly reduced level of complement in FHF may be owing to exuberant antibody response which has been shown in that disease.[14] Another possibility is defective production of complement by metabolically crippled hepatocytes. The close correlation between prothrombin index and C3 implies that impaired synthesis of coagulation proteins parallels decreased manufacture of some complement components by the liver.

It is believed that the critical element in the pathogenesis of liver cell destruction in hepatitis B virus disease is cell-mediated immunity. Accordingly the greater the cellular immune response the more pronounced the hepatic necrosis and the worse the disease. A number of tests has shown cellular immunity to be deficient in HB_sAg carriers.[3] There ought to be by this reasoning, an exuberant and aggressive cellular immune response in FHF. We have in fact shown the opposite. T- and B-cell functions, the former assessed by PHA and antigen transformation of lymphocytes and both by antigen-induced leucocyte migration inhibition,[15] were depressed. As antibody does not always correlate well with severity of clinical illness,[16] it is possible that mechanisms other than T-cells and antibodies are important in immunopathology. There is some evidence that in hepatitis B virus liver disease K cells may be crucial to the development of hepatic damage.[17]

In AH there was accentuated antigen transformation of lymphocytes which was expected, and depressed leucocyte migration inhibition which was surprising. This dissociation between different tests of T-cell function has been recorded in other viral infections and probably reflects varying involvement of separate T-cell subtypes in any particular immune reaction.

The mechanisms of impaired cell-mediated immunity in AH and FHF are probably multiple. The severe metabolic derangements in FHF must be responsible for some of the immunological abnormalities. The possible presence of serum factors other than that which has been shown to depress metabolism in polymorphononuclear leucocytes in FHF [18] may contribute to these immunodeficiencies. Such serum factors have been observed to depress T-cell function in chronic hepatitis.[19]

The sensitivity of the prothrombin index as a criterion of severe disease in FHF has been noted in a previous study from this centre [20] and by other workers.[6] Therefore if tests for C3 are not available the prothrombin index is a valuable investigation for the management of such patients.

We have shown in two polar forms of hepatitis mainly due to hepatitis B virus, that a diminution in complement components can be clinically useful, and that alterations in aspects of cell-mediated immunity are probably related to the immunopathological processes contributing to these disease states.

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