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Immune response to φX 174 in man

5. Primary and secondary antibody production in primary biliary cirrhosis

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SUMMARY In primary biliary cirrhosis, the primary immune response to the bacteriophage ϕX 174 is normal but the secondary response is significantly reduced. The reduction is primarily of IgG antibody, while IgM is proportionately less affected. These changes may be the result of a reduction in helper T lymphocyte function and may contribute to the increase in the ratio of serum concentration of IgM to IgG.

Several hypotheses have been suggested to explain the hyperglobulinaemia of liver disease. Havens *et al.* (1951) described increased responses to diphtheria toxoid in patients with cirrhosis and suggested that these patients were immunologically hyper-responsive. Additional support for this concept stems from the demonstration that in rats subjected to partial hepatectomy (Havens *et al.*, 1956) or rendered cirrhotic by carbon tetrachloride (Thomas *et al.*, 1976) the production of antibody after intravenous challenge is greater than in normal animals. This apparent increase in reactivity is in part dependent on diversion of antigen from the liver to the spleen because of a reduction in the phagocytic capacity of the liver in cirrhosis (Thomas *et al.*, 1973).

Changes in the reactivity of the lymphoid organs or maldistribution of antigen may explain the increase in γ globulin in most forms of chronic liver disease but additional factors must exist to account for the disproportionate increase in IgM concentration that is peculiar to primary biliary cirrhosis (PBC). It is established that the initial antibody response to thymus-dependent antigens is of IgM class followed later by IgG antibody. A study of the humoral immune response after standardised challenge may therefore shed some light on factors controlling IgM and IgG antibody production in this disease. We therefore subjected 12 patients with PBC to a standardised intravenous antigenic challenge with the bacteriophage ϕX 174 and measured the IgM and IgG components of the 1° and 2° antibody responses.

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Methods

PATIENTS

Twelve patients with primary biliary cirrhosis were studied. The diagnosis was dependent on a typical history with cholestatic liver function tests, a positive mitochondrial antibody test, and a compatible liver biopsy which was graded I-IV (Scheuer, 1967).

Details of histology and biochemistry results are given in Table 1. None of the patients received corticosteroids or immunosuppressive drugs in the 12 months before testing. All patients were volunteers who gave informed consent after the nature of the investigations had been explained to them.

TECHNIQUES

The method of preparing the bacteriophage has been described previously (Peacock *et al.*, 1973). Batches were tested for sterility and pyrogenicity by standard methods before injection. Patients were injected intravenously with $2{\cdot}0 \times 10^9$ plaque-forming units (PFU) of ϕX 174. Blood samples were taken before immunisation and at intervals for 28 days. The blood was allowed to clot at room temperature, the serum was removed and stored at -20°C and was inactivated for 30 minutes at 56°C before testing. Patients were given a second intravenous dose of $2{\cdot}0 \times 10^9$ PFU between the 28th

Table 1 *Biochemical and histological data*

Patient	*Sex*	*Age (yr)*	*Serum bilirubin (5-17 μmol/l)*	*Serum alkaline phosphatase (2-12 KA/100 ml)*	*Serum aspartate transaminese (10-35 U/ml)*	*IgG 50-180 IU/ml*	*IgA 70-250 IU/ml*	*IgM 50-200 IU/ml*	*Mito-chondrial antibody*	*Histological diagnosis (grade)*
2	F	63	306	200	209	308	86	388	1/1024	PBC IV
3	F	55	10	93	161	202	139	286	1/32	PBC III
4	F	53	10	50	19	140	149	724	1/512	PBC III
5	F	49	102	64	71	167	212	548	1/32	PBC III
6	F	53	24	65	67	158	258	668	1/256	PBC II
7	F	53	12	65	65	190	165	1040	1/2048	PBC IV
8	F	51	10	56	40	150	186	500	1/256	PBC II
9	F	46	39	70	162	140	116	760	1/512	PBC IV
10	F	71	51	68	150	256	236	720	1/1024	PBC III
11	F	59	73	36	74	256	428	840	1/2048	PBC III
14	F	48	17	50	106	108	165	400	1/2048	PBC II
16	F	32	20	57	130	195	258	480	1/2048	PBC I

and the 42nd day. Blood samples were taken for a further month.

Antibody was measured by assessing the capacity of a series of dilutions of serum to inactivate bacteriophage, and calculating the 50% bacteriophage neutralisation titre (SD_{50}). Serum was subjected to rate zonal centrifugation on a sucrose density gradient, to separate IgG and IgM antibodies. Antibody was measured in the fractions as previously described and IgM and IgG containing fractions were identified by immunodiffusion against commercial antisera to IgM and IgG. The levels of antibody in the secondary response were compared with those of the control normal subjects of similar age range (Peacock *et al.*, 1973).

Immunoglobulin concentrations were measured using Hoechst immunodiffusion plates.

Lymphocytes were harvested from heparinised venous blood using a Ficoll/Triosil gradient. The washed lymphocytes were then resuspended in Eagles' medium containing 10% heat inactivated fetal calf serum (Flow Labs.) and 0·02 M Hepes buffer at a cell concentration of 1×10^6 cells/ml. Cultures containing 1×10^6 lymphocytes and 50 μl of a 1/10 dilution of phytohaemagglutinin (Wellcome Labs.) were set up. The cultures were incubated for 72 hours and then 50 μci ^{14}C thymidine were added to each culture. After incubation for another four hours, the cells were harvested and the ^{14}C uptake measured. The stimulation index is calculated as follows: counts per minute per 10^6 mitogen stimulated lymphocytes/counts per minute per 10^6 unstimulated lymphocytes.

An age matched control was examined at the same time as each patient with liver disease.

Rosetting techniques were used to determine the percentages of T and B lymphocytes. Rosette tests for T cells were performed using sheep erythocytes (E) (Jondal *et al.*, 1972). Lymphocytes binding three or more sheep erythrocytes were regarded as T rosette forming cells. Rosette tests for lymphocytes bearing a C3 receptor were performed using sheep erythrocytes sensitised with IgM class rabbit antibody and human complement (EAC) (Jondal *et al.*, 1972).

The number of E and EAC cells per cu mm of blood was calculated from the above data and from an absolute lymphocyte count carried out on the peripheral blood smear. This data was obtained at the time of primary immunisation, and, in the absence of intercurrent viral infection, peripheral blood T and Fc-receptor bearing lymphocyte concentrations remained relatively constant.

Results

None of the patients had detectable levels of antibody before immunisation.

PRIMARY RESPONSE

All the patients developed a normal antibody response to the first injection of bacteriophage (Fig. 1). The range of response was large but this is also a feature of the primary response in normal individuals. The peak of the primary response occurred between seven and 21 days after immunisation (Table 2).

SECONDARY RESPONSE

Eight patients showed a variable impairment of the secondary response and four produced responses in the normal range (Fig. 2). There was no correlation between this impairment and the severity of the liver disease. Patient 2 had very severe disease and died within three weeks of the end of the study but her antibody response was only slightly depressed. On the other hand, patient 8, with only mild disease, produced a markedly depressed response. There was no correlation with the histological stage of the disease.

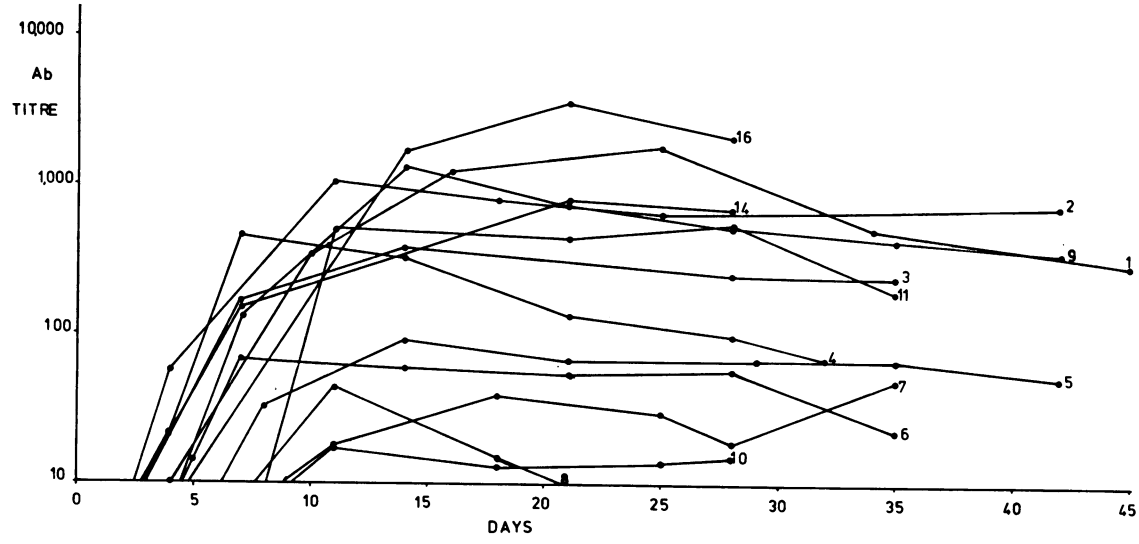

Fig. 1 *Primary response to ϕX 174.*

E AND EAC ROSETTES AND ANTIBODY PRODUCTION

E rosette forming cells were reduced in concentration in these patients but there was no correlation with either the clinical or histological grading of the disease. There was a positive correlation between the primary and secondary antibody titres and the concentration of T-cells in the peripheral blood ($r = 0{\cdot}73$, $P<0{\cdot}01$; $r = 0{\cdot}84$, $P<0{\cdot}001$).

EAC rosette forming cells were reduced in some patients and there was a positive correlation between the concentration of these cells and the magnitude of both the primary ($r = 0{\cdot}66$, $P<0{\cdot}05$) and secondary response ($r = 0{\cdot}89$, $P<0{\cdot}001$).

There was a poor correlation between PHA responsiveness and E rosetting cell concentrations.

IMMUNOGLOBULIN CLASS OF ANTIBODY DURING SECONDARY RESPONSE

Sera taken at the peak of the secondary response were examined by rate zonal centrifugation. The antibody titre recovered in the IgM and IgG containing fractions is shown in Table 3. In normal subjects (Peacock *et al.*, 1973) 80-95% of the anti-

Table 2 *Time and magnitude of primary and secondary peak titre*

Patients	*Primary peak*		*Day of 2nd injection*	*Secondary peak*		*E rosetting cells /cu mm blood*	*EAC rosetting cells /cu mm blood*	*PHA response stimulation index*
	Day	*Titre*		*Day*	*Titre*			
2	11	1 100	42	11	19 000	805	451	16·2
3	14	390	35	14	19 000	828	483	38·0
4	7	450	32	14	30	196	96	—
5	14	92	42	8	1 200	239	336	22·3
6	7	68	35	—	—	1 155	905	77·9
7	18	39	35	8	1 700	806	538	37·7
8	11	45	28	14	43	206	—	—
9	14	2 000	42	7	45 000	1 063	1 188	49·9
10	11	17	28	11	70	586	175	—
11	11	520	35	7	5 800	458	397	52·8
14	21	800	28	21	10 500	1 240	700	31·2
16	21	3 600	28	14	50 000	1 813	1 012	89·7
m ± SEM	13 ± 1·4	760 ± 308		11 ± 1·3	13 849 ± 5 465*	749 ± 149*	538 ± 109	46·1 ± 8·16*

Normal range (mean ± SEM): E 1589 ± 314 (n = 10), EAC 539 ± 135 (n = 10).

PHA stimulation index 68 ± 16 $\left(\text{Stimulation index} = \frac{\text{cpm stimulated culture}}{\text{cpm unstimulated culture}}\right)$ (n = 9)

Peak 1° − 1 660 ± 414 (n = 22)
Peak 2° − 30 333 ± 13 154 (n = 22)
*$P < 0{\cdot}01$

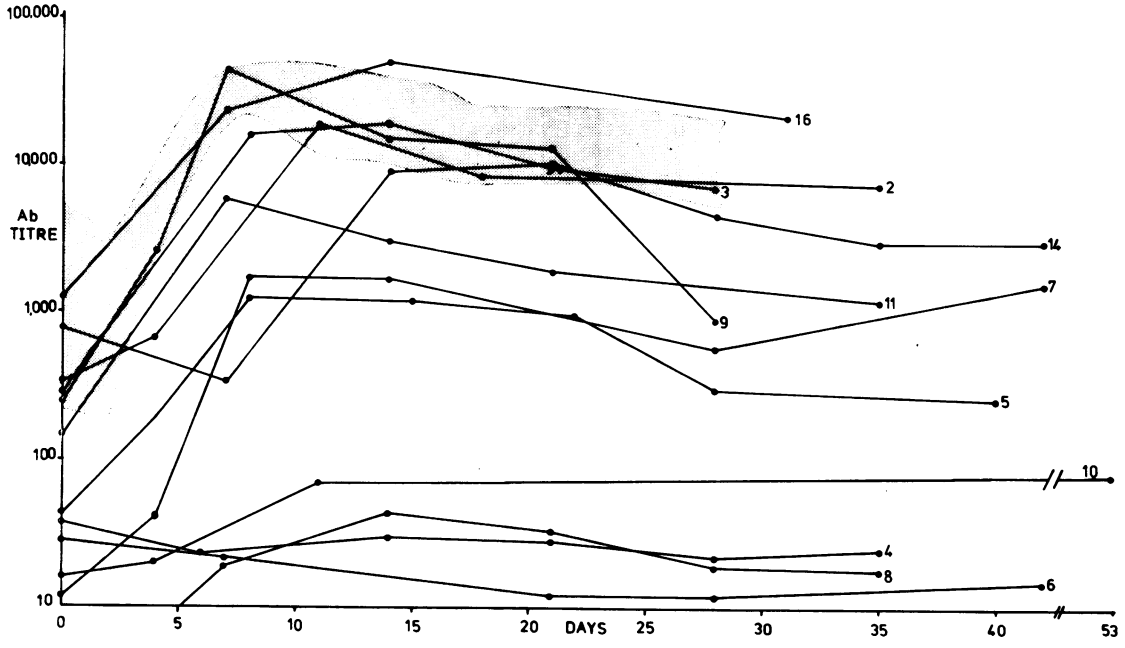

Fig. 2 *Secondary response to φX 174. Stippled area represents the normal range (mean ± 2SD) which was derived from a study of 14 normal subjects (Peacock* et al., *1973).*

body response to a second injection of antigen is found in the IgG peak, but in the patients with primary biliary cirrhosis this was significantly reduced (19-77%). The ratio of IgM to IgG antibody did not correlate with the ratio of serum IgM to IgG concentration.

Conclusions

The primary immune response to φX 174 in patients with primary biliary cirrhosis lies within the normal range but the secondary response is significantly decreased ($P < 0.01$). There is no correlation between the degree of depression of the peak response and the severity of the disease, shown by degree of cholestasis or hepatocellular necrosis.

All the patients show a marked increase in the total serum concentration of IgM (Table 3) and a moderate increase of IgG. The ratio of serum IgM to IgG is above normal in all cases. It is therefore

Table 3 *IgM and IgG antibody responses*

Patient	*Serum Ig concentrations (1U/ml)*			*Peak of secondary response*			
	M	*G*	*M:G*	*IgM ab*	*IgG ab*	*IgM:IgG ab*	*IgG (% of ab total ab)*
2	388	308	1·2	5 700	13 300	0·30	70
3	286	202	1·4	5 400	17 700	0·31	77
4	724	140	5·2	12	35	0·34	74
5	548	167	3·3	816	384	2·13	32
6	668	158	4·2	—	—	—	—
7	1 040	190	5·5	1 740	590	2·95	25
8	500	150	3·3	35	8	4·37	19
9	760	140	5·4	22 100	33 000	0·67	60
10	720	256	2·8	114	118	0·97	51
11	840	256	3·3	2 300	2 849	0·81	55
14	400	108	3·7	6 350	8 050	0·78	56
16	480	195	2·5	18 000	17 707	1·02	50
M ± SEM	613* ±63	189* ±16·9	3·5* ±0·4	5 687 ±2 271	8 521 ±3 257	1·33 ±0·39	52 ±5·8
Normal range (M ± SEM)	125 ±30	115 ±35	1·1 ±0·2	—	—	<0·25	80-95

*$P < 0.01$.

of particular interest that during the secondary response there is a higher proportion of IgM antibody than in any of the normal subjects studied. A similar defect has been described in patients with coeliac disease but the changes in serum IgM levels are less impressive (Baker *et al.*, 1975).

A number of possible explanations can be offered for this decreased secondary response. Since the response to ϕX 174 is probably thymus-dependent, a defect in either B lymphocyte or helper T lymphocyte function might underly the diminished humoral response. In some patients both B and T cell concentrations are diminished and changes in both cell populations probably contribute to the reduced antibody response. Furthermore, there is a good correlation between the peak antibody response to ϕX 174 and both T and B cell concentrations.

It is established that IgG antibody production is more dependent on the cooperation of T cells than is IgM (Mitchell, 1974). The depressed T cell function in these patients (Fox *et al.*, 1969; MacSween *et al.*, 1973) may therefore be at least partially responsible for the proportionately lower IgG compared with IgM antibody response. In addition, because of the inhibitory effect of IgG antibody on IgM antibody production (Nielson *et al.*, 1974), the reduced IgG response would lead to defective negative feedback and a protracted IgM phase of the response.

Depressed T-lymphocyte function, particularly helper cell function, in these patients is probably a result of the development of established liver disease (Thomas *et al.*, 1976). This change may result either from the complex biochemical changes occurring in these patients or an increase in suppressor T-cell function, a change which has been demonstrated in an animal model of cirrhosis probably as a result of increased antigenic challenge to the spleen (Thomas *et al.*, 1976).

The observation that levels of antibody production to an individual antigen are diminished in the presence of hyperglobulinaemia appears at first sight to be paradoxical. It has been argued that the failure of the hepatic component of the reticuloendothelial system to destroy antigen would lead to increased antibody production (Bjørneboe *et al.*, 1972; Triger *et al.*, 1972) and, indeed, this appears to be true of thymus-independent antigens (Thomas *et al.*, 1976) but, in the case of thymus dependent antigens, the change in balance between helper and suppressor T lymphocytes, brought about by the disease itself, is such that the humoral response is reduced. The lack of effect of the changed T cell function on the antibody response to thymus independent antigens may further contribute to the disproportionate increase in serum IgM concentrations, since it is the thymus independent response which is mainly of IgM type.

Whether these changes are peculiar to primary biliary cirrhosis remains to be determined. The change in balance between helper and suppressor T lymphocytes is likely to occur in all forms of chronic liver disease (Thomas *et al.*, 1976) and it seems likely that additional factors exist to account for the increased IgM concentrations in primary biliary cirrhosis.

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