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# THE IMMUNE RESPONSE TO φX174 IN MAN

## IV. PRIMARY AND SECONDARY ANTIBODY PRODUCTION IN PATIENTS WITH CHRONIC LYMPHATIC LEUKAEMIA

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SUMMARY

Ten patients with chronic lymphatic leukaemia (CLL) were injected with the antigen $\phi$X174 intravenously on two occasions to test their capacity for antibody production. Nine out of ten patients failed to develop a primary response, and five out of ten failed to develop a secondary response. Only one out of the ten of the secondary responses fell within the normal range. There was evidence of defective switching from IgM to IgG antibody in two out of three cases in which the immunoglobulin class of antibody in the secondary response was determined. The severity of the immunoparesis in CLL is related to the severity of the disease.

## INTRODUCTION

It has long been recognized that patients with chronic lymphatic leukaemia (CLL) have a striking defect in antibody production. Moreschi (1914) described a patient with CLL who, having contracted typhoid fever, failed to develop agglutinins against the infecting organism. He further found that patients with CLL had an impaired or absent agglutinin response on vaccination with *Salmonella typhi.* Many authors have since confirmed the impairment of antibody production to natural antigens in this disease (Howell, 1920; Weinstein & Fitz-Hugh, 1935; Larson & Tomlinson, 1952, 1953; Barr & Fairley, 1961; Miller, 1962).

The detailed analysis of the immune defect in CLL requires the use of more sophisticated techniques of antigenic stimulation, and these have produced contradictory results. Cone & Uhr (1964) reported ten patients who failed to produce antibody to a primary intramuscular injection of the bacteriophage $\phi$X174 or secondary antibody to diphtheria toxin. Hersh *et al.* (1970) on the other hand, in a study of thirteen patients, found that detectable antibody was produced after an injection of Keyhole limpet haemocyanin, but that the peak of antibody production was delayed and that there was a delay in switching from IgM to IgG antibody.

We have developed a method for using $\phi$X174 in the study of antibody production in humans (Peacock, Jones & Gough, 1973). Most normal subjects have no pre-existing

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TABLE 1. Clinical details of patients with chronic lymphatic leukaemia

| Patient | Sex | Age | Duration | Haematology | | Immunoglobulins | | | Spleen | Lymph nodes | Treatment | Infections | Doubling time (peripheral lymphocytes) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Hb | Lymphocytes ($\times 10^{-3}$) | Platelets ($\times 10^{-3}$) | IgG* | IgA† (mg/100 ml) | IgM‡ | | | | | |
| EW3 | F | 87 | 2 weeks | 14·2 | 9 | 142 | 1215 | 604 | 57 | − | − | — | | Stationary |
| FW4 | M | 61 | 2 months | 16·0 | 10 | 277 | 1600 | 490 | 126 | − | − | — | Chronic bronchitis | Stationary |
| JH7 | M | 79 | 10 months | 13.4 | 13 | 210 | 1700 | 250 | 120 | − | − | — | Urinary, osteomyelitis | Stationary |
| RE1 | F | 63 | 7 months | 12·8 | 38 | 210 | 980 | 260 | 110 | − | + | — | | 11 months |
| WH10 | M | 65 | 2 weeks | 13·1 | 51 | 152 | 1250 | 170 | 90 | − | + | — | | 9 months |
| RP9 | M | 73 | 1 month | 16·0 | 29 | 108 | 1400 | 380 | 88 | 7 cm | − | — | | 7 months |
| OJ5 | M | 76 | 10 months | 11·0 | 104 | 147 | 1180 | 9·7 | 64 | − | +++ | Chlorambucil 4 mg/day 1 month before and during secondary response | Herpes zoster during primary injection | 5 months |
| LM8 | F | 69 | 9 months | 11·0 | 78 | 150 | 800 | 120 | 55 | − | ++ | Chlorambucil 6 mg/day ending 1 month before second injection | | 1 month |
| FD2 | F | 62 | 4 years | 11·2 | 22 | 136 | 700 | 25 | 18 | 2 cm | +++ | Chlorambucil 6 mg/day for 1 month ending 1 month before 1st injection | Chronic bronchitis, pneumonia | 1 month |
| KW6 | F | 60 | 5 years | 7·2 | 280 | 80 | 220 | 40 | 12 | 13 cm | +++ | None for 2 years | Chest infections prior to treatment | 7 months |

* Normal value = 800–1700 mg/100 ml.
† Normal value = 140–420 mg/100 ml.
‡ Normal value = 50–190 mg/100 ml.

antibodies to this coliphage so that the primary and secondary responses can both be measured. The antigen can be given safely by intravenous injection, and the method used for measuring antibody is extremely sensitive, and relatively independent of antibody affinity. We have measured the immune response to $\phi$X174 in ten patients with chronic lymphatic leukaemia, in an attempt to resolve some of the discrepancies reported in previous studies.

*Patients*

Ten patients with CLL were studied. The diagnosis was established on the basis of a persistently raised peripheral lymphocyte count. Surface immunoglobulins and receptors for sheep erythrocytes, for the IgG Fc region, and for complement were studied in all cases and the results are to be published separately (T. J. Hamblin, in preparation). Details of the patients are given in Table 1. Seven cases were untreated during this study. Three required treatment with chlorambucil shortly before or during the course of the present study. All patients were volunteers who gave informed consent after the nature of the investigation had been explained to them. The project was approved by the Ethical Committee of the Bournemouth and East Dorset Hospital Group.

## MATERIALS AND METHODS

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 $3{\cdot}3 \times 10^9$ plaque-forming units of $\phi$X174, and blood samples were taken before immunization and at intervals for up to 6 weeks. The blood was allowed to clot at room temperature and the serum was removed and stored at $-20°C$. It was inactivated for 30 min at 56°C before use. All patients were given a second dose of $2{\cdot}2 \times 10^9$ PFU between 15 and 22 weeks after the primary injection, and blood samples were taken for a further month.

Antibody was measured by inactivating bacteriophage with serial dilutions of serum, and calculating to 50% bacteriophage neutralization titre ($SD_{50}$) (Peacock *et al.*, 1973). Serum from the secondary response of patients 1, 3 and 4 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 (Peacock *et al.*, 1973). Total immunoglobulin levels in the patients' serum were determined by radial immunodiffusion using Hoechst tripartigen immunodiffusion plates.

## RESULTS

*Pre-existing antibody*

In one patient (number 5) antibody was present in the serum at a titre of 16 before immunization. None of the remaining patients had detectable pre-immunization antibody.

*Primary response*

Only one patient (number 5) of the ten developed any detectable antibody after the first

injection of bacteriophage. The peak titre (Table 2) was 33. The results are illustrated in Fig. 1.

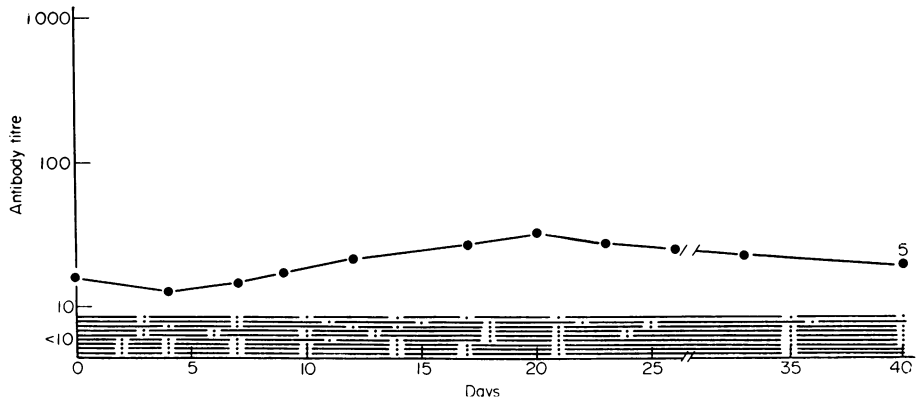


FIG. 1. Antibody levels following primary injection.

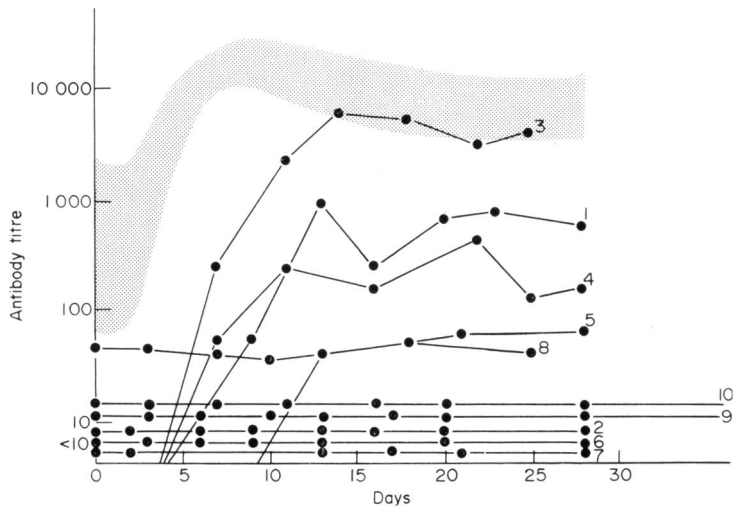


FIG. 2. Antibody levels following secondary injection. (The stippled area is the mean response ±s.d. for thirty normal subjects.)

*Secondary response*

Five out of the ten patients also failed to develop any antibodies following the second injection of bacteriophage (Fig. 2). The remaining five developed a response which was substantially lower than that of normal subjects.

*Immunoglobulin class of antibody*

Sera from the secondary response of patients numbers 1, 3 and 4 were subjected to rate-zonal centrifugation to determine whether the antibodies were of IgG or IgM class.

TABLE 2. Antibody production

| Patient | Surviving bacteriophage | Primary peak | | Interval between first and second injections (weeks) | Secondary peak | |
|---|---|---|---|---|---|---|
| | | Day | Titre | | Day | Titre |
| 3 | $2{\cdot}32\times10^4$ (day 2) | | | 22 | 14 | 5800 |
| 4 | — | | | 18 | 22 | 425 |
| 7 | — | | | 16 | — | — |
| 1 | $1{\cdot}52\times10^4$ (day 2) | | | 18 | 13 | 920 |
| 10 | — | | | 16 | — | — |
| 9 | — | | | 17 | — | — |
| 5 | Pre-existing antibody at titre of 16 | 20 | 33 | 16 | 18 | 50 |
| 8 | — | | | 15 | 28 | 62 |
| 2 | $3{\cdot}10\times10^4$ (day 2) (also $1{\cdot}5\times10^5$ on day 2 secondary) | | | 18 | — | — |
| 6 | $2{\cdot}61\times10^4$ (day 3) | | | 16 | — | — |

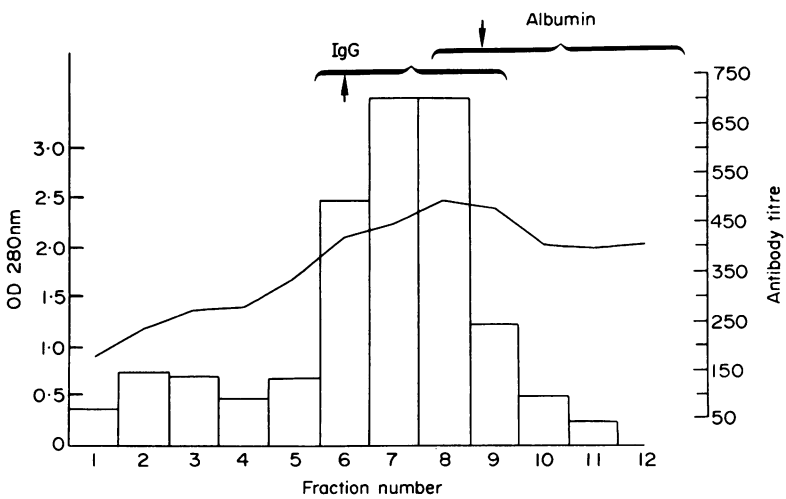


FIG. 3. Serum from peak secondary response of patient number 3. IgG and IgM separated by sucrose density gradient ultracentrifugation. Antibody mainly IgG. (——) Absorbance at 280 nm.

In patient number 3, serum taken on day 14 of the secondary response showed a predominance of IgG antibodies (Fig. 3). Patient number 1, on the other hand, had antibodies which were still predominantly of IgM class 23 days after the second injection (Fig. 4). In patient number 4, antibodies were evenly distributed between IgM and IgG on day 16. The percentage of antibodies in each of the immunoglobulin classes is summarized in Table 3.

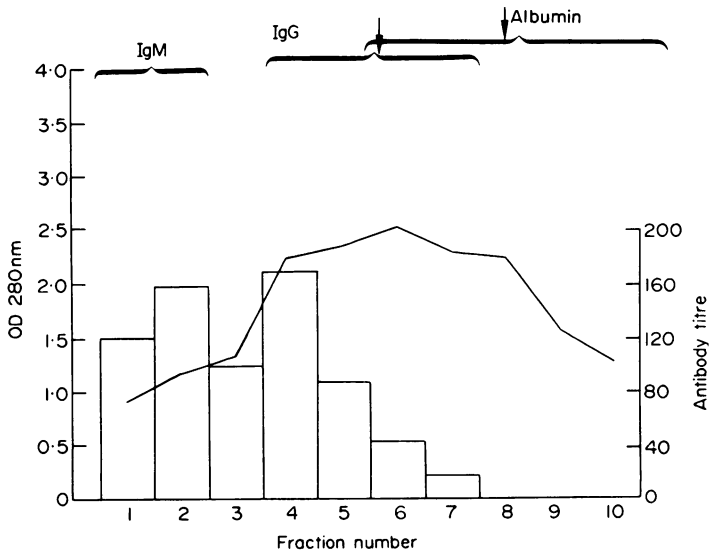


FIG. 4. Serum from peak secondary response of patient number 1. Antibody mainly IgM. (——) Absorbance at 280 nm.

TABLE 3. Immunoglobulin class of antibody. Peak secondary response

| Patient | Summed titres of antibody recovered from sucrose density gradient | | |
|---|---|---|---|
| | IgM | IgG | IgG (%)* |
| 1 | 354 | 380 | 52 |
| 3 | 484 | 2414 | 83 |
| 4 | 32 | 103 | 76 |

* Values for six normal subjects range between 80 and 95%.

*Rate of clearance of bacteriophage*

In our study of normal subjects (Peacock *et al.*, 1973) and of patients with Crohn's disease (Bucknall, Jones & Peacock, 1975), we found no viable bacteriophage in the serum after the first 24 hr. It is therefore of considerable interest that viable bacteriophage persisted until day 2 in three out of the ten patients, and until day 3 in one patient. Patient number 2, who developed no detectable primary or secondary antibody, also had viable bacteriophage 2 days after the secondary injection.

## DISCUSSION

The antibody response to $\phi$X174 is strikingly diminished in patients with CLL. Only one out of ten patients developed any detectable antibody following the first injection of bacteriophage. Out of twenty normal subjects injected with a similar dose, none failed to develop antibody. The secondary response was also profoundly depressed. Five out of ten patients failed to develop any antibody after the second injection. We have never before encountered an absent secondary response in twenty-six normal subjects and thirty patients with a variety of diseases of the liver and intestinal tract. One patient (number 3) developed a secondary response that was delayed, but normal in height. Three (numbers 1, 4 and 8) developed a much reduced secondary response, while one (number 5) who had a very poor primary response had very little further increase in antibody after the second injection.

There was evidence for delay in switching antibody production from IgM to IgG. The secondary response in case number 3 was mainly IgG (Fig. 3) but in case number 1 (Fig. 4) antibody was mainly in the IgM fractions. In case number 4, the antibody was divided evenly between IgM and IgG. The prolonged survival of viable bacteriophage is striking and correlated well with the failure of antibody production. Impaired clearance of intravenously injected bacteriophage has also been described in immunodeficient children (Ochs, Davis & Wedgwood, 1971). Further work is in hand to determine whether the delay in clearance is secondary to the impaired production of antibody or whether it represents a primary failure of antigen handling.

These results help to resolve the contradictions found in previous studies of antibody production in CLL. Cone & Uhr (1964), using the relatively less sensitive $k$ value as a measure of antibody, found no change after primary intramuscular injections of $\phi$X174 in nine patients with CLL. Only one out of ten patients developed secondary antibody after an injection of diphtheria toxoid. Hersh *et al.* (1970) injected Keyhole limpet haemocyanin into thirteen patients with CLL, and found a delay in the onset of antibody production as well as a delay in switching from IgM to IgG. They did not study the secondary response.

Our results show that the primary response to an intravenous antigen is indeed profoundly depressed in patients with CLL. There is no evidence, however, of a delayed primary response in this system. The secondary response is absent in six out of ten patients, and significantly reduced in three. One patient achieved normal levels, but after a marked delay. In two out of the three patients studied there was a significant delay in switching from IgM to IgG antibody production in the secondary response.

There is a tendency for the immunoparesis in CLL to be correlated with the severity of the disease. The patients are arranged in Table 1 and Table 2 in order corresponding with increasing severity of their disease. Case number 3, which was least ill, had the highest level of secondary antibody production. Cases numbers 2 and 6, which were clinically severely affected, with widespread lymph node involvement and splenomegaly, produced no detectable secondary antibody. The correlation is not, however, seen in all cases. Case number 7, in whom the leukaemia was clinically stationary, and who had no detectable lymphadenopathy or splenomegaly, produced no antibody.

There is also a tendency for antibody production to be inversely related to the total lymphocyte count. None of the four patients with a lymphocyte count greater than 40,000 produced any detectable antibody and patient number 3, with the highest level of antibody, had the lowest number of lymphocytes.

Although defective antibody production in CLL is now well-documented, there is very little evidence on the mechanism of immunoparesis. Despite the high numbers of leukaemic cells, substantial numbers of normal B lymphocytes can be detected in patients with this disease. Since many forms of antibody production depend on co-operation between B and T cells, it may be that the large number of immunologically ineffective cells diminishes the probability of fruitful interactions between competent cells. If this were the case, leukopheresis, by reducing the number of leukaemic cells in circulation, might restore antibody-producing capacity. This is a possibility which we are currently exploring.

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