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Research Article

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Immunologic Responses to Bacteriophage ϕ X 174 in Immunodeficiency Diseases

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ABSTRACT Immunologic responses to bacteriophage ϕ X 174 were studied in 26 patients with immunodeficiency diseases. In eight cases of infantile X-linked agammaglobulinemia, there was prolonged circulation of phage and no detectable antibody response. The remaining 18 patients cleared phage normally and produced antibodies. 10 of these patients made only IgM antibody in spite of repeated immunization; all of these have recurrent respiratory tract infections and require treatment with gamma globulin and antibiotics. Eight patients made both IgM and IgG antibody; they experience either milder or no infections, and only one requires treatment with gamma globulin.

Prolonged circulation of bacteriophage ϕ X 174 and the absence of a detectable antibody response appear to be distinguishing characteristics of X-linked agammaglobulinemia if severe combined immunodeficiency can be excluded.

INTRODUCTION

Immunodeficiency syndromes are a heterogeneous group of diseases (1)¹ which are characterized by impairment of humoral immunity, cellular immunity, or both. Most patients with impaired humoral immunity appear to make feeble antibody responses, but relatively few patients have been studied systematically (2-9).

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¹The terminology of immunodeficiency diseases used in this paper is that recommended by a working party of the World Health Organization (1). Terms likely to cause confusion are:

New name	Old name
Infantile X-linked agammaglobulinemia	Bruton's agammaglobulinemia
X-linked immunodeficiency with hyper-IgM	Dysgammaglobulinemia type I
Severe combined immunodeficiency	Swiss-type agammaglobulinemia, thymic aplasia, thymic dysplasia

Bacteriophage ϕ X 174 is a remarkably useful agent for studies of humoral immunity (10). It is a potent antigen and causes no recognized toxic effects in man (7). After intravenous administration, phage can be detected easily and enumerated in the blood by plaque count. Thus antigen clearance from the circulation can be determined. Phage neutralization is a sensitive assay for antibody and does not seem selective for one class of immunoglobulin.

The purpose of this report is to present results of studies with bacteriophage ϕ X 174 in various immunodeficiency diseases. 26 patients were immunized with bacteriophage ϕ X 174. Their antigen clearance, primary and secondary antibody response, and the sequence of antibody class, were determined and compared with normal adult volunteers.

METHODS

Subjects. 5 healthy, young adult volunteers and 26 patients with a variety of immunodeficiency diseases were studied after informed consent was obtained. The clinical and laboratory findings of these patients are characterized in Table I. Methods for studies summarized in Table I have been published (11-13). Some of these cases have been reported in part previously; references are cited in the Table. For convenience in presentation, the 26 patients are divided into four groups. Group I consists of eight patients with infantile X-linked agammaglobulinemia. Group II represents eight patients with antibody deficiency syndromes other than the infantile X-linked type. Group III is made up of five patients with ataxia telangiectasia, and group IV consists of five patients with antibody deficiency and gastrointestinal disease.

Bacteriophage ϕ X 174. The phage was grown, harvested, and purified by the method described by Uhr, Finkelstein, and Baumann (10). The purified phage was sterilized by passage through a 0.45 μ micropore filter. Sterilized phage did not cause fever or leukopenia in rabbits. Final preparations of the bacteriophage, containing between 5×10^{10} and 5×10^{11} plaque-forming units (PFU)/ml, were stored in small portions at -60°C until thawed for use. There was little decline in phage titer after storage for 6 months.

Phage clearance studies. Phage was given intravenously in a dose of 1.5×10^9 PFU/kg body wt. Blood was col-

TABLE I
Case Summaries

Type of immune defect	Case No.	Year of birth	Sex	Serum immunoglobulins* <i>mg/100 ml</i>	Blood type	Isohemoagglutinin titer	Typhoid antibody titer‡	Comments
I. Infantile X-linked agammaglobulinemia	1	1967	M	IgG 250-300 IgM <5 IgA <5	A	Anti-B = 0	O < 1:5 H < 1:5	Symptoms well controlled on gamma globulin. No plasma cells in rectal mucosa. No plasma cells or germinal centers in lymph node. Identical twin died at 8 months of age from <i>Pseudomonas</i> sepsis. Autopsy diagnosis was agammaglobulinemia. Case 6 is a maternal uncle.
	2	1960	M	IgG 20 IgM 30 IgA <5	O	Anti-A = 0 Anti-B = 0	O < 1:5 H 1:10	Recurrent infections began at 9 months of age and rheumatoid arthritis at 3 yr. No plasma cells in rectal mucosa.
	3	1960	M	IgG 200 IgM <5 IgA <5	O	Anti-A = 1:1 Anti-B = 1:1	O < 1:5 H 1:10	Severe infections since infancy. No plasma cells in rectal mucosa and no plasma cells or germinal centers in lymph node. A maternal uncle died at 9 months of age from pneumonia.
	4	1956	M	IgG 80-280 IgM <5-30 IgA <5-20	B	Anti-A = 0	O < 1:5 H < 1:5	Recurrent infections and bronchiectasis. No plasma cells in rectal mucosa or lymph node. No germinal centers in lymph node.
	5	1950	M	IgG 110-300 IgM <5-80 IgA <5	O	Anti-A = 0 Anti-B = 0	O < 1:5 H < 1:5	Recurrent bacterial infections and bronchiectasis. Convulsive disorder. Has had lobectomy. No plasma cells in rectal mucosa and no plasma cells or germinal centers in lymph node. Has eight healthy maternal uncles. Reported as case 3 in references 7 and 11.
	6	1949	M	IgG 100 IgM <5 IgA <5	O	Anti-A = < 1:2 Anti-B = < 1:2	O 1:5 H 1:10	Uncle of case 1. Frequent sino-pulmonary infections and bronchiectasis. No plasma cells in rectal mucosa.
	7	1949	M	IgG <20 IgM <5 IgA <5	O	Anti-A = 0 Anti-B = 0	O < 1:5 H < 1:5	Brother of case 8. Recurrent infections and bronchiectasis. No plasma cells in rectal mucosa. A brother died at 4 yr of age from pulmonary infection. Two maternal uncles died early in childhood, probably from infections.
	8	1947	M	IgG <100 IgM <5-60 IgA <5	O	Anti-A = 0 Anti-B = 0	O < 1:5 H < 1:5	Brother of case 7. Recurrent infections and bronchiectasis. No plasma cells in rectal mucosa.
II. Miscellaneous immunodeficiency syndromes	9	1967	M	IgG 40-100 IgM 700-105 IgA <5	O	Anti-A = 1:512 Anti-B = 1:4	O < 1:5 H 1:20	Case of X-linked immunodeficiency with hyper-IgM. Asymptomatic on gamma globulin therapy. Had <i>Pneumocystis carinii</i> pneumonia at 4 months of age proven by open lung biopsy. Good responses to Pentamidine.§ No plasma cells or germinal centers in lymph nodes. No plasma cells in rectal mucosa. An older brother died at 10 months of age from <i>P. carinii</i> pneumonia. Autopsy diagnosis was immunologic deficiency disease.

TABLE I—(Continued)

Type of immune defect	Case No.	Year of birth	Sex	Serum immunoglobulins* <i>mg/100 ml</i>	Blood type	Isohemoagglutinin titer	Typhoid antibody titer‡	Comments
	10	1964	F	IgG 800 IgM 78 IgA 30	A	Anti-B = 1:4	O < 1:5 H < 1:5	Recurrent respiratory infections since infancy. Developed cirrhosis of liver at age 3. Questionable control of infections with gamma globulin. Many plasma cells in rectal mucosa. Positive Shick test.
	11	1961	F	IgG 50-110 IgM <5-65 IgA <5-15	O	Anti-A = 0 Anti-B = 0	O < 1:5 H 1:20	Recurrent infections. No plasma cells in lymph node or rectal mucosa. Has germinal centers in lymph node. Classified as congenital agammaglobulinemia with splenomegaly. Reported as case 6 in reference 7 and case 4 in reference 11.
	12	1960	M	IgG 65-100 IgM <5-40 IgA <5	O	Anti-A = 0 Anti-B = 0	O < 1:20 H < 1:20	Brother of case 13. Recurrent upper respiratory infections and psoriasis. Does not have severe recurrent infections. No plasma cells in rectal mucosa or lymph node. No germinal centers in node. Reported as case 4 in reference 6 and reference 11.
	13	1955	M	IgG 270 IgM 15 IgA 84	O	Anti-A = 0 Anti-B = 0	O < 1:20 H < 1:20	Brother of case 12. Similar findings. Not treated with gamma globulin. Reported as case 5 in references 7 and 11.
	14	1954	F	IgG 400-600 IgM 13-130 IgA <10	A	Anti-B = 1:16	O < 1:5 H 1:10	Identical twin of case 15. Recurrent infections and bronchiectasis. No plasma cells in rectal mucosa or lymph node. No germinal centers in the node. Reported as case 1 in references 7 and 11.
	15	1954	F	IgG 330-650 IgM 15-60 IgA 5-40	A	Anti-B = 1:8	O < 1:5 H 1:5	Identical twin of case 14. Similar history and findings. Reported as case 2 in references 7 and 11.
	16	1943	F	IgG 50-110 IgM <5-65 IgA <5-15	A	Anti-A = 0 Anti-B = 0	O < 1:5 H 1:5	Recurrent infections and bronchiectasis. Good response to gamma globulin. No plasma cells in rectal mucosa.
III. Immunodeficiency with ataxia telangiectasia	17	1961	M	IgG 60-200 IgM 400-2000 IgA <5	A	Anti-B = 1:4	O < 1:5 H 1:20	Brother of case 18. Severe sino-pulmonary disease with bronchiectasis and cor pulmonale. Many IgM plasma cells in the rectal mucosa. Reported as case 7 in reference 7 and case 1 in reference 12.
	18	1960	M	IgG 80-270 IgM 330-1000 IgA <5	A	Anti-B = 1:128	O 1:5 H 1:20	Brother of case 17. Similar history and findings except does not have cor pulmonale. Reported as case 8 in reference 7 and case 2 in reference 12.
	19	1957	F	IgG 460-860 IgM 600-800 IgA <5	A	Anti-B = 1:1000	O 1:5 H 1:80	Sister of case 21. Has not had recurrent infections. Many IgM plasma cells in the rectal mucosa. Reported as case 4 in reference 12.

TABLE I—(Continued)

Type of immune defect	Case No.	Year of birth	Sex	Serum immunoglobulins* mg/100 ml	Blood type	Isohemoagglutinin titer	Typhoid antibody titer‡	Comments
	20	1956	F	IgG 600-1000 IgM 100-350 IgA 16-20	O	Anti-A = 1:128 Anti-B = 1:256	O 1:10 H 1:160	Mild sinopulmonary infections and severe psoriasis. Many IgM plasma cells in the rectal mucosa. Reported as case 5 in reference 12.
	21	1952	M	IgG 400-1000 IgM 160-280 IgA <5	A	Anti-B = 1:128	O 1:5 H 1:80	Brother of case 19. Has occasional sinopulmonary infection not requiring gamma globulin. Many IgM plasma cells in the rectal mucosa. Reported as case 6 in reference 12.
IV. Miscellaneous immunodeficiency syndromes associated with malabsorption	22	1947	F	IgG 50 IgM <5-15 IgA <5	A	Anti-B = 0	O 1:40 H 1:1280	Patient has an unusual syndrome of small stature, mental retardation, and malabsorption. Frequent colds but no recurrent bacterial infections. Serum carotene was low and vitamin B ₁₂ absorption was below normal. Small intestinal biopsy demonstrated <i>Giardia</i> but no plasma cells.
	23	1945	M	IgG 460 IgM 50 IgA <10	O	Anti-A = 1:32 Anti-B = 1:8	O 1:80 H > 1:2560	No history of infections. Had severe gastrointestinal bleeding, often requiring blood transfusion, since infancy. At laparotomy, an extensive hemangioma of the duodenum was partially removed. Nodular lymphoid hyperplasia of small intestine. Rectal mucosa contained numerous plasma cells and lymphoid follicles.
	24	1933	M	IgG 350 IgM 30 IgA <5	A	Anti-B = 0	O < 1:5 H > 1:2560	Case of nodular lymphoid hyperplasia of the small intestine and sarcoidosis. Recurrent pulmonary infections. Fully reported in reference 13.
	25	1924	M	IgG 420 IgM 80 IgA <5	A	Anti-B = 1:8	O 1:5 H 1:40	Recurrent infections and bronchiectasis. Diarrhea since age 15. Low serum carotene and fat absorption. No plasma cells in the rectal mucosa.
	26	1919	M	IgG 140-230 IgM <5-10 IgA <5	O	Anti-A = 0 Anti-B = 0	O 1:5 H < 1:5	Recurrent infections and bronchiectasis. Diarrhea began in 1969. Serum carotene and fat absorption were low. No plasma cells in the rectal or small intestinal mucosa. <i>Giardia</i> found in small intestinal biopsy specimens.

* By single radial diffusion. Most patients had many determinations over several years; many patients were treated with gamma globulin when serum was collected.

‡ By bacterial agglutination after three immunizations with typhoid vaccine.

§ Obtained from National Communicable Disease Center, Atlanta, Ga.

lected after 15 min to determine the resting phage titer; the dose was chosen to give an initial concentration of 5×10^7 PFU/ml serum. Samples were obtained at various intervals thereafter, and serum was tested for the presence of bacteriophage. The number of plaque-forming units per milli-

liter was determined by the agar overlay method (14) and serial dilution of the serum samples.

Neutralizing antibody. After phage had disappeared from the circulation, the antibody activity was determined by phage neutralization and was expressed as the rate of

inactivation or K value as derived by a standard formula (7). The lower limit of sensitivity by the usual 1-hr incubation is a K value of 0.01.

Saliva and sputum. Saliva specimens were collected from a few volunteers and selected patients after stimulation of salivary flow by chewing sour candy. Sputum was collected from case 18 during postural drainage and pulmonary therapy. The sputum specimens obtained from that case were not grossly blood tinged and had negative tests for hemoglobin.

Gel filtration. Serum and saliva proteins were separated by gel filtration on a 2.5 × 100 cm column of Sephadex G-200 (Pharmacia, Uppsala, Sweden). Sephadex G-200 was sieved (100, 200, and 220 meshes per in.), and middle-sized beads were used. The gel was expanded by repeated suspension in boiling water. The buffer for all studies was 0.1 M Tris in 0.15 M NaCl, pH 7.5. The column was adapted for upward flow using an LKB pump (LKB Produkter AB, Stockholm, Sweden) at a rate of 15 ml/hr. Fractions were collected in 6-ml portions. Protein was determined by optical density at 280 λ in a Beckman spectrophotometer, and antibody activity was assayed by phage neutralization.

Antibody susceptibility to reducing agents. Susceptibility of phage antibody to 2-mercaptoethanol was done by the method of Grubb and Swahn (15).

RESULTS

Antigen clearance. Bacteriophage disappeared from the circulation of all volunteers and most patients by the 4th day. This finding is similar to that reported previously (7). The eight cases classified as infantile X-linked agammaglobulinemia in group I had prolonged circulation of bacteriophage (Fig. 1). All had bacteriophage present in the circulation for 11 days or more. In most instances the decline in titer of bacteriophage was comparable with the rate of spontaneous inactivation of bacteriophage at

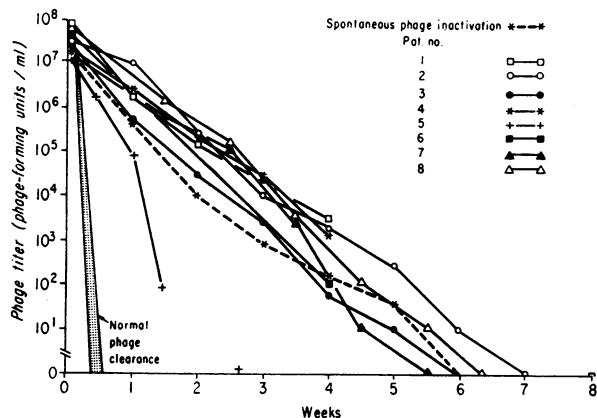


FIGURE 1 Disappearance of ϕ X 174 from the blood in normal volunteers and eight patients with X-linked agammaglobulinemia. Normal phage clearance, shown by the stippled area, is based on five adults in this study, six immunologically normal children (7), and one newborn infant (21). Cases 9-26 cleared phage in the normal time. The rate of spontaneous phage inactivation was determined on a suspension of phage in broth at 37°C.

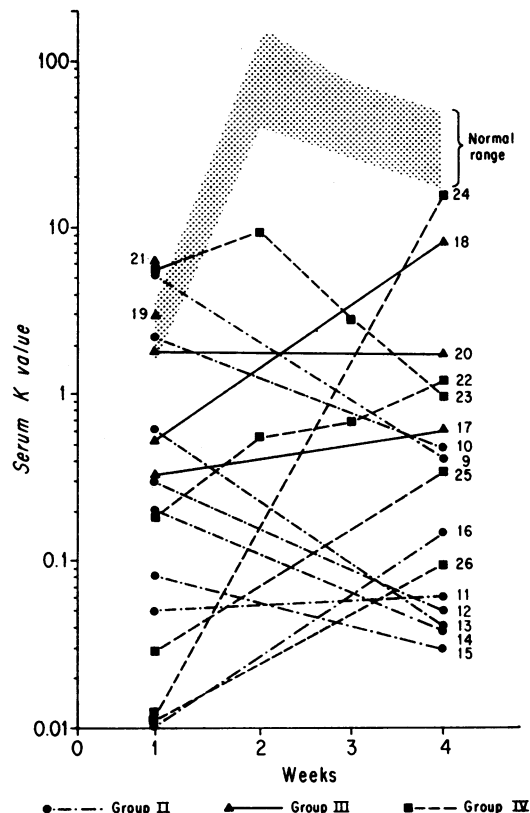


FIGURE 2 Primary antibody responses to ϕ X 174 of cases in groups II-IV. Phage was injected intravenously at time zero. Normal range is based on studies in five normal adults.

37°C. None of the other 18 patients in the study had phage present in the serum 7 days after injection.

Case 5 had phage present in his circulation 11 days after injection and none at 18 days. Phage was injected two additional times, and the clearance on both occasions was similar. This patient had a somewhat accelerated clearance of bacteriophage when compared with the other seven cases of X-linked agammaglobulinemics, but the rate of clearance was nevertheless distinctly prolonged as compared with normals or other patient groups.

Antibody response. The range of primary antibody responses to bacteriophage in five healthy adult volunteers is shown by the crosshatched area in Fig. 2. The K values shown are similar to those obtained in a previous study on six immunologically competent children (7). Highest K values were consistently reached 2 wk after immunization; titers then declined.

No antibody was detectable in the serum of any of the patients in group I after phage had disappeared from the circulation ($K < 0.01$). Primary antibody responses of the remaining 18 cases are shown in Fig. 2. At 1 wk

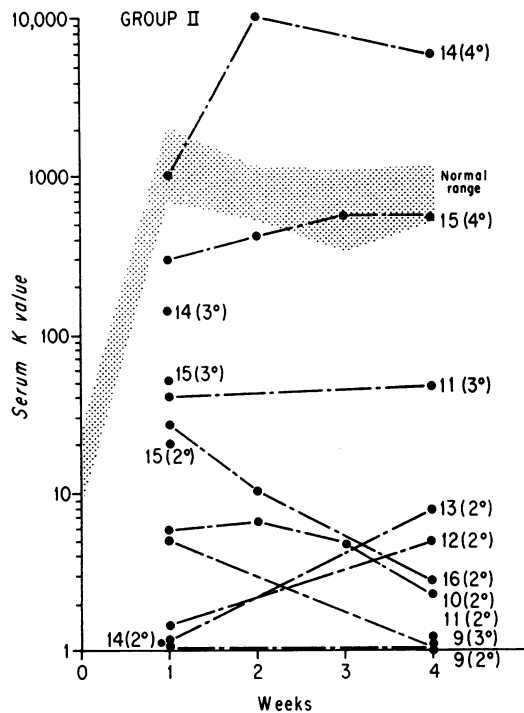


FIGURE 3 Secondary (2°), tertiary (3°), and quarternary (4°) antibody responses of patients in group II. Phage was injected at time zero. Normal range is based on studies in four adults.

after immunization six patients had K values which were in or near the normal range. Two of these cases were in group II, three were in group III, and one was in group IV. All normal volunteers showed a significantly higher K value at 4 wk than at 1 wk after immunization with bacteriophage; in contrast, the antibody titer of many patients decreased by the 4th wk. At that time none of the patients reached the antibody level of the normal controls.

The responses to secondary immunizations are shown in Figs 3-5. Some patients were given a third and two patients a fourth immunization. The normal volunteers (shown by the stippled areas) had a rapid rise in antibody; the K values declined little during the next 3 wk. Only four patients (case 18, ataxia telangiectasia; cases 22-24 in group IV) developed antibody levels after secondary immunization approaching the responses of the volunteers. All other patients had markedly diminished responses. Nevertheless, all patients who developed antibody (except for case 9) exhibited evidence of an immunologic memory; the maximum serum K value was in each instance higher after the second than after the first immunization.

Some patients developed very high serum K values after multiple immunization; for example, cases 14 and 15 (Fig. 3) after the fourth immunization, and case 18

(Fig. 4) and case 24 (Fig. 5) after a third immunization. In contrast, two siblings with ataxia telangiectasia (cases 19 and 20) had no further rise in antibody after tertiary immunization (Fig. 4).

Gel filtration of antibody. At least one specimen obtained from all patients after secondary immunization was studied by gel filtration. The first protein peak in eluates from about half of the volunteers and patients was treated with 2-mercaptoethanol. Antibody activity was completely abolished in every specimen tested, and this procedure was then discontinued. Antibody activity found in the first protein peak will be referred to as IgM, and antibody activity in the second peak will be referred to as IgG.

All volunteers had only IgM antibody at 1 wk after primary immunization (Fig. 6). 4 wk after primary immunization most antibody was still IgM although the volunteers had begun to make some IgG at that time. After secondary immunization all healthy volunteers made predominantly IgG antibody with little or no IgM antibody detectable (Fig. 6).

Of the 18 patients who made detectable antibody, 10 made only IgM antibody, and 8 made both IgM and IgG antibodies after two or more immunizations with phage (Table II). Only cases 19 and 23 made IgG antibody comparable with healthy adults.

As examples, results of gel filtration of specimens from three patients in group IV are shown in Fig. 7.

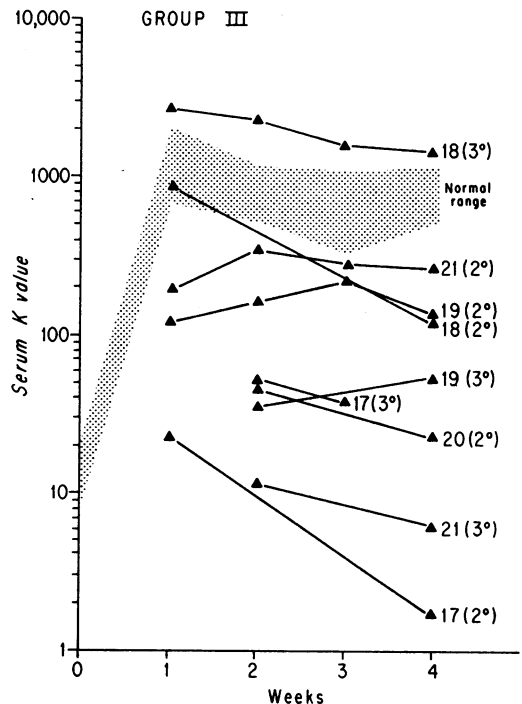


FIGURE 4 Secondary (2°) and tertiary (3°) antibody responses of patients in group III.

Case 26 made only IgM antibody. Case 22 made both IgM and IgG antibodies with IgM predominating. Case 23 had a normal response of mainly IgG antibody.

Antibody activity in saliva. Simultaneously obtained specimens of saliva and serum from the five normal volunteers and eight patients were studied for antibody activity using gel filtration. Antibody of both IgM and IgG mobility was found in saliva. In every instance the antibody in saliva eluted in the same position as that found in the serum. Salivary antibody levels, however, never approached those of serum when compared on the basis of protein concentration.

DISCUSSION

Antibody activity in this study was calculated from the rate of phage inactivation or K value (7, 10). This method of assay is based on the assumption that phage neutralization is a first-order reaction (14), an assumption now known to be incorrect. Because phage neutralization departs from first-order kinetics after 90% or more of the phage has been neutralized, two new methods of calculating antibody activity have been developed (16, 17). The K value method has greater convenience and simplicity. Our study is primarily concerned with large differences in antibody concentrations, and the results would not be changed significantly by using other methods of calculation.

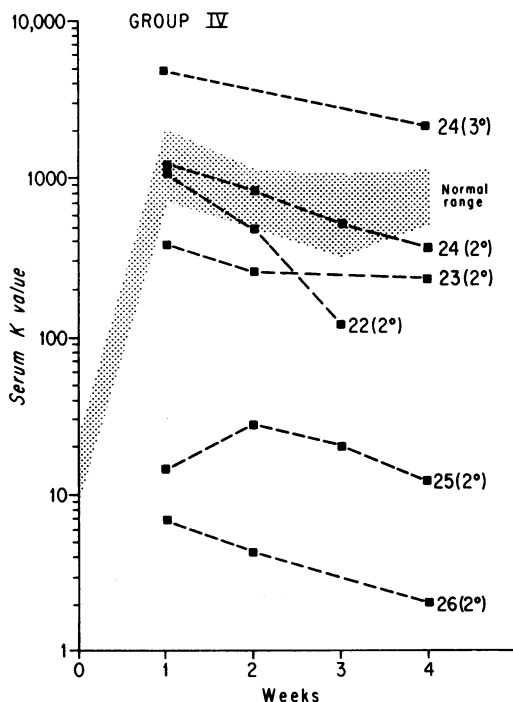


FIGURE 5 Secondary (2°) and tertiary (3°) antibody responses of patients in group IV.

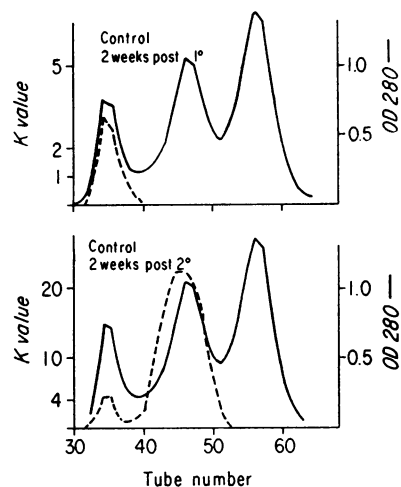


FIGURE 6 Eluates of Sephadex G-200 column chromatography of serum from a normal adult after primary (1°) and after secondary (2°) immunization. Protein concentration is indicated by solid line, antibody activity by dashed line. IgM elutes in the first peak, IgG in the second peak. Note differences in K value, scale on the left.

Antibody eluted in the first protein peak on Sephadex G-200 was assumed to be IgM; antibody in the second peak was assumed to be IgG. The assumption that the first peak contained only IgM antibody is supported by the susceptibility of the antibody activity to treatment with 2-mercaptoethanol. The possibility cannot be excluded that some of the antibody activity found in the second peak was due to immunoglobulins other than IgG, especially IgA.

The relative amounts of antibody assigned to IgM or IgG class will depend to some extent on the method of antibody assay (18). For example, hemagglutination assays detect primarily IgM antibody. Exclusive use of such a method may lead to erroneous conclusions about the sequence of appearance of antibody activity in IgM and IgG. Phage neutralization is not selective for IgM or IgG, and the sequence of an early IgM and later IgG antibody response is well established (6, 7).

Clinical and laboratory findings in patients of group I are compatible with the diagnosis of infantile X-linked agammaglobulinemia (1, 4, 8). All had prolonged circulation time (mean 30.4 days, range 11–42 days) of bacteriophage after intravenous injection. In sharp contrast, normal controls and the remaining 18 patients cleared the phage by 4 days after injection. Furthermore, none of the patients in group I made detectable antibody. We have previously seen such prolonged circulation of phage only in a case of severe combined immunodeficiency (19). Prolonged circulation of phage and absence of a detectable antibody response appear to be specific and reliable diagnostic criteria of X-linked agammaglobulinemia, if severe combined immunodeficiency can be

TABLE II
Correlation of Immunoglobulin Class of Phage Antibody after Two or More Immunizations with the Need for Gamma Globulin Therapy and Its Effect

Group	Case No.	No. of phage injections	Immunoglobulin class of phage antibody*		Treatment with gamma globulin	
			IgM	IgG	Needed‡	Effect
II	9	3	+	0	Yes	Good
	10	2	+	++	Yes	Fair
	11	3	+++	0	Yes	Good
	12	2	++	+	No	No effect when tried
	13	2	++	+	No	No effect when tried
	14	4	++++	0	Yes	Good
	15	4	++++	0	Yes	Good
	16	2	++	0	Yes	Good
III	17	3	+++	0	Yes	Fair
	18	3	++++	0	Yes	Fair
	19	3	+	+++	No	—
	20	2	++	+	No	—
	21	3	+++	+	No	—
IV	22	2	+++	+	No	—
	23	2	+	+++	No	—
	24	2	++++	0	Yes	Good
	25	2	++	0	Yes	Good
	26	2	+	0	Yes	Good
Controls (five normal adults)		2	+	++++	—	—

* Concentration and proportions of IgM and IgG antibody were estimated from results of gel filtration. No antibody is represented by 0; high concentrations of antibody are indicated by + + + +.

‡ By clinical impression on the basis of severity and frequency of infections.

excluded. Available evidence (20, 21) indicates that antigen clearance and antibody response to bacteriophage ϕ X 174 are normal in newborn infants. Thus it should be possible to establish or exclude a diagnosis of X-linked agammaglobulinemia in the immediate neonatal period. Such a test would be most useful in a study on an infant born into a family with an affected sibling.

The findings on the 18 patients who cleared bacteriophage and developed antibody are summarized in Table II. The immunoglobulin class of the antibody is represented quantitatively. Only eight of the patients had demonstrable IgG antibody after two or more injections of bacteriophage. Seven of these eight cases have either milder or no infections in comparison with the severity of the difficulties experienced by the other patients in this series. Only one of the eight (case 10) has received gamma globulin until recently, and the therapeutic benefit seemed questionable. Of the patients who were either unable to make detectable antibody (8 with X-linked agammaglobulinemia) or who developed antibody only of the IgM class (10 with various immunodeficiencies),

all have recurrent infections and require gamma globulin and frequent antibiotic therapy.

The severity of infections and the need for gamma globulin therapy in this study are closely correlated to the ability of patients to synthesize IgG antibody. It seems most likely that this relationship reflects the degree of immunologic impairment; patients who cannot synthesize IgG antibody represent a group which is more severely immunologically impaired. On the other hand, it is possible that a feeble IgG antibody response is a significant factor in defense of patients with immunodeficiency diseases.

Of particular interest is case 9, a patient with X-linked immunodeficiency with hyper-IgM similar to one reported by Gleich, Uhr, Vaughan, and Swedlund (6). Both patients appear to have a relatively intact mechanism to synthesize IgM and a severe defect of the IgG system. Case 9 was the only patient in this study who made only IgM antibody and did not exhibit immunologic memory.

The prolonged circulation of phage in infantile X-linked

agammaglobulinemia was remarkable and unexpected. Because the disappearance rate of phage in several patients paralleled the spontaneous inactivation rate of phage at 37°C, we presume that these patients do not even have a nonimmune mechanism for clearance of this antigen. Bacteriophage ϕ X 174 is a large protein and is known to remain primarily in the intravascular space after intravenous injection (10). Nakamura, Spiegelberg, Lee, and Weigle (22) have shown that large globular proteins tend to remain in the intravascular space. By remaining primarily intravascular, ϕ X 174 may have little contact with fixed phagocytic cells of the lungs, liver, and spleen. Leukocytes in the blood may be unable to phagocytose ϕ X 174 efficiently in the absence of antibody. If one assumes that the only defect in infantile X-linked agammaglobulinemia is absence of antibody, then one would conclude that phage can only be removed from the circulation in man by an immune process. Further investigations are needed on these points.

The various types of antibody deficiencies in our patients closely resemble some animal models of immunodeficiency. Warner, Uhr, Thorbecke, and Ovary (23) showed recently that hormonal bursectomy completely ablates antibody response in some chickens and impairs the production of IgG antibody in others. Bacteriophage

ϕ X 174 circulated for over 21 days in some of the hormonally bursectomized birds, and no antibody response could be detected. Other bursectomized chicks made IgM antibody only. Immunosuppressive drugs such as 6-mercaptopurine (24), cytosine arabinoside (25), methotrexate (25, 26), and L-asparaginase (27) have been shown to suppress the IgG antibody response to a much greater extent than the IgM antibody response. In adult rabbits, 6-mercaptopurine impairs mainly the IgG response; in young rabbits, however, both IgG and IgM antibody responses are suppressed (28). Some treated animals became completely agammaglobulinemic and made no antibodies. Two theories of the differentiation of antibody-producing cells can account for observations made in experimental animals and in our patients. If different immunoglobulins are produced in separate cell lines (29), then the IgM-producing cell line must be more resistant to insult. In a more attractive hypothesis, Cain, Cooper, Van Alten, and Good (30) proposed that cells producing all classes of immunoglobulins are products of the same cell line at different stages of differentiation. A stem cell produces first IgM cells and then differentiates into IgG- and IgA-producing cells. Interruption of this sequence at different steps would produce many of the currently recognized antibody deficiencies that are seen in the animal models and in human immunodeficiency diseases.

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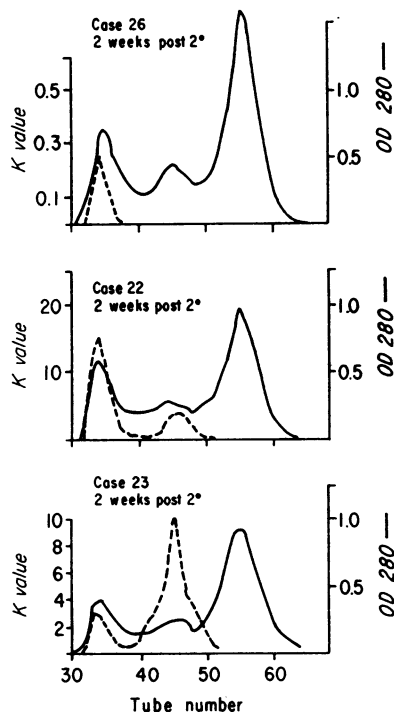


FIGURE 7 Sephadex G-200 column chromatography of serum from three patients of group IV after secondary (2°) immunization. Case 26 shows antibody activity in the first peak only, whereas cases 22 and 23 have activity in both peaks.

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