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THE IgM AND IgG RESPONSE TO *BORDETELLA PERTUSSIS* VACCINATION AND INFECTION

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SUMMARY. Ultracentrifugation was used to separate IgG from IgM in serum samples from children after pertussis vaccination or infection and the fractions were examined by indirect haemagglutination (IHA) and complement-fixation (CF) tests. IHA detected pertussis-specific IgM and IgG but CF detected only IgG which appeared to be of a different subclass from the IHA IgG. The IgM titres were higher after infection than after vaccination but the reverse tended to apply to the IgG titres. Little IgM or CF IgG was detected 5 months after a dose of vaccine, but the IHA IgG persisted longer. Vaccinated children who were subsequently infected showed IgM and CF IgG responses similar to those of unvaccinated, infected children but the IHA IgG titres reached much higher levels.

INTRODUCTION

Indirect haemagglutination (IHA) and complement fixation (CF) antibodies are produced after vaccination or infection with *Bordetella pertussis*. The IHA titres are generally higher than the CF titres and the behaviour of the two antibodies is different: after vaccination, IHA titres of up to 1280 can persist for 5 or more years but the CF titres fall to less than 10 in about 8 months; after infection, IHA usually shows a greater rise in titre than CF but occasionally only the CF titre rises (Macaulay, 1979). These two tests may, therefore, detect different antibodies, possibly different types of immunoglobulin, because both tests detect antibody to the serotype antigens 1, 2 and 3. Because vaccinated children sometimes become infected with *B. pertussis*, it would be useful if antibody produced during infection could be differentiated

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from that induced by vaccination. Dolby and Stephens (1973) separated IgM from IgG antibody in pooled sera from infected or vaccinated children on Sephadex G-200 and tested the fractions for agglutinin, bactericidal antibody and haemagglutinin. They found indications that the antibody response to vaccination was different from that due to infection; for example agglutinin and bactericidal antibody were present mainly in the IgG fraction 1 month after a second dose of vaccine but were present in equal amounts in the IgG and IgM fractions 2–9 weeks after infection. Because the response of individual children to infection varies widely (Macaulay, 1979) the results obtained with pooled sera are difficult to interpret and some of the differences may be obscured. In this study ultracentrifugation was used to separate IgM from IgG antibody in serum samples taken from children after vaccination or infection with *B. pertussis*, and the fractions were tested for IHA and CF antibody.

Materials and methods

Sera. Fourteen serum samples were available from children, aged 7 months–3 years, who had received pertussis vaccine. They had been taken for virological investigations and the Health Department supplied the vaccination history of the children; six samples were taken 4 weeks–5 months after a second dose of vaccine and eight samples 3 weeks–21 months after a third dose had been administered.

Thirteen serum samples, taken during the course of the investigation of children with whooping cough, were also available; seven samples were from five unvaccinated children, aged 3 months–5 years, and six samples were from four vaccinated children, aged 3–11 years. In three cases from each group the diagnosis was confirmed by the isolation of *B. pertussis*, serotype 1,3; otherwise, confirmation of infection was by an eightfold or greater rise in the IHA titre.

Sucrose-density-gradient centrifugation. Sucrose-density gradients were prepared in cellulose nitrate centrifuge tubes by layering 0·9-ml volumes of sucrose solutions, strengths 37·5%, 31·25%, 25·0%, 18·75% and 12·5% (w/v) in the order given and leaving the tubes at room temperature for 5 h to equilibrate. The serum was diluted with an equal volume of dextrose-gelatine-veronal buffer, *p*H 7·3 (Cruickshank *et al.*, 1975) and inactivated at 55°C for half an hour; 0·5 ml of this dilution was layered on top of a sucrose gradient, and the tubes were balanced with liquid paraffin. They were then centrifuged at 8°C in a swing-out rotor on an MSE Superspeed 65 Mk 2 ultracentrifuge for 17 h at 40 000 rpm (approx. 135 000 *g*). As soon as the tubes were removed from the centrifuge the bottom of each was pierced with a needle mounted to restrict penetration and twelve fractions of 28 drops each were collected. Fractions 2–11 were tested for pertussis antibody by IHA and CF tests (Macaulay, 1979). The immunoglobulin classes in fractions from 15 sera were detected by double diffusion in agar with antisera specific for human IgG, IgA and IgM (Wellcome Reagents Ltd).

Results

Immunoglobulins detected in serum fractions

Double diffusion in agar detected IgM in fractions 2–4, IgG in fractions 4–11 and IgA in fractions 4–9. Thus IgG and IgA were not separated but it is unlikely that either of the two serological tests used detected IgA because IgA does not fix complement (Stanworth and Turner, 1973) and Thomas (1975) found that his indirect haemagglutination test did not detect pertussis IgA in

nasal secretions. Therefore, in the results that follow, the antibody detected in fractions 4–11 is assumed to be IgG.

IgM and IgG response to vaccination and infection

CF antibody was never detected in fractions 2–4, the IgM fractions, but IHA antibody could be detected in any fraction. Figures 1–3 show the results on the 27 sera, with the titres in fractions 3 and 8 representing the IgM and IgG titres respectively; the titres are given as reciprocals of the initial dilution because one of the tests is a CFT. There was no significant difference in the response to a second or third dose of vaccine and these results are not distinguished.

The highest IgM titre after vaccination was 16, six weeks after the third dose, and 5 months or more after the last injection the titre was $\leqslant 2$ (fig. 1). The IgM response after infection was similar whether or not the child had previously been vaccinated; 2 weeks after the onset of cough the IgM titre was 4, and from 3 to 6 weeks the titre was 16–512. The serum taken at 7 weeks had a titre of 8, but the lack of antibody in the IgG fractions (table) in this serum suggests that this is the beginning of a late antibody rise rather than a fall in IgM titre.

Fig. 2 shows that the IHA IgG titres after a second or third dose of vaccine were much higher than the IgM titres; titres of 512–8192 were detected during weeks 3–8, and even in the sample taken 21 months after a third dose of vaccine the IHA IgG titre was 64. The IHA IgG response after infection depended on whether the child had previously been vaccinated. In vaccinated children there was an early rise in IHA IgG and titres of 64–1024 were seen 3 and 5 weeks after the onset of cough, while unvaccinated children showed no IHA IgG until after 5 or 6 weeks when titres of 4 or 8 were detected.

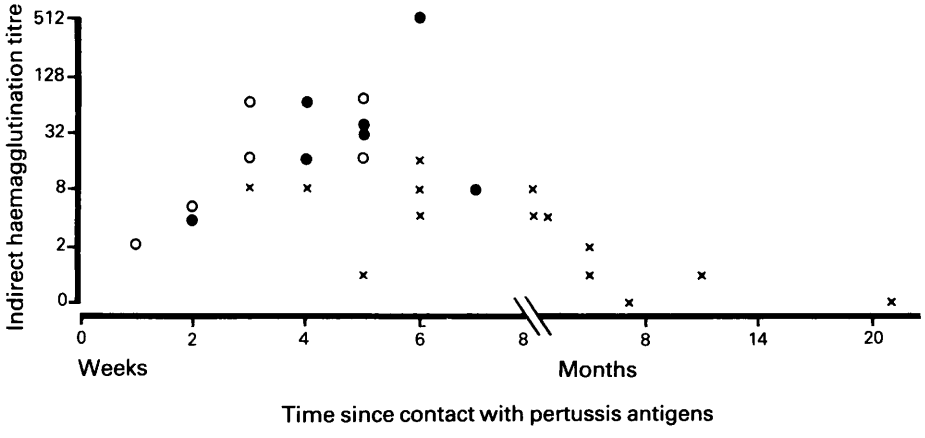

FIG. 1.—Indirect haemagglutination IgM titres in fraction 3 of serum from unvaccinated children with whooping cough (•), vaccinated children with whooping cough (o), and children who received two or three doses of pertussis vaccine (×).

TABLE

Antibody titres in the IgG fractions of five sera from unvaccinated children with whooping cough

Fraction no.	Titre at indicated time after onset of cough and by indicated test									
	4 weeks		5 weeks		5 weeks		6 weeks		7 weeks	
	IHA	CF	IHA	CF	IHA	CF	IHA	CF	IHA	CF
4	8	0	16	0	16	0	256	0	8	0
5	4	0	16	0	16	0	128	0	2	0
6	4	0	16	0	8	0	64	0	2	0
7	2	N	4	0	4	2	8	0	2	0
8	0	2	0	2	4	4	8	2	0	0
9	0	N	0	0	N	4	4	2	0	2
10	0	0	0	0	0	0	N	0	0	2

IHA = indirect haemagglutination test; CF = complement-fixation test. N indicates that antibody was detected only in the undiluted fraction.

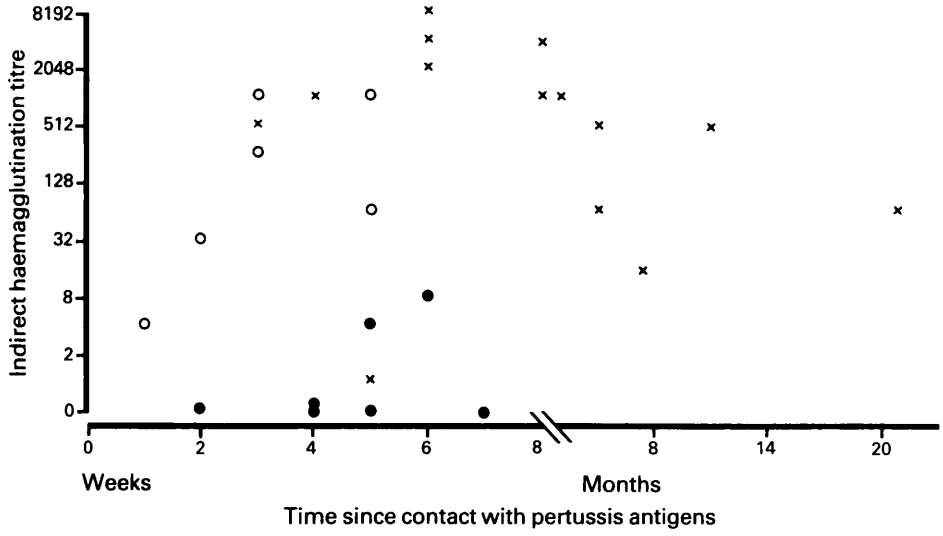

FIG. 2.—Indirect haemagglutination IgG titres in fraction 8 of sera indicated by symbols as in fig. 1.

The CF IgG was at much lower titres in all children (fig. 3). Titres of up to 16 were found 3–8 weeks after the last dose of vaccine but by 5 months, antibody was detected only in the neat fraction, if at all. After infection the titres were even lower than after vaccination; the maximum titre of 4 was detected at 5 weeks. The CF IgG titres after infection showed no significant difference between vaccinated and unvaccinated children at least up to 7 weeks after onset.

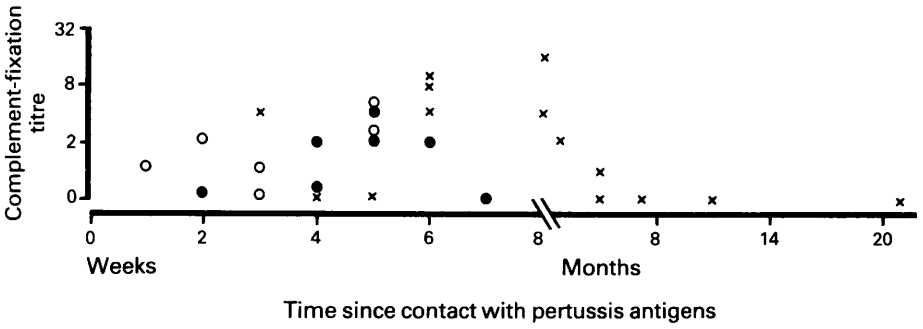

FIG. 3.—Complement-fixation IgG titres in fraction 8 of sera indicated by symbols as in fig. 1.

Evidence that CF and IHA antibodies are in different subclasses of IgG

IHA IgG persists for longer than CF IgG but only the CF titre rose in three cases of whooping cough investigated previously (Macaulay, 1979). These results cannot be explained by the difference in sensitivity of the two tests because even in the ultracentrifuged fractions the titres of the two tests were not related. For example, two sera taken 1 or 2 months after a dose of vaccine had an IHA titre of 1024 in fraction 8, while in one case the CF titre was 16, the highest seen in any fraction, and CF antibody was not detected in the second. The difference could either be due to the two tests detecting antibody to different antigens, even though both tests give titres with type specific sera, or it could be due to the two tests detecting different subclasses of IgG. The ultracentrifugation results provide some evidence for the latter suggestion because CF antibody tended to be found in the fractions with the higher numbers. The difference was most clearly seen in sera from unvaccinated children with whooping cough. The table shows the titres in the IgG fractions of the five sera from such children where IHA antibody was detected mainly in fractions 4–7 and CF antibody in fractions 7–10.

DISCUSSION

Interpreting the serological response to infection is difficult because of the number of antigens present in bacteria, the different classes of antibody that may be produced, and the variation in the response of patients to infection. It is generally accepted that IgM fixes complement, but the CF test used here did not detect IgM to *B. pertussis*. Both IgG and IgM are active in the pertussis bactericidal test, which is complement dependent (Dolby and Stephens, 1973). However, Dolby and Dolby (1969) found that the IgM fraction was a hundred times less active in the bactericidal test than the IgG fraction. In the present study the CF titres were not high and IgM complement fixation may have been too low to be detected. An alternative explanation is that antibodies to different antigens are involved in the two tests. Bactericidal antibody is directed against a lipopolysaccharide antigen, present in strains with little

typing antigen (Ackers and Dolby, 1972), and the CF test used here gives low titres with pertussis typing sera (Macaulay, 1979).

Subclasses of IgG vary in their ability to fix complement and the ultracentrifugation results suggest that CF IgG behaves differently from IHA IgG because it is a different subclass of IgG and not because of a difference in the sensitivity of the two tests. Bradstreet *et al.* (1972) compared an indirect fluorescence (IF) antibody test which distinguished IgG and IgM by means of complement fixation. They considered that the correlation between IF for IgG and complement fixation was sufficient to indicate that IgG was detected by both, even though the titres in the two tests did not always run parallel, and suggested that the discrepancies could have been due to the different IgG components in the sera.

Vaccinated children with whooping cough may show a strong IgM response because infection provides a stronger antigenic stimulus than vaccination or because different serotypes are involved in the two responses. The vaccines that these children received probably contained little antigen 3 (Abbott, Preston and Mackay, 1971) and in three cases the infection was with a type 1,3 strain. Thus the IgG response could have been to antigens 1 and 2 to which the children had already been immunised while the IgM response was to antigen 3. Whatever the explanation, the strong IgM response after infection suggests a method for diagnosing *B. pertussis* infection on one serum sample in vaccinated and in unvaccinated children. A CF titre of more than 20 in whole serum is suggestive of whooping cough in a child who has not been in contact with pertussis antigens within 7 months of the test (Macaulay, 1979) but because the CF test detects IgG, this level may not be reached until late in the disease. IHA IgM rises earlier than CF antibody and a titre of 8 or more in ultracentrifuged fraction 3 supports the diagnosis of whooping cough in a children who has not been in contact with pertussis for more than 5 months. If paired sera are available, a rise in either CF or IHA antibody supports the diagnosis without the need for ultracentrifugation.

When paired sera from children with suspected whooping cough were tested, IHA gave a rise in titre more often than CF but the CF titre could rise without any rise in the IHA titre (Macaulay, 1979). The ultracentrifugation results suggest that early rises in unvaccinated children are due to IgM antibody and that IHA can detect this, while the marked rise in titre in vaccinated children with whooping cough is due to stimulation of the persisting IHA IgG antibody. CF titres can rise independently of the IHA titre because CF detects an antibody different from IHA antibody.

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