

Low IgG4 concentrations in infants with *Haemophilus influenzae* type b infections

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Haemophilus influenzae type b causes considerable morbidity and mortality in infants and young children. Immunity to this organism has been attributed in part to the formation, and increase with age, of antibodies to the capsular polysaccharide of the bacteria. A degree of immunodeficiency could explain why some infants and young children develop invasive haemophilus disease. In this study we investigated immunocompetence in ten infants with invasive haemophilus disease. We found normal lymphocyte mitogen responses, neutrophil iodination and bactericidal and fungicidal capacities in this group. While we found no deficiencies of any of the immunoglobulin classes, three patients had low concentrations of IgG4 and two of them had low concentrations of IgG2 as well. These findings suggest that in some infants who develop haemophilus infections, the measurement of IgG subclasses may reveal immune defects that would not otherwise be apparent.

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Haemophilus influenzae is a common pathogen in young children. It is the most common cause of bacterial meningitis in the first few years of life, causing deaths and neurological sequelae. Invasive *H. influenzae* disease is rare after age four or five years, presumably due to the acquisition of protective levels of opsonic antibodies to *H. influenzae* type b capsular polysaccharide (Hib CPS) (1). It is not clear why some apparently normal young children develop invasive *H. influenzae* disease and others do not. Classical immunoglobulin deficiency has not been a common finding in infants with *H. influenzae* infection. Recently, with the development of assays for IgG subclass measurements, the spectrum of recognisable types of hypogammaglobulinaemia has been broadened. Nevertheless, the definition of IgG subclass deficiency is difficult because reference ranges vary from laboratory to laboratory depending on techniques, antisera and control populations used, and for IgG4, because many laboratories do not use techniques sensitive enough to separate low normal and subnormal concentrations (2, 3). Ideally, each laboratory should assess patient values according to its own reference range.

It is generally agreed that antibodies to the capsular polysaccharide of Hib are protective. The

demonstration of impaired production of functional IgG subclass antibodies to Hib would define a specific defect in immunity. However, normal IgG subclass responsiveness to infection at different ages has not been defined to the extent that subnormality can be reliably detected (4). Conversely, reliable age-normal reference ranges for IgG subclasses have been established in a number of laboratories and provide a useful basis for screening for possible immunodeficiency. We investigated immunocompetence in children with invasive *H. influenzae* disease including the evaluation of IgG subclass concentrations measured by a sensitive ELISA technique according to reference ranges obtained in the same laboratory with the same technique.

Patients and methods

Patients – We studied 10 infants being treated at the Adelaide Children's Hospital for serious invasive culture-proven Hib infections. Their ages ranged from 4 to 16 months. Nine of the children has suffered at least one episode of Hib meningitis and one had suffered two episodes. Another child had suffered two episodes of meningitis, but in only one of these was Hib isolated. One child had

Table 1. Clinical details of patients with Hib disease

Patient No.	Age (months)	Clinical Details
1	4	Hib meningitis × 2
2	6	Hib meningitis
3	8	Hib meningitis
4	9	Hib meningitis and pneumonia
5	10	Hib meningitis
6	11	Hib meningitis
7	11	Hib meningitis
8	12	Hib septicaemia
9	14	Hib meningitis × 1 (meningitis × 2)
10	16	Hib meningitis, shigella gastroenteritis, boils

had Hib septicaemia without meningitis. A summary of clinical details is given in Table 1.

Methods – Serum IgA, IgG, IgM and the complement components C3 and C4 were quantitated by rate nephelometry (ICS, Beckman, Palo Alto). Serum IgE was measured by radioimmunoassay (Phadebas, Prist, Pharmacia, Sweden). Total haemolytic complement activity was measured by the lysis of sheep erythrocytes coated with rabbit haemolysin (5).

Serum immunoglobulin G subclasses were measured by an ELISA method using the isotype specific monoclonal antibodies, NL16 (Oxford, Unipath), HP6014, SJ33 and SK44 (Miles-Bioyeda) for IgG1, IgG2, IgG3 and IgG4 respectively (3, 6) which have been shown to be suitable for IgG subclass measurement by ELISA (7). Serum sam-

ples for IgG subclass quantitation were all run in duplicate. On each ELISA plate a quality control serum was also run in duplicate. The IgG subclass standard serum (WHO 67/97) was run in triplicate.

Lymphocytes and neutrophils were purified from approximately 5–10 ml of heparinized blood by the rapid one-step method involving centrifugation of blood on hypaque-ficoll medium (8). T cells were enumerated by spontaneous rosetting with sheep red cells (9). B cells were enumerated by fluorescence using FITC-labelled goat anti-human immunoglobulin (10). Lymphocyte responses to phytohaemagglutinin (PHA), pokeweed mitogen (PMW) and concanavalin A (Con A) were measured by a radiometric microculture technique (11). Neutrophil iodination was measured by a microassay procedure (12), fungicidal capacity by reduction of *Torulopsis glabrata* colony counts (13) and bactericidal capacity by reduction of *Staphylococcus aureus* colony counts (14).

Controls – Sera from 292 healthy subjects from the general community and from immunisation clinic attenders, obtained after informed consent, were used to establish age-normal reference percentile ranges for IgA, IgG, IgM for the IgG subclasses and complement components C3 and C4. In patients, isotype concentrations below the 5th percentile were considered low. Direct questioning together with written questionnaires, was used to ensure that control subjects were not excessively infection-prone (2, 15).

Table 2. IgG Subclass Concentrations

Patient		IgG subclass concentration (g/l)			
Age (mo)	No	IgG1	IgG2	IgG3	IgG4
4–6	RR	(1.79–6.24)	(0.33–1.80)	(0.08–0.49)	(0.006–0.29)
	M	(3.67)	(0.72)	(0.21)	(0.06)
4	1	7.82↑	0.96	0.48	0.043
6	2	11.90↑	0.84	0.51	0.010
7–12	RR	(1.97–6.53)	(0.36–1.96)	(0.08–0.51)	(0.009–0.33)
	M	(3.91)	(0.80)	(0.22)	(0.07)
8	3	2.35	0.68	0.49	0.037
9	4	4.74	0.57	0.28	0.016
10	5	3.82	0.84	0.44	0.230
11	6	3.49	1.28	0.34	0.290
11	7	3.47	0.18↓	0.30	0.007↓
12–24	RR	(2.07–6.69)	(0.38–2.05)	(0.09–0.52)	(0.010–0.36)
	M	(4.05)	(0.85)	(0.23)	(0.08)
12	8	2.53	0.27↓	0.21	0.008↓
14	9	3.71	0.38	0.31	0.007↓
16	10	8.36↑	0.67	0.41	0.014

RR = reference range, i.e. 5th and 95th percentile range for age

M = median

Table 3. Mean IgG Subclass Concentrations

Age (months)	IgG Subclass Concentration			
	IgG1	IgG2	IgG3	IgG4
4-9				
Patients	2.80	0.83	0.49	0.019
Controls	2.91	0.56	0.29	0.055
(p)	(ns)	(ns)	(p < 0.01)	(p < 0.05)
10-24				
Patients	4.23	0.60	0.34	0.093
Controls	3.78	0.91	0.30	0.169
(p)	(ns)	(ns)	(ns)	(ns)

Because of practical and ethical constraints on using children as controls for cellular studies, adult controls, bled on the same morning as the patients, were used. Previous studies in our laboratory using 7 to 8 year olds showed no significant difference between these children and adults in proportions of T and B cells or in responses to PHA, PWM and ConA (Ferrante, unpublished). Falcao et al. (15) have shown no significant differences in T cell percentages between children and adults.

Statistics – The χ^2 test or the Student's t test were used, as appropriate.

Results

None of the patients had reduced concentrations of IgG, IgA or IgM or reduced total haemolytic complement activity. No deficiencies of serum C3 or C4 were found in the 8 patients in whom these were quantitated. Two (p. 1 and p. 5) had raised IgA concentrations. Three (p. 1, p. 2 and p. 10) had raised IgG concentrations (p < 0.001) and in two of them, the IgM concentration was raised also. Two patients had raised IgE concentrations.

IgG subclass concentrations are shown in Table 2. Three patients had IgG1 concentrations above the 95th percentile for age (p. 1, p. 2 and p. 10). None had reduced IgG1 concentrations. Two patients had IgG2 concentrations below the fifth percentile (p. 7 and p. 8) and one at the fifth percentile (p. 14). All but two (p. 7 and p. 8) had IgG3 concentrations at or above the mean for age. None had low IgG3 concentrations. Three patients (p. 7, p. 8 and p. 9) had IgG4 concentrations below the 5th percentile for age (p < 0.001). None had raised IgG4 concentrations. All but two (p. 5 and p. 6) had IgG4 concentrations well below the median for age. In each of the children who had a low level of IgG4, IgG2 was low or borderline-low.

The mean concentrations of IgG subclasses in patients and in age matched control children are

compared in Table 3. There was a higher mean IgG3 concentration (p < 0.01) and a lower mean IgG4 concentration (p < 0.05) in the 4-9 month old patients.

The cellular immune function studies showed that patients differed from controls in that they had lower percentages of T cells in their peripheral blood (52.5 ± 2.8 and 61.0 ± 2.7 , respectively; p < 0.01). There were no significant differences in B cell percentages, in lymphocyte responsiveness to mitogens or in neutrophil quantitative iodination, bactericidal or fungicidal activity.

Discussion

The immune response to *H. influenzae* includes the production of antibodies to Hib CPS. Antibodies, together with complement, act as opsonins to promote killing of the organism by phagocytic cells. In our patients, lymphocyte proliferative responses to mitogens and neutrophil, bactericidal, fungicidal and iodination activities were normal. The main abnormalities observed were in immunoglobulin isotype concentrations. None of the patients had a deficiency of IgA, IgG or IgM and four had raised levels of at least one of these isotypes, probably reflecting a normal response to recent infections. Three of the patients had low concentrations of IgG4, two of these also having a low IgG2 and one a borderline-low IgG2 concentration. Of further interest, however, is the finding that IgG4 was either low or low normal in most of our ten patients with serious Hib disease. Low concentrations of the other IgG subclasses were not present in a significant number of patients. This is unexpected in view of the common belief that since antibodies to Hib CPS are predominantly of IgG1 or IgG2 subclasses (17-20), deficiencies of these isotypes are most likely to be associated with Hib infections. Studies, to date, have shown disparate results and clarification is needed.

Some studies have indicated that patients with low concentrations of IgG2 may have low concentrations of antibodies to Hib CPS, either in the non-immunized state or after immunization (21-23) but others have not found a correlation between IgG2 concentrations and anti Hib CPS concentrations (19). It appears that majority of IgG2-deficient patients may not be deficient in Hib CPS antibodies (24) and that subjects who respond poorly to Hib immunization are not generally IgG2-deficient (25, 26). Heiner et al. (27) found a correlation between IgG1 rather than IgG2-deficiency and impaired production of antibodies to Hib CPS.

Recently, several investigators have sought to clarify the divergent data by measuring the concen-

trations of antibodies to Hib CPS in the IgG1 and IgG2 isotypes. IgG1 rather than IgG2 has been the predominant isotype found in both children (17) and adults (18, 19). These studies have not measured IgG3 or IgG4 antibodies to Hib CPS. Shackelford and Granoff (17) also showed that some IgG2-deficient patients had impaired production of both IgG1 and IgG2 antibodies to Hib CPS. Since, therefore, a deficiency of IgG2 can be associated with a restricted deficiency of antibody production which may also affect IgG1, it is not unlikely that a deficiency of IgG4, similarly, could reflect an antibody deficiency affecting isotypes other than IgG4, i.e. IgG4-deficient patients could have impaired production of IgG1 and/or IgG2 antibodies to Hib CPS.

Concentrations of IgG4 may be a more sensitive indicator of a subtle defect in antibody production in response to a restricted group of antigens than are concentrations of IgG2. This may help to explain why various studies have given conflicting results concerning IgG2 concentrations and Hib infections. In those studies showing a correlation between IgG2 and Hib CPS antibodies in which IgG4 also was measured, this, as well as IgG2, was low. Herrod et al. (28) found that none of 10 children who failed to respond to Hib immunization were IgG2-deficient, but two were IgG4-deficient. Also, for only IgG4 were mean values much lower in non-responders than in responders.

Hetherington (29) suggests opsonic antibodies to the outer membrane protein of Hib are more important than anti-Hib CPS antibodies in defence against invasive Hib disease and this may explain the lack of correlation between this condition and IgG1 and IgG2 concentrations. The isotypes of these antibodies are not known, although anti-protein antibodies are often predominantly IgG1 and IgG3 and sometimes also IgG4 (30). Antibodies of the IgG4 isotype, *per se*, may be important, or more likely, since IgG4 constitutes a very small percentage of total IgG, low IgG4 concentrations may reflect impaired ability to produce protein antibodies of other isotypes.

An alternative explanation of the possible significance of low IgG4 concentrations in patients with systemic Hib infections may be that IgG4, like IgA, is important as a secretory immunoglobulin and that subjects with low serum concentrations of this isotype may also have low secretory concentrations which predispose them to infection with certain organisms gaining entry through the respiratory mucosa. High concentrations of IgG4 have been found in bronchial fluid (31) and secretory IgG subclass production has been reported (32, 33).

The significance of lower T cell proportions in patients compared with normal subjects is difficult to evaluate, especially since our results showed that the lower proportions were seen evenly amongst the patients irrespective of the presence of an IgG subclass deficiency. Nevertheless, it is interesting to note that most patients had IgG4 concentrations well below mean for age.

Our study suggests that some infants who contract invasive Hib disease, when compared with normal children of the same age, may have a defect in immunoglobulin production that may predispose them to this condition. While it is possible that the IgG4 deficiency may be a result rather than a cause of the infection, this is unlikely as blood was taken during the course of the illness, before a resultant defect in IgG4 production would have had time to become apparent. Serial studies in individual patients are needed to determine whether such a defect is permanent or transient.

Further studies of specific antibody production and of the opsonic effectiveness of different antibodies in both patients with invasive Hib disease and control subjects may lead to a better understanding of the nature of this defect, and help to clarify the relationship between immunodeficiency and proneness to Hib disease.

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