Evaluation of test immunisation in the assessment of antibody deficiency syndromes

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Abstract

Antibody responses after immunisation with pneumococcal polysaccharide did not correlate with the severity and frequency of infections in 22 patients with severe hypogammaglobulinaemia, when these were measured by a Farr radioimmunoassay. Five "healthy" patients with severe hypogammaglobulinaemia not only failed to make antipneumococcal polysaccharide antibody, when measured by radioimmunoassay, but also had very low or unrecordable antibody responses to Escherichia coli and failed to produce antibody when immunised with tetanus toxoid. Some of these subjects, however, did make small amounts of IgM antipneumococcal polysaccharide antibody when this was measured by an enzyme linked immunosorbent assay, while others retained some ability to produce IgM or IgA or both in their saliva.

These findings show that the measurement of serum antibody responses after immunisation, with the possible exception of IgM antibodies to polysaccharides, is unlikely to be helpful in assessing the requirement for gammaglobulin replacement therapy in patients with hypogammaglobulinaemia.

Introduction

Hypogammaglobulinaemia is now more frequently diagnosed because of routine measurement of serum immunoglobulins in patients with recurrent infections. Nevertheless, there are still only about 300 recognised patients with clinical disease due to severe "primary" antibody deficiency in Great Britain, although there are many more with clinically milder disease due to partial deficiencies of antibody production. Secondary antibody deficiency is relatively common in patients with lymphoproliferative diseases, particularly chronic lymphatic leukaemia.[1] Although patients with primary hypogammaglobulinaemia have subtle T lymphocyte abnormalities, they have normal numbers of circulating T lymphocytes and do not suffer from the opportunistic infections—for example, *Pneumocystis carinii*, toxoplasmosis—which characteristically occur in patients with severe cellular immunodeficiency. The major clinical problem in these patients is recurrent bronchitis due to *Haemophilus influenzae*, cystitis and arthritis due to mycoplasmas, and intestinal infection with giardia or campylobacters.[2] The tendency to such infections is considerably reduced by treatment with pooled gammaglobulin.

Experience in managing about 150 patients with profound primary hypogammaglobulinaemia has shown that there is a very poor correlation between serum immunoglobulin concentrations and the severity of symptoms. Thus a patient with a serum IgG concentration of 25 IU/ml may have more severe and frequent infections than a patient whose concentration is unrecordable. It would be of considerable practical value to have a more critical test of humoral immunity, because this could then be used as a more accurate guide to the dosage and frequency of gammaglobulin replacement therapy required for individual patients. Measuring specific antibody values after test immunisation should be a better way of assessing humoral immunity. Antigens, however, vary greatly in their ability to induce antibody responses, and it is not clear whether the ideal test antigen should be a "strong" or a "weak" stimulant. There are also other important practical considerations: the vaccine should be very safe and the antibodies relatively easy to measure by radioimmunoassay or enzyme linked immunosorbent assay (ELISA). Also the vaccine should be freely available, which in practice means that it must be commercially prepared. Polyvalent pneumococcal polysaccharide vaccines and tetanus toxoid fulfil the above criteria. This paper attempts to define the best way of measuring antibodies to these antigens with a view to correlating the magnitude of the response with frequency and severity of infections.

Subjects and methods

The following groups of subjects were immunised.

Group 1 comprised 18 normal healthy volunteers aged 27-56 years (mean 39).

Group 2 comprised 22 selected patients with primary hypogammaglobulinaemia. These patients were separated into three subgroups according to the severity of their symptoms and requirement for gammaglobulin replacement therapy (table I). All were followed up for at least three years.

There was no evidence of lymphoproliferative malignancy in any of the patients except for two in group 2*a*; one had mycosis fungoides at the time of study and the other developed a lymphoma one year later. The lymphomas in both these patients appeared to have developed on a background of primary hypogammaglobulinaemia.

All but two of the patients had received standard tetanus toxoid vaccination in early childhood. None had previously been given pneumococcal vaccines.

Vaccination procedures—The subjects were immunised with a commercially prepared pneumococcal polysaccharide vaccine (Pneumovax) containing 50 μg of each of the following serotypes: 1, 2, 3, 4, 6, 8, 9, 12, 14, 19, 23, 25, 51, and 56 (USA nomenclature). The vaccine (0·5 ml) was injected subcutaneously in the deltoid region; serum samples were collected before immunisation and 14 days later. All samples were kept at −20°C until the antibodies were assayed. Some of the patients were subsequently immunised with 0·5 ml adsorbed tetanus toxoid, BP 40 IU (Burroughs Wellcome), by deep subcutaneous injection, serum samples being taken before and 14 days later. For those patients receiving weekly gammaglobulin replacement therapy gammaglobulin injections were withheld for two weeks after the vaccination so as to minimise the effect of passively administered antibody.

Antibody assays—A previously described radioimmunoassay (Farr technique) was used to measure total antipneumococcal polysaccharide antibodies.[3] A haemagglutination assay[4] and an ELISA technique were also used to test for type 3 pneumococcal polysaccharide antibody in a few patients. The ELISA technique was based on that described by Melville-Smith *et al*,[5] the plastic assay plates (NUNC) being coated with purified pneumococcal polysaccharide type 3, 50 μg/ml, in a carbonate buffer. When measuring IgM antibodies the test serum was diluted in neat horse serum to minimise non-specific binding. IgG and IgM antibodies to tetanus toxoid were

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measured by solid phase radioimmunoassay.[6] Natural antibodies to *Escherichia coli* were measured by a haemagglutination technique.[7]

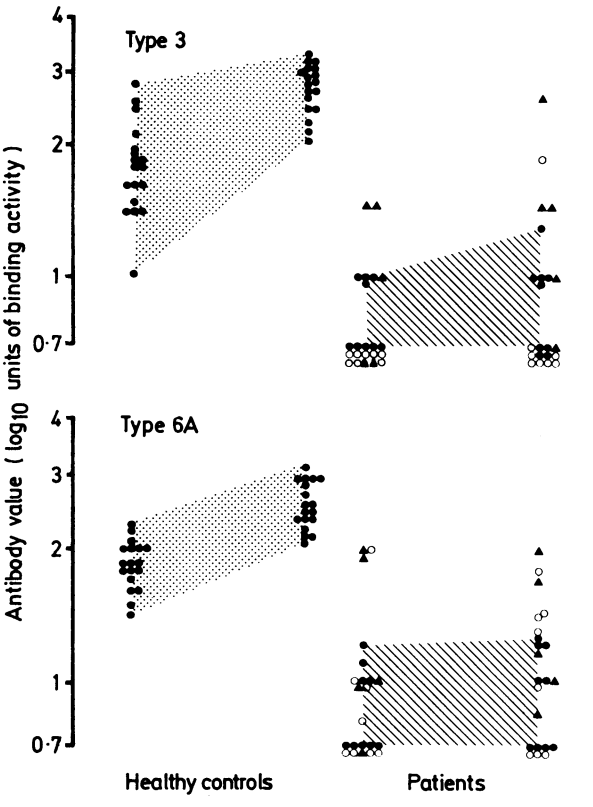
Saliva was collected by asking the patient to expectorate into a tube. IgA immunoglobulin in saliva was measured by a radioimmunoassay method previously described.[8] Saliva IgM and IgG values were measured by a solid phase radioimmunoassay similar to that used for antitetanus toxoid antibodies.

Results

All 18 healthy controls showed a rise in antibodies to type 3 or 6A pneumococcal polysaccharide, or both, indicating that it is not necessary to measure antibodies to all the components of this vaccine when using it to test for gross abnormalities in humoral immunity (figure). The figure also shows the pneumococcal polysaccharide antibody responses in the patients with hypogammaglobulinaemia. The majority showed negligible responses to both type 3 and type 6A pneumococcal polysaccharide; only one patient with mild symptoms produced a rise in type 3 antibody to within the normal range. There was no tendency for patients with less severe symptoms to produce higher antibody titres.

Further studies were done on six symptom free patients in subgroup 2*c* (who were not receiving gammaglobulin replacement therapy) to test whether they had a generalised defect in antibody production or one that was confined to producing immunoprecipitable antibody to pneumococcal polysaccharide. Five showed some evidence of haemagglutinating activity against type 3 pneumococcal polysaccharide after immunisation, although the titres were very low and the haemagglutination "equivocal" in three patients (table II). Three of these patients showed a significant rise in IgM antibody when measured by ELISA, although none of the patients in subgroup 2*c* showed a significant rise in IgG antibody. Only one patient in subgroup 2*c* showed unequivocal evidence of haemagglutinating activity against *E coli* pooled antigen; and of the five who were immunised with tetanus toxoid, only one produced a significant IgM response.

The mean saliva immunoglobulin concentrations in six severely symptomatic patients in subgroup 2*a* were: IgG 0·3 (range 0·03-0·6),



Antibody responses to type 3 and type 6A pneumococcal polysaccharide in 18 normal healthy subjects and 22 patients (● subgroup 2*a*, ▲ subgroup 2*b*, ○ subgroup 2*c*) with hypogammaglobulinaemia. Shaded areas denote range of response in normal healthy subjects and those patients with severe symptoms of hypogammaglobulinaemia (subgroup 2*a*).

TABLE I—*Details of 22 patients with primary hypogammaglobulinaemia (group 2) separated into subgroups according to severity of symptoms and need for replacement therapy*

Subgroup	Mean age in years (range)	No	Mean serum immunoglobulin concentrations (IU/ml) (range)*			Clinical features
			IgG	IgA	IgM	
2*a*	40 (21-56)	9	26 (5-46)	<7	<12 (<12-35)	Recurrent upper and lower respiratory tract infections requiring weekly gammaglobulin replacement therapy
2*b*	37 (26-72)	5	20 (9-30)	<7 (<7-14)	12 (<12-24)	Mild (mainly upper) respiratory tract infections requiring gammaglobulin every 2 weeks or less
2*c*	34 (19-59)	8	22 (<1-46)	<7	<12 (<12-24)	No respiratory symptoms for >2 years. Previous tendency to respiratory infections in 5 patients. Hypogammaglobulinaemia discovered by chance in 1 patient and during investigation of arthritis or diarrhoea in 2 patients. No therapy

*Normal values: IgG 60-133, IgA 35-261, IgM 47-295 IU/ml.
Conversion: IU to mass values—IgG: 1 IU ≈ 80·4 μg. IgA: 1 IU ≈ 14·2 μg. IgM: 1 IU ≈ 8·47 μg.

TABLE II—*Results of studies in six patients in subgroup 2c*

Case No	Haemagglutinating antibodies (reciprocal of titre)			ELISA† pneumococcal polysaccharide 3 antibodies (U/ml)				Tetanus toxoid antibodies (μg/ml)‡				Saliva immunoglobulin (IU/ml)	
	E Coli	Type 3 pneumococcal polysaccharide		Before		After		Before		After			
		Before	After	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgA	IgM
17	Neg	Neg	16*	60	31	74	218	0·2	1·3	12	1·3	0·05	0·01
18	8*	2	8	<4	63	<4	154	<0·2	<0·2	<0·2	<0·2	<0·007	0·27
19	Neg	4	32	183	62	135	435	0·5	0·3	1·5	0·3	<0·007	0·02
20	Neg	4	16*	80	31	69	44	ND	ND	ND	ND	ND	ND
21	8*	Neg	8*	<4	31	<4	44	0·2	0·3	<0·2	0·3	0·52	0·79
22	Neg	Neg	Neg	148	31	74	31	2·7	4·9	2·7	7·4	<0·007	0·39
Normal values	>64	No data						7-13	1·2-2·5	83-160	1·8-3·6	1·6-9·2	<0·06

ND = Not done.
*These results equivocal because only partial haemagglutination observed.
†ELISA results expressed as U/ml, compared with postimmunisation serum sample from normal subject that was arbitrarily assigned to have 1000 U/ml.
‡Normal range for tetanus toxoid responses obtained from 30 healthy subjects not immunised in past four years. All figures for specific antitetanus antibody concentrations obtained by comparing test sera with affinity purified standard.[3]

IgA 0·9 (<0·01-4·0), and IgM 0·4 (<0·01-2·1) μg/ml. The concentrations in 10 healthy or mildly symptomatic patients in subgroups 2b and 2c were: IgG 0·7 (range 0·1-4·3), IgA 0·8 (<0·01-7·4), and IgM 1·6 (<0·01-6·7) μg/ml. Analysis by Student's t test showed no significant differences in saliva immunoglobulin concentrations between subgroup 2a and subgroups 2b and 2c.

Discussion

This paper attempts to define the most direct way of testing for clinically significant defects in humoral immunity in patients with recurrent infections. Our results show that the interpretation of a failure to respond to certain antigens is not straightforward and that further work is needed to establish a clinically useful test. The surprising finding is that there are severely hypogammaglobulinaemic subjects who make very little antibody when test immunised but nevertheless remain in reasonably good health. These subjects fail to produce precipitable antibody after immunisation with pneumococcal polysaccharide, although some of them produce an IgM response which can be measured by a "solid phase" (ELISA) technique. This suggests that they can make only low affinity IgM antibodies, which implies that the response is clonally restricted.

The measurement of antibodies to type 3 and type 6A pneumococcal polysaccharide after immunisation by "liquid phase" radioimmunoassay is clearly unsuitable as a guide to the necessity or frequency of gammaglobulin replacement therapy in patients with hypogammaglobulinaemia. We found no difference in the amount of antibody measured in this way between patients with either severe or moderate symptoms of recurrent infection. Unfortunately, serum was not available to test patients in subgroup 2a with frequent infections for antibodies to type 3 pneumococcal polysaccharide using the ELISA technique. Further work is needed to test whether the difference between hypogammaglobulinaemic patients with mild and severe symptoms critically depends on the amount of IgM produced to polysaccharide antigens. This possibility is plausible because the peripheral blood B lymphocytes from some patients with primary hypogammaglobulinaemia can make variable amounts of IgM immunoglobulin in vitro, although their cells usually fail to make specific antitetanus IgM or IgG antibody despite the presence of normal T cell "help."[9] One possible explanation for these in vitro abnormalities is that the antigen presenting cell is abnormal and that antibody responses to tetanus toxoid are critically dependent on such cells. On the other hand, recent work on in vitro antibody production to pneumococcal polysaccharide shows that the accessory cell requirements are different.[10] These in vitro findings may help explain why some hypogammaglobulinaemic subjects retain the in vivo ability to make IgM antipneumococcal polysaccharide but fail to show a response to tetanus toxoid.

Cellular immunity, as measured by cytotoxic responses, numbers of circulating T cells, or delayed hypersensitivity skin reactions, appears to have no influence on the severity of infections in hypogammaglobulinaemic patients; most children with X linked hypogammaglobulinaemia who have normal or even enhanced cellular immunity are particularly prone to infections.[2]

There are likely to be other factors which determine the severity and frequency of infections in hypogammaglobulinaemic patients. Local antibody production at mucosal surfaces may vary among patients, and this possibility is supported by the presence of significant amounts of IgA or IgM or both in the saliva of four of the "healthy" hypogammaglobulinaemic patients in subgroup 2c. When analysed as a group, however, there was no significant difference in the saliva immunoglobulin concentrations between healthy or mildly symptomatic patients and those with severe symptoms. Nevertheless, possibly more refined methods for quantifying immunoglobulins and specific antibodies in saliva might be helpful in distinguishing these groups.

We have shown that a pronounced deficiency in antibody production does not necessarily lead to recurrent infections in subjects living in the relatively clean British environment. Probably some of these subjects remain in good health because they can make IgM antibody, at least to polysaccharides. We have also shown that measuring IgG or IgM antibodies to tetanus toxoid after immunisation is unlikely to be helpful in assessing the gammaglobulin requirements for hypogammaglobulinaemic patients. Testing the response to pneumococcal polysaccharide may be more useful, provided that a suitable solid phase immunoassay is used to measure IgM antibody.

Now that antibodies are easy to measure with ELISA techniques, probably we shall find many clinical situations where antibody production is severely depressed despite normal concentrations of serum immunoglobulins. For example, some patients having major surgery or suffering from chronic bronchitis fail to produce antitetanus antibodies when immunised.[11 12] Lymphoproliferative diseases, particularly chronic lymphatic leukaemia, are also often associated with a failure to respond to immunisation.[1] The unanswered question is whether the depression of antibody production is clinically important, and whether this deficiency should be corrected with gammaglobulin replacement therapy. Carefully controlled trials will be needed to avoid treating large numbers of apparently immunodeficient patients with expensive gammaglobulin concentrates.

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