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Clinical and Immunologic Characteristics of Healthy Children with Subnormal Serum Concentrations of IgG2[1]

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ABSTRACT. To understand the relevance of subnormal serum concentrations of IgG2, we measured IgG2 in serum of 575 healthy children and identified 11 with concentrations >2 SD less than the mean for age. The levels of IgG2 present were similar to those found in symptomatic children with IgG2 subclass deficiency associated with antibody deficiency. The 11 children ranged in age from 1 to 14 y (mean = 5.7). Detailed clinical information was available on 10 of the 11 children and each was matched for age with two controls. The median number of visits/y to the doctor for infectious illnesses was identical for the two groups (1.0). Nine of the children with subnormal IgG2 were followed for 1 to 5 y (mean = 2.3). All nine children had normal serum concentrations of IgA, IgG1, IgG3, and IgG4 but seven had persistently subnormal or low-normal serum IgG2 concentrations. One of these seven children also had a subnormal serum concentration of IgG, and one had subnormal IgM. Antibody responses to Haemophilus b polysaccharide vaccine were normal in five of six who were immunized. In vitro secretion of Ig by mitogen-stimulated peripheral blood mononuclear cells was measured in six of seven children with persistently subnormal or low-normal IgG2; five showed decreased secretion of IgG2, and two of the five also had subnormal secretion of IgG1 and IgG3. An important implication of this study is that the subnormal concentrations of serum IgG2 found in infection-prone children are not a sufficient explanation for their increased susceptibility to infection. The healthy children with low serum concentrations of IgG2 differ from symptomatic children with subnormal IgG2 in that the former have otherwise normal serum Ig concentrations and have normal antibody responses to Hib PS vaccine. (*Pediatr Res* 27: 16–21, 1990)

Abbreviations

Hib PS, Haemophilus influenzae type b capsular polysaccharide
Hib PS-D, Haemophilus b diphtheria toxid conjugate
PBL, peripheral blood mononuclear cells

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The relation between the IgG2 subclass of human Ig and protective immunity against infectious diseases is incompletely understood. The observation that antibodies produced in response to some polysaccharide antigens are often relatively restricted to IgG2 (1, 2) has suggested a special role for IgG2 in protective immunity to infections caused by polysaccharide encapsulated organisms. For example, a relationship between the slow maturation of serum IgG2 concentrations and the poor antibody responses of normal children to polysaccharide antigens has been suggested (3–5). Further, many reports have described children and adults with subnormal serum concentrations of IgG2 who experience recurrent sinusitis, pneumonia, and/or bacteremic infections caused by polysaccharide encapsulated bacteria (6–11). However, skepticism regarding a simple relationship between serum IgG2 concentrations and protective immunity has arisen following reports of several healthy subjects with no detectable serum IgG2 who have a deletion of the constant region gene for IgG2 (12, 13).

To understand better the clinical relevance of subnormal serum concentrations of IgG2 in children, we identified a group of children in primary care facilities with serum IgG2 concentrations more than 2 SD less than the mean for age. We present the results of a detailed review of the clinical histories of these children and the results of a prospective study of their serum Ig concentrations, antibody response to Hib PS, and *in vitro* secretion of IgG subclasses after mitogen stimulation.

MATERIALS AND METHODS

Subjects. After obtaining informed, written consent from parents, serum samples for measurement of IgG2 were obtained from 575 healthy children, ages 7 to 203 mo, at the time of visiting their pediatrician at a prepaid health care group program for well-child care, or when admitted for elective surgery to St. Louis Children's Hospital. Surgical patients were undergoing orthopedic procedures, hernia repair, or eye muscle surgery. Children were excluded if they had multiple anomalies or a history of frequent infections (children with myringotomy tubes, or more than six physician visits annually for infection). Of the 575 samples, 483 were from venous blood, and were used to establish the normal ranges of IgG2. Of these, 10 (2.1%) had subnormal serum IgG2 concentrations (>2 SD less than mean for age) (Table 1). The median age and dispersion of ages of the children with subnormal IgG2 did not differ from those of children with normal IgG2 concentrations. Inasmuch as results of assays for IgG2 gave 10–20% lower values on capillary compared to venous samples (Shackelford PG, unpublished data), results of measurement of IgG2 in capillary samples were not used to establish the normal ranges. However, we used capillary samples to identify additional healthy children younger than 18

mo of age with subnormal IgG2. Among 92 children screened using capillary samples, one was found to have subnormal serum IgG2 that was subsequently confirmed on venous blood. Thus, during the study period between September 1985 and March 1987, 11 apparently healthy children with subnormal serum IgG2 concentrations were identified. Parents of nine of the children consented to further immunologic evaluation and these children have been followed through May 1988.

Clinical History. Detailed clinical histories were obtained on 10 of the 11 children with subnormal serum IgG2 concentrations and also for two age-matched controls from the same pediatrician's practice. Criteria for inclusion of the controls were identical to those used for children with subnormal IgG2. For each child, both the medical records were examined and a parent was interviewed. Comparisons were made of the number of visits to the pediatrician for infectious illnesses during the 12-mo intervals before the initial serum sample, and before the last available sample. Reasons for visits to the pediatrician were categorized as upper respiratory infection (including pharyngitis), otitis media, gastroenteritis, sinusitis, lower respiratory infection, and "other".

Vaccine Response. Children were immunized subcutaneously with 25 μg of Hib PS prepared by Praxis Biologics (Rochester, NY). Postimmunization serum was obtained 1 to 3 mo after immunization. Normal values for serum antibody concentrations after immunization were based on previously published values (14), and on contemporaneous study of 36 healthy children vaccinated with Hib PS. These 36 children ranged in age from 2.0 to 12.4 y (mean = 3.5), and had normal serum IgG2 concentrations. Their antibody responses were indistinguishable from the previously published values and therefore the data were combined.

Assays. Ig. Serum IgG, IgA, and IgM were measured by nephelometry (Beckman Instruments, Fullerton, CA). Serum concentrations of IgG1, IgG2, IgG3, and IgG4 were measured using murine MAb and a particle concentration fluorescence immunoassay as previously described (15). The IgG1-specific murine MAb (HG11) was produced in our laboratory (16). The IgG2-specific antibody (HP6008, GOM1) was purchased from ICN Immunobiologicals, Lisle, IL. G2m(23)-positive and G2m(23)-negative myeloma proteins are detected equally by this antibody (15). The IgG3-specific antibody (HP6066) was obtained from Dr. M. E. Conley (Philadelphia, PA). An IgG4-specific antibody, HP6011, was purchased from Unipath Limited, Bedford, U.K. As previously described (15), the subclass assays are highly specific and sensitive. The interassay coefficient of variation ranged from 7 to 14%. However, to assess changes in a serum IgG subclass concentration in a subject over time, all samples from an individual were rerun in the same assay.

Table 1. *IgG_2 subclass concentrations in children without history of increased infections*

Age (mo)	No. tested	Geometric mean (mg/mL)	Normal bounds*	No. with IgG_2 >2 SD less than geometric mean
7–11	25	0.38	0.13–1.09	0†
12–23	47	0.76	0.26–2.19	1
24–35	47	0.99	0.42–2.31	1
36–59	80	1.11	0.37–3.34	3
60–83	85	1.29	0.43–3.89	1
84–107	56	1.51	0.52–4.42	2
108–131	50	1.81	0.66–4.96	1
132–203	93	2.35	0.83–6.62	1

* Normal bounds were determined by taking antilog of (mean logarithm ± 2 SD of logarithms).

† One subject was identified in this age group from the 92 subjects <18 mo screened by measurement of IgG_2 in capilllary blood. The low concentration of IgG_2 in the capillary sample was confirmed in a subsequent sample from venous blood (see text).

To assess the stability of serum IgG2 levels in normal children, we measured under code IgG2 concentrations in paired sera obtained 1 to 2 mo apart from 19 children with a mean age of 3.02 y. The mean ± SD of the difference in the paired values was 4.3 ± 14%. The direction of variation was random; values decreased in 12 children and increased in seven.

G2m(23) allotype assay. G2m(23) allotype was determined with an assay method similar to the IgG2 assay. Latex particles coated with a G2m(23)-positive myeloma protein, SAG2 (kindly provided by Dr. Robert Kyle, Rochester, MN), were incubated with SH21 (ICN Immunobiologicals, Lisle, IL), a murine MAb specific for the G2m(23)-positive allotype, and an appropriately diluted serum sample. G2m(23) protein in the sample inhibits binding of SH21. The amount of SH21 bound to latex particles was determined with fluorescein-conjugated goat antibody to mouse Ig. The G2m(23) assay was initially standardized with purified SAG2 protein that gave a value of 1.8 g/L of G2m(23)-positive IgG2 for a pool of human serum from 1638 blood donors. This pool was subsequently used as the standard for all G2m(23) assays. The ratio of concentrations of G2m(23)-positive IgG2 to total IgG2 in a sample was used to determine its G2m(23) allotype. All samples with a G2m(23)-positive/IgG2 ratio less than 0.2 were called G2m(23) negative, and samples with a ratio more than 0.2 were called G2m(23) positive. Of 141 G2m(23)-positive samples, the G2m(23)/IgG2 ratio was 0.62 ± 0.19 (mean ± SD) and the range was 0.29 to 1.18. Samples negative for the allotype were always less than the sensitivity of the assay (0.1 g/L) and, thus, usually had ratios far less than 0.2.

Antibody assays. Total antibody to Hib PS was measured using a radioactive antigen binding assay with ^{125}I-labeled antigen (17). The antibody concentrations, in μg/mL, were determined from a standard curve using dilutions of the U.S. Office of Biologics serum reference pool estimated to contain 80 μg/mL of total antibody in undiluted serum.

ELISA were used to measure IgG1 and IgG2 antibodies to Hib PS. IgG3 ,and IgG4 antibody concentrations were not measured because in most individuals they represent only a minor component of anti-Hib PS antibodies (18). For measurement of IgG1 and IgG2 antibody concentrations, microtiter plates were coated with polysaccharides conjugated to poly-L-lysine, test sera were titrated in the plate, and binding of subclass-specific antibody was detected with biotinylated conjugates of secondary antibodies as previously described (19). The anti-IgG1 reagent was MAb HG11 (16). To detect IgG2 antibodies, a mixture of two MAb was used (HP6014 and HP6008 from ICN, Immunobiologics, Lisle, IL) to achieve optimal sensitivity whereas minimizing the light chain preference of HP6014 (15).

Enumeration of leukocyte populations. PBL isolated by Ficoll separation were stained and enumerated by flow cytometry as described (20). To enumerate subpopulations among lymphocytes, nonlymphocytes were "gated out" using forward and 90° scatter signals. We used MAb Leu 4, Leu 3, Leu 2, Leu 11b and B1 for CD3, CD4, CD8, CD16, and CD20 antigens, respectively.

In vitro subclass secretion. In vitro secretion of IgG subclasses by isolated human PBL was performed as previously described (16). In brief, cells were recovered from 20 mL of heparinized blood using Ficoll-Hypaque and Percoll sedimentation, washed, and cultured in 48-well cluster plates (Costar Plastics, Cambridge, MA). The 0.8 x 10^6 cells were cultured for 8 d in 0.75 mL of RPMI 1640 (GIBCO, Grand Island, NY) supplemented with endotoxin-free heat-inactivated FCS (GIBCO) (final concentration 10%), 100 μg/mL of streptomycin, 100 μg/mL of penicillin, 2 mM L-glutamine, and 1% tryptic soy broth (Difco Laboratories, Detroit, MI). For stimulation, each well contained staphylococcal protein A (0.005%) and pokeweed mitogen (0.033%). The mitogen doses chosen induced optimal secretion of the IgG subclasses (16).

ELISA were used to measure concentrations of IgG1, IgG2, and IgG3 in the supernates. Dynatech Immulon plates (Chantilly, VA) were coated with 10 μg/mL (120 μL) of MAb specific for either IgG1 (HG11), IgG2 (HP6016), or IgG3 (HP6066).

Uncoated sites were blocked with 1% BSA diluted in PBS and 0.5% Tween 20. Appropriately diluted culture supernates were added and titrated in serial 4-fold dilutions and incubated at room temperature for 3 h. After washing, bound IgG subclasses were detected with an alkaline phosphatase-conjugated goat antihuman IgG and p-nitrophenylphosphate as the substrate. IgG subclass concentrations in the unknown samples were calculated by comparison of the absorbance of the unknown with that of a set of myeloma proteins and dilutions of a standard serum pool.

Statistical Analysis. The normal bounds for IgG2 in various age groups were determined by taking the antilog of (mean logarithm ± 2 SD of the logarithms). At least 25 subjects were included in each age group. A Mann-Whitney test was used to compare frequency of visits for infectious illnesses.

RESULTS

Clinical history. Of the 11 healthy children with subnormal serum IgG2 concentrations, we were able to contact 10 to obtain detailed clinical information and were able to follow them over a period of 1.0 to 5.1 y (mean = 2.3). One child, age 14.1 y (subject 4), had a history of pneumococcal meningitis at age 3 mo and four episodes of pneumonia treated as an outpatient, before the age of 5 y. However, this child is included because he had been free of serious or frequent infections for the following 9 y. None of the other nine children had a history of serious infections, and they did not visit their physician more frequently for infectious illnesses during the 12 mo preceding the initial serum sample than did the 20 age-matched control subjects (median number of visits for infectious illnesses = 1.0/y for both groups). During follow-up, the children with subnormal IgG2 concentrations remained clinically healthy, and during the last year of follow-up they did not visit their physicians more frequently than the age-matched healthy control children (1.5 visits/y versus 2.0 visits/y, $p > 0.05$).

G2m(23) allotype. G2m(23) is an allotype on IgG2 molecules found predominantly in Caucasians (21). The frequency of this allotype in Caucasians is approximately 57–76% (17, 22). In previous studies, Caucasian adults who were negative for G2m(23) had lower serum IgG2 concentrations than those who were G2m(23) positive (3, 23). In a recent study of 8015 blood donors, 14 of the 15 individuals with IgG2 concentrations 3 SD less than the mean were G2m(23) negative (expected approximately 6), $p < 0.01$ by Fisher Exact test (Nahm MH, unpublished data). G2m(23) was determined in sera from 10 of the 11 children (Table 2) and was present in four of the eight Caucasian children tested (expected approximately three). Thus, in the children, this frequency is not significantly different from that expected in healthy Caucasian subjects.

Ig. Sufficient sera were available from the initial screening samples to determine serum IgG, IgA, and IgM concentrations in eight of the 11 children, and IgG1, IgG3 ,and IgG4 in nine of the children (Table 2). Three of the children had abnormal values: one child (subject 7) had subnormal concentrations of IgG and, possibly, IgA, one child (subject 1) had subnormal concentrations of IgG1 and IgG4, and one child (subject 6) had a subnormal IgG1 concentration.

The parents of nine of the 11 children consented to have follow-up immunologic evaluation of their child. These evaluations were performed at a median interval of 1.9 y after obtaining the initial serum sample (range 1.0 to 5.1 y). As shown in Figure 1 and Table 3, five of the nine children had persistently subnormal IgG2 concentrations at the time of follow-up. Of the remaining four children, two had low-normal IgG2 values and two had normal IgG2 concentrations. One of the children with a low-normal IgG2 value (subject 4) also had a subnormal IgG concentration, and one with a subnormal IgG2 value (subject 7) had subnormal IgM, but all nine had normal serum concentrations of IgA, IgG1, IgG3, and IgG4 (Table 3).

Response to Hib PS vaccine. Six of the nine children were immunized subcutaneously with 25 μg conventional Hib PS vaccine prepared by Praxis Biologics (Rochester, NY) (Table 3). One additional child (subject 4) was inadvertently immunized intramuscularly with 0.5 mL of Hib PS-D vaccine (Connaught, Swiftwater, PA) containing 25 μg of capsular polysaccharide (24). The two unimmunized children (subjects 2 and 9) had prevaccine serum antibody concentrations of 0.7 and 1.2 μg/mL, concentrations within the normal range for children of this age (14). At the time of immunization, all six of the children who received polysaccharide vaccine had subnormal serum concentrations of IgG2; the seventh child, who received conjugate (subject 4, Table 3), had low-normal serum IgG2 when he was immunized. Six of the seven immunized children had normal total anti-Hib PS responses to immunization. Of these, four had detectable IgG1 responses (> 0.5 μg/mL) and three had detectable IgG2 responses (> 0.3 μg/mL) (Table 3). This pattern of IgG subclass responses is similar to that observed previously in children immunized with either Hib PS or Hib PS-protein conjugate vaccines (25). Only one of the immunized children (subject 7) had a subnormal

Table 2. *Initial immunologic and clinical features of apparently healthy children with low IgG2 concentrations*

					Ig concentrations (mg/mL)						
Subject	Age (y)	Race*	Gm (23)	Visit/y†	G_1	G_2	G_3	G_4	G	A	M
1	4.6	B	−	1	1.78‡	0.32‡	0.17	0.007‡	ND§	ND	ND
2	8.3	W	+	1	6.82	0.21‡	0.47	0.25	7.70	0.59	1.71
3	3.3	W	+	4	6.66	0.30‡	0.54	0.39	8.24	0.62	2.10
4	14.1	W/AI	+	2	4.14	0.61‡	0.21	0.20	6.86	1.00	1.41
5	2.9	W	−	1	9.72	0.26‡	0.39	0.09	10.20	1.33	2.40
6	4.1	W	−	3	2.68‡	0.34‡	0.12	0.01	5.12	0.52	0.66
7	1.3	B	−	2	ND	0.22‡	ND	ND	3.06‡	<0.40‖	0.45
8	0.9	W	+	0	ND	0.12‡	ND	ND	ND	ND	ND
9	9.5	W	−	0	5.08	0.55‡	0.25	0.18	9.85	1.07	0.80
10	7.3	B	ND	0	5.07	0.46‡	0.39	0.34	ND	ND	ND
11	6.3	W	−	NC¶	6.73	0.42‡	0.14	0.63	9.68	1.45	1.16

*B, Black; W, Caucasian; AI, American Indian.

†Visits to the physician for infection during the 12 mo before the initial blood sample.

‡ Value low for age.

§Not done because of insufficient quantity of serum.

‖Insufficient sample to repeat at lower dilution.

¶ Unable to contact patient to obtain detailed clinical information.

Table 3. *Follow-up immunologic and clinical features*

Subject	Interval (y)*	Age (y)	Visit/(y)†	Ig concentration (mg/mL) G_1	G_2	G_3	G_4	G	A	M	Antibody to Hib PS (μg/mL) Age (y)‡ Vaccine	Total Pre	Total Post	IgG_1	IgG_2
1	2.6	7.2	0	8.65	1.69	0.29	0.21	11.20	1.56	1.30	4.4	ND§	6.3	<0.5	<0.3
2	5.1	13.4	0	6.44	2.39	0.57	0.12	13.00	0.84	3.60	13.4‖	1.2‖	ND	<0.5	0.6
3	1.6	4.9	2	7.97	0.38¶	0.48	0.24	7.51	0.70	1.98	3.0	ND	17.5	4.4	0.7
4	1.5	15.6	1	4.01	1.07¶	0.26	0.33	5.23**	0.99	1.07	15.8	0.9	35.0††	6.7	2.3
5	1.6	4.5	0	8.43	0.22**	0.28	0.08	9.57	0.81	1.78	4.8	0.2	80.0	16.8	2.7
6	2.5	6.6	3	5.58	0.37**	0.23	0.03	8.58	1.40	0.80	4.1	ND	7.5	3.7	<0.3
7	1.0	2.3	1	3.51	0.34**	0.34	0.70	4.77	0.41	0.41**	2.0	0.1	0.1**	<0.5	<0.3
8	1.9	2.8	3	6.92	0.37**	0.43	0.10	9.06	0.55	1.09	2.4	ND	0.8	<0.5	<0.3
9	2.5	12.0	3	6.61	0.83**	0.28	0.29	9.45	1.25	0.64	2.5‖	0.7‖	ND	<0.5	<0.3

* Interval between initial serum sample and follow-up.
† Visits to the physician for infection during the 12 mo before obtaining the follow-up blood sample.
‡ Subjects 1, 3, 5, 6, 7, 8 had subnormal serum concentrations of IgG2 when immunized; subject 4 had a low-normal serum IgG2 concentration.
§ Not done because of insufficient quantity of serum, or vaccine not given.
‖ Vaccine not given.
¶ Value near lower limit of the normal bounds.
** Value low for age.
†† Hib PS-D vaccine given.

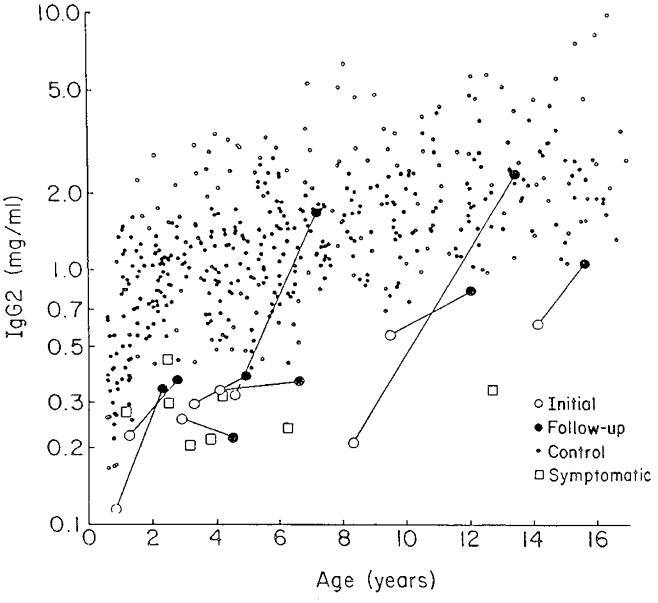

Fig. 1. Initial and follow-up serum IgG2 concentrations of apparently healthy children with low serum IgG2. Values for 483 controls with serum IgG2 in the normal range are shown. For comparison, values of serum IgG2 in eight infection-prone children with low serum concentrations of IgG2 and antibody deficiency are shown.

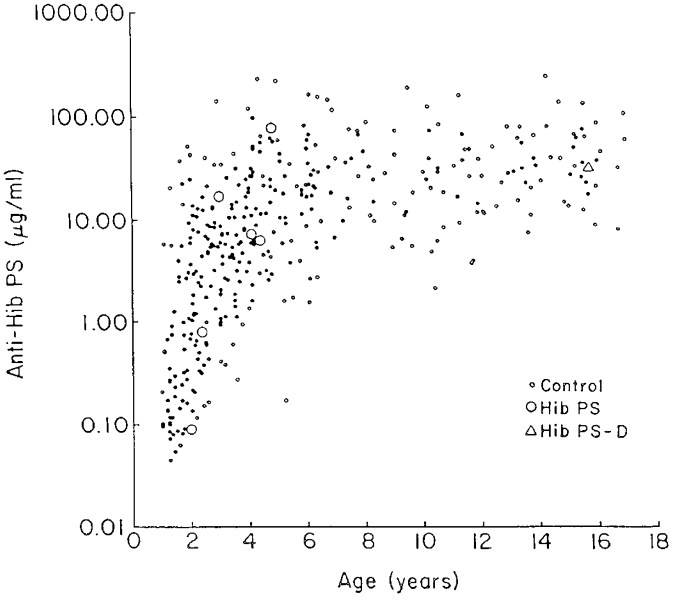

Fig. 2. Total anti-Hib PS antibody concentrations in serum obtained 1 to 2 mo after immunization with Hib PS vaccine in 386 healthy controls 18 mo to 17 y of age and six children with low serum IgG2. One subject with low serum IgG2 was immunized with Hib PS-D conjugate vaccine.

serum antibody response to immunization (Fig. 2). This child also had persistently subnormal concentrations of serum IgG2, low-normal serum IgG, subnormal serum IgM, and a decreased percentage of B cells in peripheral mononuclear cells (see below). Subject 7 was subsequently immunized with one dose of Hib PS-D conjugate vaccine and developed 2.8 μg/mL of total anti-Hib PS antibody in serum obtained 1 mo later. However, there was no measurable IgG1 or IgG2 component in this response as determined by ELISA.

Lymphocyte studies. Lymphocyte populations and *in vitro* secretion of Ig by mitogen-stimulated PBL were measured in seven of the nine children, six of whom had persistently subnormal or low-normal serum IgG2 (Table 4). For each assay, two age-matched control children with normal serum IgG2 concentrations were tested concurrently. All seven children tested had normal distributions of T lymphocyte subpopulations (data not shown). Two children had normal secretion of IgG1, IgG2, and IgG3: subject 1, whose serum IgG2 concentration had increased to normal (Table 3) and, of note, subject 7 who had a subnormal percentage of B cells (Table 4), persistently subnormal serum IgG2 concentrations, and a poor response to Hib PS vaccine (Table 3). The other five children, all of whom had subnormal or low-normal serum IgG2 concentrations, showed decreased secretion of IgG2, and two of the five also had decreased secretion of IgG1 and IgG3 (Table 4).

DISCUSSION

The recent availability of assays to measure accurately human IgG subclasses, and the development of Ig preparations that can

Table 4. *In vitro secretion of Ig by mitogen-stimulated B cells*

Subject	Age(y)	Ig (ng/mL/8 × 10^5 cells) IgG$_1$	IgG$_2$	IgG$_3$	%B cells*
1	7	10 805	454	312	12.4
3	5	ND†	16‡	ND	15.5
4	16	2 735‡	175‡	323‡	8.2
5	5	15 070	38‡	497	12.3
6	7	5 638	52‡	287	8.6
7	2	5 478	179	249	3.6‡
8	3	2 462‡	37‡	123‡	8.6
Controls (range)					
$n = 7$	10-adult	6646–17 408	357–3050	500–1698	4.5–11.9
$n = 10$	2–9	4200–42 000	120–510	210–900	6.1–14.7

* Enumeration of leukocyte populations performed on isolated mononuclear cells.

† Not done because of technically unsatisfactory assay.

‡ Below normal range.

safely be administered intravenously, have heightened the interest of clinicians in the clinical syndromes referred to as "selective IgG subclass deficiencies." Our studies of 11 apparently healthy children, selected on the basis of serum IgG2 concentrations >2 SD less than the mean for age, showed that subnormal serum IgG2 does not necessarily reflect a functional immunodeficiency. By careful history and clinical follow-up, only one child had any evidence of increased susceptibility to infection and, in that child, the serious infections had occurred 9 y before study. These observations are noteworthy considering the fact that the low serum IgG2 concentrations of some of these children were in a range observed in infection-prone children with IgG2 subclass deficiency associated with antibody deficiency (Fig. 1). Further, the clinical histories of the infection-prone children differed strikingly from the histories of the subjects in this study. Most of the infection-prone children had recurrent sinusitis and/or pneumonia requiring visits to their physicians every 4 to 6 wk; one child had four bacteremic *H. influenzae* type b infections. In the present study, we could not attribute the subnormal serum IgG2 concentrations in the asymptomatic children to variation of regulation of total serum IgG2 concentration by genes linked with the G2m(23) locus because only four of the eight Caucasian children tested were G2m(23) negative, a frequency not significantly different from that expected in the general population (17, 22).

On follow-up immunologic evaluation of nine of the 11 children, two experienced increases of IgG2 to the normal range, and seven had persistently subnormal or low-normal IgG2. The two who developed normal IgG2 concentrations had no other immunologic abnormalities and probably represent a benign variant of transient hypogammaglobulinemia of infancy. Of the seven children with persistently subnormal or low-normal serum IgG2 concentrations, five had normal levels of all other Ig, and four of five tested had normal quantitative and qualitative antibody responses to conventional Hib PS vaccine. Subject 9, who had not been immunized, had a total anti-Hib PS of 0.7 μg/mL that would be in the high normal range for an unimmunized child of 2.5 y (14). Subject 4, who inadvertently received Hib PS-D conjugate instead of Hib PS, showed high total and IgG antibody responses. These results are in marked contrast to those of infection-prone children with persistently subnormal or low-normal serum concentrations of IgG2 (25a). The latter consistently had very poor antibody responses to Hib PS, and many also had abnormal levels of other Ig isotypes.

In view of the apparent excellent health of the children in the present study, and their generally normal serum Ig, and normal responses to Hib PS vaccine, we were surprised to find that mitogen-stimulated *in vitro* production of IgG2 was decreased in five of seven children studied. Thus, this in vitro finding suggests that the low serum concentration of IgG2 in these children may reflect decreased production of IgG2 in response to mitogenic or other nonspecific stimuli occurring *in vivo*. It should be noted that the *in vitro* abnormality seen in these children was milder as well as qualitatively different from that seen by us in infection-prone children with subnormal levels of serum IgG2 (25a). Peripheral blood mononuclear cells from infection-prone children produce much lower amounts of IgG2, and also produce extremely small amounts of IgG1 and IgG3. In contrast, PBL from the children in our study produced IgG2 only slightly less than normal and, in most children, their abnormality was limited to IgG2.

Patients deficient in terminal complement components experience sporadic but severe meningococcal infections (26, 27). Also, despite apparent health in most IgA-deficient patients, there is an increased incidence of autoimmune disease among IgA-deficient individuals (28, 29). Thus, we plan to follow the clinical status of these apparently healthy children with subnormal IgG2 levels over a longer period of time.

Finally, an important implication of this study is that a subnormal concentration of serum IgG2 does not necessarily reflect a deficiency in specific antibody responses, and is not a sufficient explanation for increased susceptibility to infection. Children with subnormal serum concentrations of IgG2 should not be treated with intravenous Ig without further evaluation, particularly a careful clinical assessment and demonstration of abnormal specific antibody responses.

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