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Severity of infections in IgA deficiency: correlation with decreased serum antibodies to pneumococcal polysaccharides and decreased serum IgG2 and/or IgG4

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SUMMARY

In order to define abnormalities of humoral immunity which determine susceptibility to respiratory tract infections in IgA-deficient adults, serum IgG subclass concentrations, and serum concentrations of pneumococcal antibodies and *Haemophilus influenzae* type B (Hib) antibodies sera from IgA-deficient adults with and without susceptibility to respiratory tract infections were compared. Infection susceptibility was not related to the degree of IgA deficiency, but was related to deficiency of IgG4 and, to a lesser extent, IgG2, as well as to low basal serum concentrations of pneumococcal polysaccharide antibodies. The combination of IgG2 and/or IgG4 deficiency and a non-protective basal serum concentration of antibody against two or more pneumococcal polysaccharides was present in the serum of six of 12 (50%) patients with severe infections, but only one of 44 (2%) patients without infections. Furthermore, the preservation of antibody responses against the most immunogenic pneumococcal polysaccharide type 3, but not against the less immunogenic types 7F, 9N and 14, in patients with severe infections suggested that abnormalities of pneumococcal polysaccharide antibody responses might include defects of affinity maturation.

Keywords IgA deficiency IgG subclasses anti-pneumococcal polysaccharide antibodies respiratory tract infections B cell differentiation disorder

INTRODUCTION

IgA deficiency is the characteristic manifestation of a terminal B cell differentiation disorder which is thought to result from an immunoregulatory abnormality determined in part by, or associated with, MHC genes [1,2]. In some IgA-deficient individuals, this immunoregulatory abnormality is associated with additional abnormalities of antibody-mediated immunity, including IgE deficiency [3], an imbalance of serum IgG subclass concentrations characterized by subnormal concentrations of IgG2 and/or IgG4, and elevated concentrations of IgG1 and IgG3 [4–6], and by impaired antibody responses against both protein and polysaccharide antigens [4,7–8].

The increased frequency of respiratory tract infections found in some IgA-deficient individuals is apparently related to factors other than the IgA deficiency, because many IgA-deficient individuals are asymptomatic. The abnormalities which underlie this propensity to infection are, however, largely undefined except that IgG2 deficiency is associated with an increased frequency of infections in IgA-deficient individuals (reviewed in [9]), possibly because it is a manifestation of an immune defect which results in impairment of antibody responses against polysaccharide antigens [10]. Quantitative deficiency of IgG2 *per se* is, however, not likely to account entirely for the increased number of infections in infection-prone, IgA-deficient individuals because other abnormalities, such as selective deficiency of polysaccharide-specific IgG2 antibodies, are also recognized [8]. Because IgG4 is sometimes undetectable in the serum of IgA-deficient individuals, IgG4 deficiency may be an additional predisposing factor. However, the significance of undetectable serum IgG4 in the presence or absence of IgA deficiency can be interpreted only with careful reference to the method employed for quantification of IgG subclasses. For example, IgG4 is undetected by radial immunodiffusion in up to 20–30% of normal individuals [11].

To determine which abnormalities of antibody-mediated immunity are associated with an increased propensity to

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respiratory tract infections, we obtained sera from a group of 66 symptomatic or asymptomatic IgA-deficient adults. Serum concentrations of IgG subclasses and antibodies to the capsular polysaccharides of *Haemophilus influenzae* type B (Hib) and *Streptococcus pneumoniae* were quantified in an ELISA. A newly developed, highly sensitive immunoradiometric assay was used to measure serum IgG subclass concentrations to assess the relevance of IgG subclass deficiencies to infection in IgA-deficient adults.

PATIENTS AND METHODS

IgA-deficient individuals

IgA-deficient adults were identified from three sources: (i) hospital patients referred because of an increased susceptibility to respiratory tract infections; (ii) hospital patients incidentally found to have IgA deficiency during the course of investigations for another medical problem; and (iii) blood donors found to be IgA-deficient during a screening programme to identify IgA-deficient donors. Most had undetectable IgA (< 30 mg/dl) by nephelometry; all had IgG concentrations of $\geqslant 500$ mg/dl. Sera were available from 66 individuals, of whom two probably had drug-induced IgA deficiency and one had IgA deficiency demonstrated during investigations of graft-*versus*-host disease (GVHD). Clinical information was obtained on all individuals by interview and/or review of hospital notes by one of us (M.A.H.F.) and infection history collected according to a standard protocol. With respect to respiratory tract infections, these individuals were classified as having either (i) no abnormal susceptibility to infections ($n = 47$); (ii) susceptibility to mild respiratory tract infections ($n = 6$); or (iii) susceptibility to severe respiratory tract infections ($n = 13$). Severe infections were defined as abnormally recurrent and/or persistent infections of the upper and/or lower respiratory tract with purulent sputum or nasal discharge associated with systemic manifestations often requiring the use of antibiotics. All patients with severe infections had clinical evidence of bacterial infections which were confirmed radiologically and/or microbiologically in most patients. In contrast to severe infections, mild infections were characterized by upper respiratory tract infections without systemic symptoms, and were often difficult to differentiate from symptoms of allergic airways disease.

Age information was available for 65 of the 66 patients. At the time of these studies, patients ranged in age from 15 to 80 years. Mean age was 42 ± 16 years (± 1 s.d.); median age was 37 years. Age distribution: five patients, 15–19 years; 10 patients, 20–29 years; 18 patients, 30–39 years; 13 patients, 40–49 years; eight patients, 50–59 years; seven patients, 60–69 years; three patients, 70–79 years; one patient, 80 years.

Total serum IgA concentrations

All specimens are screened for total serum IgA concentration by nephelometry. The sensitivity of IgA concentration determination by nephelometry is 6 mg/dl. The intra- and inter-assay coefficients of variation range from 2% to 4% and 5% to 7%, respectively. Following nephelometric screening, the serum concentrations of IgA were measured by solid-phase enzyme immunoassay. The IgG fraction of anti-IgA MoAbs (M26012) (Oxoid, USA, Inc., Columbia, MD) were covalently attached to CM Trisacryl M Ion Exchange Beads (IBF Biotechnics, Savage, MD). Test sera were incubated with the antibody-coated beads at dilutions of 1:10 000 in 0·05% Tween-20 in 0·01 M PBS pH 7·4 (PBS-$T_{0·05\%}$) for 2 h at room temperature with shaking. After washing five times with PBS-$T_{0·05\%}$, alkaline phosphatase-labelled goat anti-human IgA (American Qualex, La Mirada, CA), diluted 1:1000 in PBS-$T_{0·05\%}$ was added and incubated for 2 h at room temperature. After washing with PBS-$T_{0·05\%}$, substrate (1 mg/ml para-nitrophenyl phosphate in 0·05 M sodium carbonate pH 9·6, with 0·01 mg/ml $MgCl_2$) was added to each well and incubated until the standard (100 mg/dl purified human IgA; The Binding Site, San Diego, CA) reached OD of $1·000 \pm 0·100$ at 405 nm. IgA concentrations were determined from standard curves constructed using log *versus* log, plotting OD_{405} on the ordinate *versus* concentration in mg/dl on the abscissa. The sensitivity of IgA concentration determination by ELISA is 10 μg/dl. The intra- and inter-assay coefficients of variation for this assay range from 8% to 12% and 10% to 15%, respectively. The internally validated reference range for serum IgA (70–315 mg/dl) was similar to that reported in the literature [12].

Total IgG subclass concentrations

Serum IgG subclass concentrations were measured by an immunoradiometric assay. IgG fractions of MoAbs to each of the four IgG subclasses (IgG1, MoAb NL16; IgG2, MoAb HP6014; IgG3, MoAb ZG4; IgG4, MoAb RJ4) (Oxoid, USA) were covalently bound to CM Trisacryl M Ion Exchange Beads (IBF Biotechnics). Test sera, diluted in 0·1% Tween in PBS (PBS-$T_{0·1\%}$) at 1:25 000 for IgG1, 1:5000 for IgG2, 1:5000 for IgG3, and 1:25 000 for IgG4, were incubated with the antibody-coated beads for 1 h at room temperature with vigorous shaking. After washing with 1% Tween 20 in PBS, the beads were incubated at room temperature for 1 h with goat anti-human IgG (Irvine Scientific, Santa Ana, CA) labelled with ^{125}I ($2–2·2 \times 10^6$ ct/min per ml), then washed with PBS-$T_{0·1\%}$. Radioactivity was counted on Hydragamma 16 Kallestad (Chaska, MN). The concentration of each serum IgG subclass was calculated from a standard curve plotted as log *versus* log (ct/min on the ordinate *versus* concentration in mg/dl on the abscissa) using eight serial dilutions of human IgG (WHO Reference Pool, CDC Control no. 67–97). The sensitivity for IgG subclass concentration determination by this method is 1 pg/ml. The intra- and inter-assay coefficients of variation range from 2% to 9% and 3% to 12%, respectively. The internally validated reference ranges for serum IgG subclasses were similar to those reported in the literature [9], except that the lower limit of the range of IgG4 established with 300 healthy adults was 3 mg/dl.

Pneumococcal and H. influenzae *b polysaccharide antibodies*

Validation of assays for IgG antibodies to *Strep. pneumoniae* polysaccharides is reported in detail elsewhere [13]. Pneumococcal polysaccharides of serotypes 3 (3), 7F (51), 9N (9) and 14 (14) by Danish (American) nomenclature (Lederle Laboratories, Pearl River, NY) and *H. influenzae* type b (Hib) polysaccharides (Lederle Labs), diluted to 2–5 μg/ml in 0·05 M carbonate buffer pH 9·6, were incubated at 37°C overnight in the wells of Immulon I polystyrene plates (Dynatech, Chantilly, VA). Plates were washed with 0·01% Tween 20 in PBS (PBS-$T_{0·01\%}$). Test sera, diluted 1:70 for the pneumococcal polysaccharide antibodies assay and 1:10 for the Hib polysaccharide antibodies assay with 1% bovine serum albumin (BSA; Sigma

Chemical Co., St Louis, MO) in PBS-$T_{0\cdot01\%}$ (BSA-PBS-$T_{0\cdot01\%}$), were incubated for 0·5 h at room temperature with vigorous shaking. After washing, antibody was detected using alkaline phosphatase-labelled goat anti-human IgG, heavy and light chain specific, $F(ab')_2$ (American Qualex) diluted 1:4000 with BSA-PBS-$T_{0\cdot01\%}$. Antibody concentrations were calculated from standard curves constructed using log *versus* log, plotting OD_{405} on the ordinate *versus* concentration on the abscissa (ng of antibody nitrogen per ml (ng Ab N/ml) for pneumococcal and ng of protein per ml (ng/ml) for Hib antibodies). The internally validated reference range for protective serum concentrations of antibodies to pneumococcal polysaccharides (200–300 ng Ab N/ml) and Hib (150 ng/ml) were similar to values reported in the literature [13–16].

Three known positive sera, calibrated against standards employed in a standardized radioimmunoassay (courtesy of Dr Gerald Schiffman), were used as references for the pneumococcal polysaccharide antibodies assay. Two-fold serial dilutions of a positive specimen calibrated against the FDA CBER standard (Dr Carl Frash, Bethesda, MD) were used as standards in the Hib polysaccharide antibodies assay.

Statistical analysis

Statistical analyses were performed using the Wilcoxon rank sum test or, where indicated, Fisher's exact test.

RESULTS

Severity and frequency of respiratory tract infections are not correlated with decreased serum concentrations of IgA

Serum IgA concentrations were $<0\cdot001$ mg/dl in 71% (47/66) of the IgA-deficient adults examined in this study. Serum IgA concentrations varied from 0·02 to 30·8 mg/dl in the remaining 29% (19/66) individuals. When the patients were divided into three groups (severe, recurrent infections; mild infections; no increased susceptibility to infection), there was no statistically significant difference in the proportion of individuals with IgA concentrations $<0\cdot001$ mg/dl in the three groups of patients, i.e. each group had a similar frequency of individuals with IgA concentrations $<0\cdot001$ mg/dl (data not shown).

Concomitant IgG4 deficiency correlates with severity of infections in IgA-deficient individuals

Serum concentrations of each of the IgG subclasses were quantified by a sensitive immunoradiometric assay. Although IgG1 concentrations were greater than the upper value of the reference range (950 mg/dl) in 47% (31/66) of the IgA-deficient individuals examined, there were no statistically significant differences in IgG1 average serum concentrations among the three groups (data not shown). Similarly, although IgG3 concentrations were >90 mg/dl in 11% (7/66) and <20 mg/dl in 8% (5/66) of the IgA-deficient individuals, there were no significant differences in average serum concentrations of IgG3 among the three groups (data not shown). Thus, neither IgG1 nor IgG3 concentrations appear to correlate with increased frequency and severity of respiratory tract infections in IgA-deficient adults.

In contrast, the average serum concentration of IgG2 was lower in the group with severe infections compared with the group with no increased susceptibility to infection ($P < 0\cdot05$); there was no statistically significant difference between

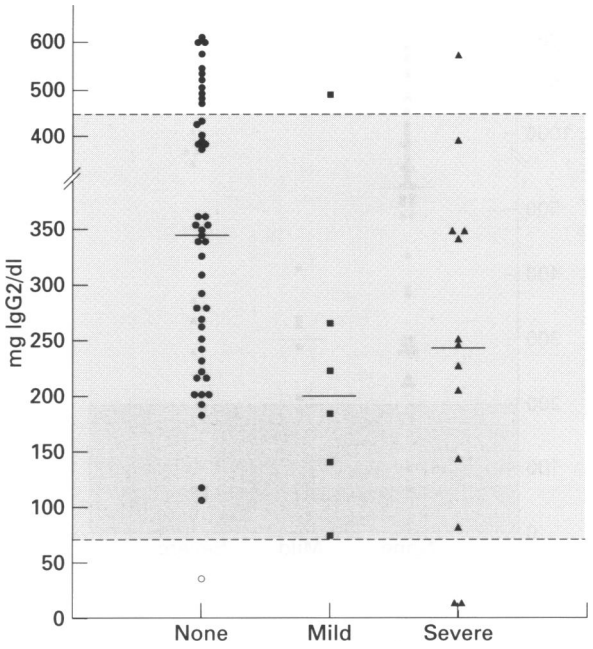

Fig. 1. Serum IgG2 concentrations in IgA-deficient patients with severe infections, mild infections or no increased susceptibility to infections. The adult reference range is shaded and the single patient with graft-*versus*-host disease (GVHD) is indicated by an open circle. The median values are indicated by the bars.

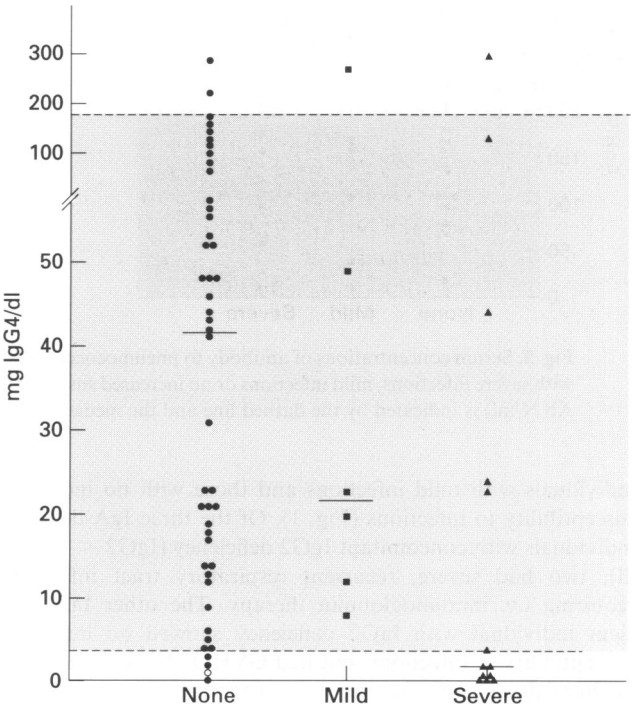

Fig. 2. Serum IgG4 concentrations in IgA-deficient patients with severe infections, mild infections or no increased susceptibility to infections. The adult reference range is shaded and the single patient with graft-*versus*-host disease (GVHD) is indicated by an open circle. The median values are indicated by the bars.

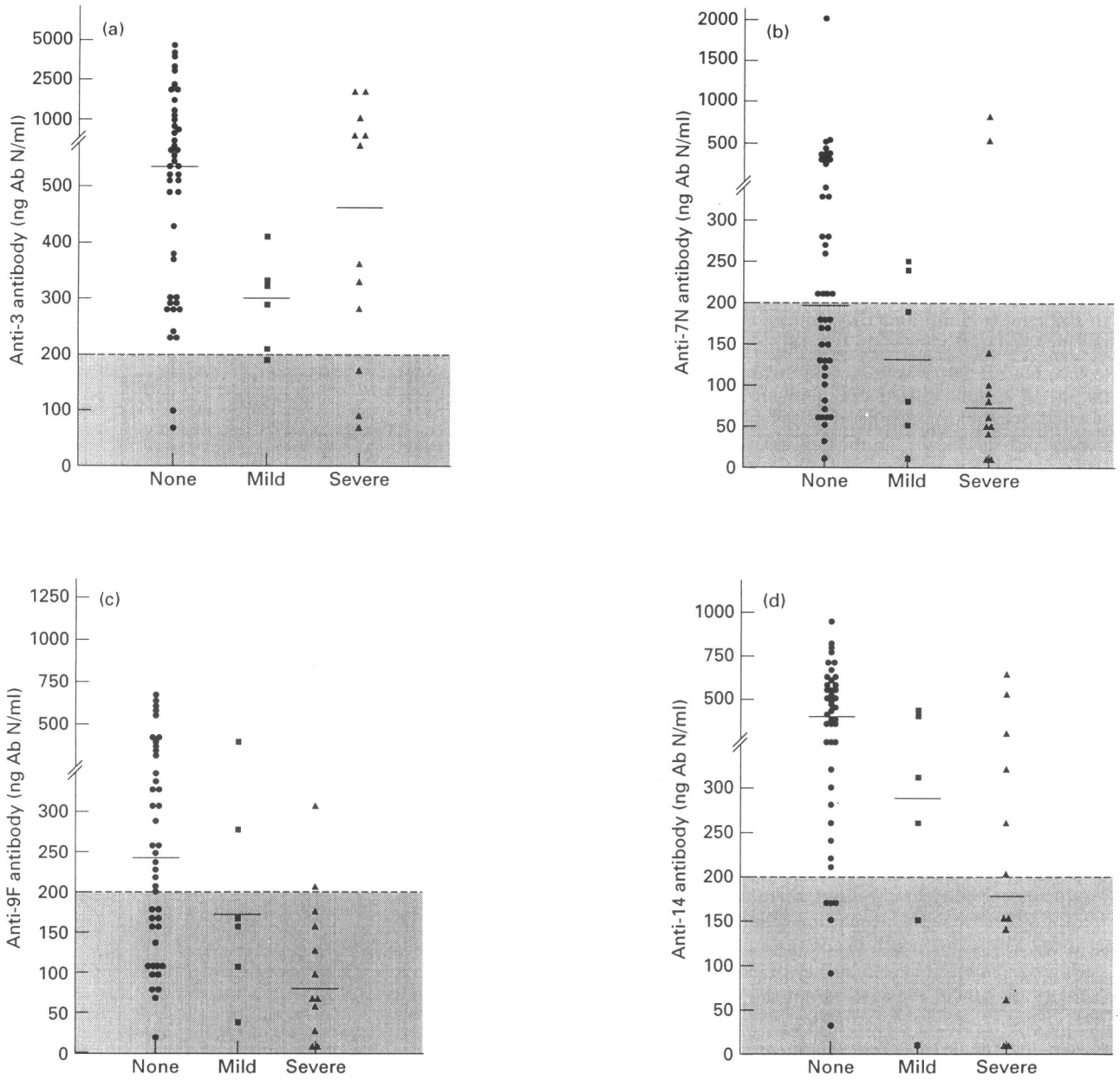

Fig. 3. Serum concentrations of antibody to pneumococcal polysaccharide serotypes 3 (a), 7N (b), 9F (c), 14 (d) in IgA-deficient adults with severe infections, mild infections or no increased susceptibility to infections. The non-protective antibody concentration (< 200 ng Ab N/ml) is indicated by the dashed line and the median values for each group are indicated by the bars.

individuals with mild infections and those with no increased susceptibility to infections (Fig. 1). Of the three IgA-deficient individuals with concomitant IgG2 deficiency (IgG2 < 70 mg/dl), two had severe, recurrent respiratory tract infections requiring i.v. immunoglobulin therapy. The other IgA-deficient individual with IgG2 deficiency showed no increased susceptibility to infections, but had GVHD. Thus, 15% (2/13) of individuals with severe, recurrent infections were also deficient for IgG2.

Similarly, the average serum concentration of IgG4 was also decreased in the group of individuals with severe infections compared with the group with no increased susceptibility to infection ($P < 0{\cdot}02$; Fig. 2). There was no statistically significant difference in the average concentration of serum IgG4 between the group with mild infections and that with no increased susceptibility to infections. Of the 10 IgA-deficient individuals with concomitant IgG4 deficiency (< 3 mg/dl), seven had severe infections. Thus, 54% (7/13) individuals with severe infections also were deficient for IgG4. Furthermore, all three individuals with IgG2 deficiency were also severely IgG4-deficient, with serum concentrations of < 1 mg IgG4/dl.

Non-protective concentrations of pneumococcal, but not Hib polysaccharide antibodies correlate with severe, recurrent respiratory infection

To assess the antibody response to encapsulated organisms in

Table 1. IgG subclass deficiency and non-protective basal serum concentrations of pneumococcal polysaccharide antibodies are associated with severe respiratory tract infections in IgA-deficient adults

Propensity to infections	Serum IgG2 and/or IgG4 deficiency (%)	Non-protective (< 200 ng/ml) serum concentration of antibody to pneumococcal polysaccharides (%)				IgG2 and/or IgG4 deficiency and non-protective serum concentration of antibody to two or more pneumococcal polysaccharides (%)
		3	7	9	14	
Non ($n = 47$)*	4 (9)	2 (5)	22 (50)	17 (39)	6 (14)	1 (2)§
Mild ($n = 6$)	0	1 (17)	4 (67)	4 (67)	2 (33)	0
Severe ($n = 13$)†	7 (54)‡	3 (25)	10 (83)‡	10 (83)‡	6 (50)‡	6 (50)‡

* Only 44 sera available for pneumococcal polysaccharide antibody assays.
† Only 12 sera available for pneumococcal polysaccharide antibody assays.
‡ $P < 0{\cdot}05$ (Fisher's exact test) compared with individuals with no increased propensity to infection.
§ One individual with graft-*versus*-host disease (GVHD).

patients with IgA deficiency, serum concentrations of antibody against Hib and pneumococcal polysaccharide antigens were quantified by ELISA. The average concentrations of serum Hib antibodies in the three groups of IgA-deficient individuals were not significantly different (data not shown). Only two individuals had non-protective concentrations (< 150 ng/ml) of Hib antibodies. Both of these individuals had IgG2 and/or IgG4 deficiency and non-protective serum concentrations of antibodies against all four serotypes of pneumococcal polysaccharide. One had severe infections and the other showed no increased susceptibility to infections, but had GVHD.

In contrast, the average serum concentrations of antibodies against the pneumococcal polysaccharides 7F, 9N and 14 (the less immunogenic serotypes), but not 3 (the most immunogenic serotype) [13–14,17–18], were lower in the group of IgA-deficient individuals with severe infections compared with the group with no increased susceptibility to infection ($P < 0{\cdot}02$, 7N; $P < 0{\cdot}01$, 9F and 14; Figs 3a–d; Table 1). The average concentration of serum antibodies against pneumococcal polysaccharide type 3 was lower only in patients with mild infections ($P < 0{\cdot}01$; Fig. 3a); only one individual, however, had a non-protective serum concentration (< 200 ng Ab N/ml). Although non-protective basal concentrations of serum antibodies to the less immunogenic pneumococcal polysaccharides types 7F, 9N and 14 were common in all groups, the frequencies of non-protective concentrations of antibodies to each of the four serotypes (i.e. including serotype 3, the most immunogenic) were greater in the group with mild infection than in the group with no increased incidence of infections, and greatest in the group with severe, recurrent infections (Table 1).

IgG2 and/or IgG4 deficiencies concomitant with non-protective concentrations of pneumococcal polysaccharide antibodies correlate with increased frequency and severity of respiratory tract infections in IgA-deficient adults

Although IgG2 and/or IgG4 deficiencies were most common in IgA-deficient individuals with severe, recurrent respiratory infections (Table 1), approximately 9% (4/47) of IgA-deficient individuals with no increased susceptibility to infections also had IgG2 and/or IgG4 subclass deficiency. The data were therefore analysed to determine whether combined abnormalities of IgG subclasses and pneumococcal polysaccharide antibodies better differentiate individuals with severe infections from the others. The frequency of non-protective serum concentrations of antibody against two or more pneumococcal polysaccharides in individuals without concomitant IgG2 and/or IgG4 deficiency did not differ significantly between the group with severe infections and the group with no increased susceptibility to infection ($P = 0{\cdot}24$; data not shown). Irrespective of infection history, the average serum concentrations of pneumococcal antibodies were lower in individuals with combined IgG2 and IgG4 deficiency than in individuals with IgG4 deficiency alone for serotypes 3 (248 *versus* 733 ng Ab N/ml), 7F (43 *versus* 220 ng Ab N/ml), 9N (28 *versus* 175 ng Ab N/ml) and 14 (55 *versus* 304 ng Ab N/ml). Notably, the combination of IgG2 and/or IgG4 deficiency and non-protective serum concentrations of antibodies against two or more pneumococcal polysaccharides was significantly more frequent ($P < 0{\cdot}05$) in individuals with severe infections compared with individuals with no increased susceptibility to infections (50% (6/12) *versus* 2% (1/47), respectively; Table 1). Combined IgG2 and/or IgG4 deficiency and non-protective concentrations of pneumococcal polysaccharide antibodies were not found in the group with only mild infections.

DISCUSSION

The increased susceptibility to infections in some individuals with IgA deficiency is not related to the severity of the IgA deficiency alone. Rather, it is related to additional disorders of humoral immunity. Although decreased concentrations of IgG2 and possibly IgG3 in IgA-deficient individuals with increased susceptibility to infections have been reported [9], IgG subclass deficiencies do not correlate with frequent and severe infections in most IgA-deficient individuals (reviewed in [3]). Although we noted that serum concentrations of IgG2 are lower in individuals with severe infections compared with individuals with no increased susceptibility to infections (244 *versus* 346 mg/dl), IgG2 deficiency was present in no more than 20% of IgA-deficient individuals with severe infections. In our group of 66 IgA-deficient adults, IgG4 deficiency (15%) was more common than IgG2 (5%) or combined IgG2/IgG4 deficiency (5%). Average concentrations of IgG4 were lower in the group of individuals with severe infections compared with the group with no increased susceptibility to infection (2 *versus* 42 mg/dl). Of the individuals with severe infections, 54%

(7/13) had serum concentrations of IgG4 below 3 mg/dl. Thus, there is a strong correlation between concomitant IgG4 deficiency and frequent, severe respiratory infections in IgA-deficient individuals.

The mechanism by which low concentrations of serum IgG2 and/or IgG4 might contribute to a predilection to infections in some IgA-deficient individuals is not known. IgG2 deficiency is theorized to predispose IgA-deficient individuals to infections because antibodies to polysaccharide antigens are often of the IgG2 subclass [19]. As IgA-deficient individuals with severe infections are often infected by encapsulated bacteria, a deficiency in this class of antibodies may result in more frequent, severe infections. However, most IgA-deficient individuals, including many with severe infections, do not have concomitant IgG2 deficiency. In our study, only 15% (2/13) of IgA-deficient individuals with severe infections were IgG2-deficient. Furthermore, the IgG2 subclass by no means contains all serum antibodies to polysaccharide antigens; IgG2 deficiency may be compensated for by other immunoglobulin classes or IgG subclasses [20,21]. As our results indicate, severe infections in IgA-deficient individuals are more strongly correlated with a deficiency of IgG4 than of IgG2 (54% *versus* 15%).

Although 54% of IgA-deficient individuals with severe respiratory infections had concomitant IgG2 and/or IgG4 deficiency, 46% did not. These individuals, however, often had non-protective concentrations of serum antibodies against one or more pneumococcal polysaccharides. Other studies have demonstrated deficient or aberrant antibody responses against polysaccharide antigens in IgA-deficient individuals [8,21–23]; none, however, has shown their relationship with frequent, severe infections in IgA-deficient adults as clearly as in this study. We find that serum concentrations of Hib antibodies are not indicative of severe infections in IgA-deficient adults, possibly because naturally occurring anti-Hib antibodies are predominantly of the IgG1 subclass in normal and IgG subclass-deficient adults [24]. However, increased frequencies of non-protective concentrations of pneumococcal polysaccharide antibodies (serotypes 3, 7F, 9N and 14) are progressively more common in IgA-deficient patients with mild and severe infections. The frequency of non-protective concentrations of antibodies to all three of the less immunogenic pneumococcal serotypes (7F, 9N and 14) [13–14,17–18] was significantly increased in the group with severe infections compared with the group with no increased susceptibility to infection. When concomitant IgG2 and/or IgG4 deficiency is accompanied by non-protective concentrations of pneumococcal polysaccharide antibodies, a strong correlation with frequent, severe infections in IgA-deficient adults is observed. Whereas only 2% (1/47) of the IgA-deficient individuals with no increased incidence of infection had non-protective concentrations of antibodies to two or more pneumococcal polysaccharides concomitant with IgG2 and/or IgG4 deficiency, 50% (6/12) of the patients with severe, recurrent infections had the combined abnormalities. Whether these combined abnormalities are both necessary and sufficient to explain the immunological defect which makes some IgA-deficient individuals more prone to severe infections than others is unknown. It is notable that the combination of IgG2 and/or IgG4 deficiency and non-protective concentrations of pneumococcal polysaccharide antibodies was found in both of the two individuals whose infections were of such severity as to require treatment with i.v. immunoglobulin.

It should be noted that serotype-specific polysaccharides, which may contain some non-type-specific C-polysaccharide antibody contamination, are currently preadsorbed with C-polysaccharide to avoid the possibility that these assays detect non-type-specific IgG. However, because C-polysaccharide was not commercially available at the time of these studies, our reference and patient sera were analysed without preadsorption with C-polysaccharide. Nevertheless, there is no reason to believe that healthy IgA-deficient patients react differently to non-type-specific C-polysaccharide than IgA-deficient patients susceptible to infections, i.e. the differences noted between the healthy and susceptible patients are probably not due to the presence of non-type-specific antibodies.

IgG2 and/or IgG4 deficiency may represent an extreme B cell maturational defect which affects IgG subclass switching in the later stages of B cell maturation and differentiation. In less extreme cases, it is possible that only the process of somatic hypermutation (point mutations, additions and deletions in the immunoglobulin VJ and VDJ variable domain exons) which controls affinity maturation of antibodies might be defective [25,26]. The frequency of non-protective concentrations of the less immunogenic serotypes (types 7F, 9N and 14) in those individuals with mild and severe infections was consistently greater than the frequency of non-protective concentrations of the most immunogenic serotype studied (type 3), suggesting an inability to generate a mature, high-affinity antibody response to certain antigenic stimuli, i.e. specific pneumococcal polysaccharide antigens.

Although IgG4 and/or IgG2 deficiency and non-protective basal concentrations of antibodies to two or more pneumococcal polysaccharides discriminated between patients with and without severe infections, only 50% of patients with severe infections had this combination of abnormalities. However, evaluation of antibody responses in other ways may have demonstrated discriminating abnormalities in the other patients with severe infections. For example, testing the ability to mount an immune response to a polyvalent pneumococcal vaccine may be clinically useful. In future studies, quantification of basal and post-immunization concentrations of both total IgG and IgG subclasses specifically reactive with pneumococcal polysaccharides of varying immunogenicity may be helpful in defining other abnormalities in IgA-deficient individuals with severe infections, particularly those who do not have IgG2 and/or IgG4 deficiency accompanied by subnormal basal concentrations of serum antibodies to pneumococcal polysaccharides. Furthermore, as demonstrated in children immunized with conjugated Hib vaccine, the measurement of antibody affinity rather than quantity may be more closely related to the bactericidal activity of antibodies [27]. It may therefore be informative to measure the affinity of pneumococcal antibodies before and after immunization.

In conclusion, we have shown that approximately 50% of IgA-deficient adults with an increased susceptibility to respiratory tract infections have abnormally low concentrations of serum IgG2 and/or IgG4 accompanied by low basal concentrations of serum antibodies to specific pneumococcal polysaccharides. Thus, the assessment of IgA-deficient individuals with an increased susceptibility to infections requires several investigations, including assays of IgG subclasses and basal serum concentrations of serum antibodies to pneumococcal polysaccharides. Further studies are needed to assess the

clinical utility of quantifying antibody responses before and after immunization with pneumococcal polysaccharides and protein antigens, e.g. tetanus toxoid.

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