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# Subclass Restriction Pattern of Antigen-Specific Antibodies in Donors with Defective Expression of IgG or IgA Subclass Heavy Chain Constant Region Genes

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We have developed a method for the measurement of the IgG and IgA subclass distribution of antigen-specific human antibodies. The controls for the specificity of the assay include the use of a number of monoclonal human antibodies and sera from individuals with deletions of particular immunoglobulin heavy chain constant region genes. The system was used to determine the shift in immunoglobulin subclass patterns of specific antibodies against a variety of protein and polysaccharide antigens in individuals with a regulatory deficiency of a given IgG or IgA subclass. Normally, the pattern is quite distinct and antibodies against protein antigens are mainly of the $IgG_1$ subclass, whereas antibodies against polysaccharide antigens are mainly of the $IgG_2$ subclass. The results on serum from an $IgG_1$ deficient donor suggested that $IgG_3$ and $IgG_4$ appear to compensate for a lack of $IgG_1$, whereas isolated deficiencies of $IgG_3$, $IgG_4$, or $IgA_2$ do not markedly influence the expected distribution of specific antibodies. In $IgG_2$-deficient individuals a more complex pattern was observed where antibodies against protein antigens were retained, whereas levels of antibodies against polysaccharide antigens could vary markedly between donors, which appeared to be dependent on whether the $IgG_2$ deficiency was an isolated defect or combined with $IgG_4$/IgA deficiency. However, all the $IgG_2$-deficient donors had a skewed pattern of anti-polysaccharide antibodies with a shift to $IgG_1$ to $IgG_3$. 

## INTRODUCTION

Human immunoglobulins are composed of five structurally distinct classes with different biological properties. IgG and IgA may also be further subdivided into subclasses, and each are suggested to have a specialized function. Serum levels of each class and subclass appear to be independently regulated during ontogeny and adult levels of IgM, $IgG_1$, and $IgG_3$ are reached at an early age, whereas $IgG_2$, $IgG_4$, and IgA may not reach adult levels until adolescence (1). This particular maturation pattern is also reflected in the immunoglobulin class and subclass pattern of specific antibodies in infants which may be restricted to IgM, $IgG_1$, and $IgG_3$, a finding which is compatible with a successive utilization of heavy chain constant region genes, with an initial use of the most $J_H$ proximal heavy chain constant region genes.



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Previous studies in man have suggested a marked subclass restriction of antigen-specific antibodies where protein antigens mainly give rise to IgM and $IgG_1$ antibodies with minor contributions of antibodies of the $IgG_3$, $IgG_4$, and $IgA_1$ subclasses (for review see (2)). Antibodies against carbohydrate antigens, on the other hand, are normally restricted to IgM, $IgG_2$, and possibly also $IgA_2$ in adult donors (for review see (2)). A markedly different pattern is seen in children who normally express $IgG_1$ antibodies against polysaccharide antigens (3, 4), possibly reflecting the immaturity of the immune system. Recent data suggest that different $V_H$ genes may be expressed in the various subclasses and that $IgG_1$ antibodies against carbohydrate antigens display a lower mean affinity than an $IgG_2$ antibody with a corresponding specificity (5).

Selective deficiency of one immunoglobulin class is a well-known form of immunodeficiency first recognized more than 2 decades ago. Immunoglobulin subclass deficiency, however, has only recently been recognized as a distinct disease entity but deficiencies of isolated IgG as well as IgA subclasses have already been described. In the "common" form of $IgG_2$ deficiency, the corresponding heavy chain constant region gene is retained in the genome (6). Due to what appears to be a regulatory dysfunction (7), antibodies against selected polysaccharide antigens are largely absent. However, in donors where the deficiency is due to a deletion of the $IgG_2$ constant region gene, anti-carbohydrate antibodies are present in all tested individuals (8, 9) and are, as anticipated, restricted to the $IgG_1$ and/or $IgG_3$ subclasses. In this paper we describe the complex shifts of the subclass pattern of specific antibodies in $IgG_1$, $IgG_2$, $IgG_3$, $IgG_4$, or $IgA_2$ subclass deficient individuals.

## MATERIALS AND METHODS

*Sera.* Sera from normal blood donors and from patients with known IgG or IgA subclass deficiencies (for references see Tables 3 and 6) were collected and stored at −30°C until used. IgG subclass levels were determined in immunodiffusion assays utilizing monoclonal reagents (Unipath, London, England) or polyclonal rabbit antisera (kindly performed by Dr. V. Oxelius, Department of Pediatrics, Lund's Hospital, Sweden).

*Antigens.* Tetanus toxoid and diphtheria toxoid were gifts from Dr. M. Fall-Persson, National Bacteriological Laboratory, Stockholm, Sweden. Outer membrane protein from *Haemophilus influenzae* was given to us by Dr. I. Allen, Oxford University, Oxford, England. Alphatoxin and teichoic acid from *Staphylococcus aureus* were obtained from Dr. R. Möllby, National Bacteriological Laboratory, Stockholm, Sweden. Pneumococcal capsular polysaccharides (serotypes 3, 6A, and 8) were gifts from MSD, Mechelen, Belgium. Capsular polysaccharide from *H. influenzae* type B was given to us by Dr. R. A. Insel, Department of Pediatrics, Rochester, New York. NP, coupled to bovine serum albumin, was a gift from Professor O. Mäkelä, Helsinki, Finland.

*ELISA.* All antigens were coated on polystyrene microtiter plates at a concentration of 2–10 μg/ml. The subclass distribution of specific antibodies was determined as described previously in detail (10). Briefly, after incubation of the serum samples (diluted 1:100) overnight on antigen-coated plates, commercially avail-

able monoclonal antibodies against the various human IgG subclasses (Table 1) were added in optimal concentrations. Rabbit anti-mouse Ig (DAKO Immunoglobulins, Copenhagen, Denmark) was thereafter added. After incubation for 4 hr, alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma Chemical Co., St. Louis, MO) was added and the plates were again incubated overnight. After washing, substrate was added and the plates were incubated for 10–20 min. Absorbance was measured at 405 nm using a Titertek multiscan (Elflab OY, Helsinki, Finland). Values are given as net absorbance (subtracting the absorbance value obtained in wells where only patient serum was omitted).

## RESULTS

### *Specificity of Anti-Subclass Reagents*

In order to assess the subclass distribution of specific antibodies it was necessary to determine the specificity of the available monoclonal antibodies against the various subclasses (Table 1). In our system, purified myeloma proteins have previously been shown to be unsuitable for this purpose due to contamination with irrelevant subclass proteins (10). We therefore employed a different approach utilizing cell lines producing human monoclonal antibodies with a known specificity (Table 2) or sera from individuals with homozygous deletions encompassing the immunoglobulin subclass constant region genes (Table 3). Some of the antibodies displayed a low degree of undesired cross-reactivity with inappro-

TABLE 1
MONOCLONAL ANTI-SUBCLASS REAGENTS USED IN SPECIFICITY CONTROLS

| Monoclonal | Specificity[a] | Designation | Dilution | Mouse isotype | Source | Distributor |
|---|---|---|---|---|---|---|
| 1 | IgG1 | Bam 09 | 1:100 | IgG1 | R. Jefferis | Unipath |
| 2 | | Bam 15 | 1:1000 | IgG1 | R. Jefferis | Unipath |
| 3 | | HP6001 | 1:1000 | IgG1 | C. B. Reimer | CDC |
| 4 | | HP6055 | 1:1000 | IgG1 | C. B. Reimer | CDC |
| 5 | | SG-11 | 1:1000 | IgG1 | —[b] | Yeda |
| 6 | | 2C7 | 1:500 | IgG1 | O. Mäkelä | —[c] |
| 7 | IgG2 | Bam 10 | 1:2000 | IgG1 | R. Jefferis | Unipath |
| 8 | | Bam 14 | 1:100 | IgG1 | R. Jefferis | Unipath |
| 9 | | HP6002 | 1:5000 | IgG1 | C. B. Reimer | CDC |
| 10 | | HP6014 | 1:10000 | IgG1 | C. B. Reimer | CDC |
| 11 | | SH-22 | 1:1000 | IgG1 | — | Yeda |
| 12 | IgG3 | Bam 08 | 1:8000 | IgG1 | R. Jefferis | Unipath |
| 13 | | HP6047 | 1:1000 | IgG1 | C. B. Reimer | CDC |
| 14 | | SJ-33 | 1:1000 | IgG1 | — | Yeda |
| 15 | | 2F5 | 1:1000 | IgG1 | O. Mäkelä | — |
| 16 | IgG4 | Bam 16 | 1:12000 | IgG1 | R. Jefferis | Unipath |
| 17 | | SK-44 | 1:1000 | IgG1 | — | Yeda |
| 18 | | — | 1:1000 | IgG1 | J. Thorell | Pharmacia |

[a] For details of the monoclonal anti-subclass reagents used see ref. 11.
[b] Purchased directly from the respective companies.
[c] Not commercially available.

TABLE 2
HUMAN MONOCLONAL ANTIBODIES USED FOR SPECIFICITY CONTROL OF ANTI-SUBCLASS REAGENTS

| Monoclonal | Subclass | Light chain | Specificity | Source | Reference |
|---|---|---|---|---|---|
| A | IgG1 | κ | Tetanus toxoid | R. F. Tiebout | 12 |
| B | IgG1 | λ | Hepatitis B surface antigen | E. A. M. Stricker | 13 |
| C | IgG1 | κ | Diphteria toxoid | R. A. Insel | 14 |
| D | IgG2 | λ | Hemophilius influenzae, outer membrane lipopolysaccharide | B. R. Brodeur | 15 |
| E | IgG2 | κ[a] | NP[b] | M. S. Neuberger | 16 |
| F | IgG2 | λ | Hemophilus influenzae type B polysaccharide | R. A. Insel | 14 |
| G | IgG3 | λ | Tetanus toxoid | R. F. Tiebout | 17 |
| H | IgG4 | κ[a] | NP[b] | M. S. Neuberger | 16 |
| I | IgA1 | κ | Pneumococcal polysaccharide type 8 | M. Steinitz | 18 |

[a] The antibody is a genetic construct where the heavy chain variable gene and the entire kappa-light chain is of mouse origin.
[b] 4-hydroxy-3-nitrophenacetyl caproic acid.

priate subclasses when used at concentrations aimed at achieving comparable optical densities (HP 6001 and HP 6002), whereas others were highly cross-reactive (SG-11, SH-22, HP 6047, and SK-44) (Table 4). Additional antibodies were only weakly reactive (Bam 09 and Bam 14) and could thus not be relied upon. For simplicity, one satisfactory monoclonal directed against each subclass was selected for further use. These antibodies (Bam 15, Bam 10, Bam 08, and Bam 16) showed a high degree of specificity (Table 5) and, with the possible exception of Bam 10, a high degree of sensitivity (0.1–10 ng/ml, the latter figure referring to Bam 10). Cross-reactivity was tested using a range of concentrations of both the human monoclonal antibodies (0.0001–3 μg/ml) and the sera from donors with heavy chain constant region gene deletions (diluted 1:10–1:1000) (data not shown).

*Subclass Restriction of Antigen-Specific Antibodies*

In an $IgG_1$-deficient child, antibodies against protein antigens were present

TABLE 3
SUBCLASS DEFICIENT SERA USED FOR SPECIFICITY CONTROL OF ANTI-SUBCLASS REAGENTS

| Donor | Ig subclass deficiency | Source | Reference |
|---|---|---|---|
| TAK 3 | G1-A1-G2-G4 | G. Lefranc | 19 |
| EZZ | G1-A1-G2-G4 | G. Lefranc | 20 |
| T 17 | A1-G2-G4 | A. N. Helal | 21 |
| SAF | A1-G2-G4 | A. O. Carbonara | 22 |
| FRO | A1-G2-G4 | A. O. Carbonara | 22 |
| DEM | A1-G2-G4 | A. O. Carbonara | unpublished |
| ROS[a] | G2-G4 | A. O. Carbonara | unpublished |

[a] ROS is heterozygous with different types of deletions on the two haplotypes (A1, G2, G4, E/G2, G4, E).

and restricted to the $IgG_3$ and $IgG_4$ subclasses (Table 6) (data not shown on antibody patterns against tetanus toxoid, diphtheria toxoid, and pneumococcal polysaccharides types 3 and 19F). Antibody levels against most tested carbohydrate antigens were relatively low and these antibodies were mainly of the $IgG_2$ and $IgG_3$ subclasses. Individuals with $IgG_2$ deficiency almost invariably also lack $IgG_4$ and frequently also IgA. In these donors, antibodies against protein antigens displayed the expected IgG subclass pattern (specific $IgG_4$ antibodies were not always present in normal sera). Levels of antibodies against polysaccharides were however markedly reduced. The low amounts observed (Table 6) were restricted to the $IgG_1$ and $IgG_3$ subclasses. Two patients had a selective deficiency of $IgG_2$. In both these sera, marked levels of $IgG_1$ and $IgG_3$ antibodies against polysaccharide antigens were present.

In $IgG_3$- or $IgG_4$-deficient individuals the expected subclass profile of specific antibodies was found with a few minor exceptions. These include the relative, although not total, lack of antibodies of the deficient subclass, the absence of $IgG_2$ antibodies against teichoic acid in BAR 15, and the shift of antibodies against outer membrane protein from the anticipated $IgG_3$ subclass to $IgG_4$ in donor C.A.

### *Antibody Patterns in $IgA_2$ Subclass Deficient Donors*

In both (30–33) individuals tested, $IgA_1$ antibodies against most antigens were present in the serum (data not shown). The first patient (30, 31) also lacks IgG and

TABLE 4
CROSSREACTIVITY PATTERN OF ANTI-SUBCLASS REAGENTS TESTED ON HUMAN MONOCLONAL ANTIBODIES[a]

| Anti-subclass monoclonal | Specificity | A IgG1 | B IgG1 | C IgG1 | D IgG2 | E IgG2 | F IgG2 | G IgG3 | H IgG4 | I IgA1 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | IgG1 | 0.21 | 0.30 | 0.02 | 0.10 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 |
| 2 | IgG1 | 0.82 | 0.52 | 0.35 | 0.00 | 0.01 | 0.01 | 0.01 | 0.00 | 0.00 |
| 3 | IgG1 | 0.31 | 0.61 | 0.57 | 0.04 | 0.04 | 0.04 | 0.18 | 0.02 | 0.03 |
| 4 | IgG1 | 0.14 | 0.64 | 0.06 | 0.03 | 0.24 | 0.03 | 0.10 | 0.36 | 0.02 |
| 5 | IgG1 | 0.18 | 0.40 | 0.17 | 0.02 | 0.04 | 0.01 | 0.05 | 0.28 | 0.50 |
| 6 | IgG1 | 0.64 | 0.73 | 0.08 | 0.00 | 0.00 | 0.00 | 0.05 | 0.00 | 0.02 |
| 7 | IgG2 | 0.00 | 0.00 | 0.03 | 0.15 | 0.21 | 0.71 | 0.02 | 0.00 | 0.00 |
| 8 | IgG2 | 0.07 | 0.11 | 0.05 | 0.07 | 0.05 | 0.07 | 0.11 | 0.05 | 0.00 |
| 9 | IgG2 | 0.19 | 0.35 | 0.06 | 0.40 | 0.89 | 1.11 | 0.03 | 0.18 | 0.02 |
| 10 | IgG2 | 0.04 | 0.06 | 0.02 | 0.72 | 0.81 | 1.14 | 0.05 | 0.01 | 0.24 |
| 11 | IgG2 | 0.39 | 0.37 | 0.09 | 0.91 | 0.49 | 1.20 | 0.65 | 0.20 | 0.00 |
| 12 | IgG3 | 0.00 | 0.00 | 0.01 | 0.02 | 0.00 | 0.02 | 0.90 | 0.00 | 0.00 |
| 13 | IgG3 | 0.07 | 0.03 | 0.04 | 0.02 | 0.19 | 0.00 | 1.02 | 0.21 | 0.03 |
| 14 | IgG3 | 0.00 | 0.00 | 0.03 | 0.02 | 0.02 | 0.01 | 1.13 | 0.00 | 0.00 |
| 15 | IgG3 | 0.00 | 0.00 | 0.04 | 0.00 | 0.02 | 0.01 | 1.23 | 0.00 | 0.00 |
| 16 | IgG4 | 0.00 | 0.00 | 0.03 | 0.00 | 0.05 | 0.02 | 0.03 | 0.90 | 0.00 |
| 17 | IgG4 | 0.28 | 0.00 | 0.00 | 0.00 | 0.25 | 0.01 | 0.28 | 0.97 | 0.10 |
| 18 | IgG4 | 0.00 | 0.01 | NT[b] | 0.00 | NT | NT | 0.01 | NT | 0.00 |

[a] The human monoclonals were tested on their respective antigens (see Table 2) and results are given as net absorbance after 10–20 minutes.

In the experiments given above, A, B, E, G, H and I were tested at a concentration of 0.1 ug/ml and the remaining human monoclonals at a 1:10 (D) or a 1:100 dilution (C and F) of culture supernatant.

[b] Not tested.

TABLE 5
CROSS REACTIVITY PATTERN OF ANTI-SUBCLASS REAGENTS TESTED ON SERA FROM INDIVIDUALS WITH MULTIPLE HEAVY CHAIN CONSTANT REGION GENE DELETIONS[a]

| Anti-subclass monoclonal | Specificity | TAK3 | EZZ | SAF | DEM | ROS |
|---|---|---|---|---|---|---|
| 2 | IgG1 | 0.00 | 0.00 | 1.04 | 0.96 | 0.94 |
| 7 | IgG2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12 | IgG3 | 0.79 | 0.96 | 0.30 | 0.96 | 0.05 |
| 16 | IgG4 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

[a] Net absorbance after 20 minutes. All sera were diluted 1:10 and tested on microplates coated with teichoic acid. For information on the various constant region gene deletions see Table 3.

the subclass distribution pattern of specific antibodies could therefore only be assayed in the second donor. The data suggest a normal utilization of the various IgG subclasses (data not shown).

## DISCUSSION

The immunoglobulin subclass pattern of antibodies against a given antigen is usually quite stable and follows a predictable course during ontogeny. Previous studies in man have suggested a high degree of flexibility in the utilization of various constant region genes since individuals lacking the heavy chain constant region gene for $IgG_2$ will usually still be able to mount an $IgG_1$ or $IgG_3$ restricted antibody response against polysaccharide antigens (8, 9). The VDJ-C joining process does however appear to be strictly controlled and the affinity of these antibodies is, as predicted (8), markedly lower than that of the corresponding antibodies of the $IgG_2$ subclass (5), suggesting the use of different sets of $V_H$ genes.

In order to correctly assess the subclass distribution of antibodies it is imperative that appropriate reagents are used. Thus, monoclonal anti-subclass reagents may vary in their degree of cross-reactivity depending on the method used (11) and the utilization of a sensitive method such as ELISA therefore requires a strict control of the specificity of the mouse antibodies utilized. As outlined in the first paragraph of Results, it is quite clear that only a minority of the monoclonal anti-subclass reagents available are selective enough to be of any value in our system. The novel use of human monoclonal antibodies and sera from patients with different forms of immunoglobulin genes deletions was a prerequisite for the analysis, since purified myeloma proteins often contain considerable amounts of contaminating Ig of the other subclasses and thus are unsuitable for stringent specificity controls (10).

$IgG_1$ deficiency is a rare condition and only five cases have as yet been described in the literature (23, 34). Our data quite clearly imply that lack of $IgG_1$ may be compensated for an apparent increase in the amounts (as judged by absorbance values) of $IgG_3$ and $IgG_4$ antibodies. The influence on the antibody repertoire against polysaccharide antigens is, however, due to the immaturity of both the patient described in this paper and the patient previously tested (34), not altogether assessable. Young children normally display mainly low affinity (5)

TABLE 6

Ig SUBCLASS DISTRIBUTION OF SPECIFIC ANTIBODIES IN IgG SUBCLASS DEFICIENT DONORS[a]

| Donor | Age | Deficiency[b] | References[c] | α-toxin | | | | HI outer membrane protein | | | | Teichoic acid | | | | Pneumococcal polysaccharide 6A | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | IgG1 | IgG2 | IgG3 | IgG4 | IgG1 | IgG2 | IgG3 | IgG4 | IgG1 | IgG2 | IgG3 | IgG4 | IgG1 | IgG2 | IgG3 | IgG4 |
| C.v.B. | 5 | IgG1 | 23, unpublished[d] | 0.00 | 0.01 | 0.19 | 0.05 | 0.00 | 0.03 | 0.79 | 0.19 | 0.00 | 0.00 | 0.03 | 0.01 | 0.00 | 0.08 | 0.05 | 0.02 |
| S.G. | adult | IgG2 | 24, 25 | 1.45 | 0.14 | 0.17 | 0.16 | 1.32 | 0.05 | 0.21 | 0.05 | 0.57 | 0.00 | 0.00 | 0.00 | 0.23 | 0.00 | 0.01 | 0.00 |
| U.L. | adult | IgG2 | 25, 24 | 0.74 | 0.01 | 0.03 | 0.02 | NT[f] | NT | NT | NT | 0.07 | 0.02 | 0.18 | 0.00 | 0.09 | 0.02 | 0.09 | 0.00 |
| S.E. | adult | IgG2 | unpublished | 0.52 | 0.01 | 0.02 | 0.02 | NT | NT | NT | NT | 0.36 | 0.01 | 0.03 | 0.00 | 0.03 | 0.02 | 0.01 | 0.00 |
| BAR 2 | 15 | IgG3 | 26, 27 | 1.02 | 0.01 | 0.05 | 0.79 | 0.62 | 0.02 | 0.01 | 0.04 | 0.02 | 0.08 | 0.00 | 0.00 | 0.05 | 0.03 | 0.02 | 0.02 |
| BAR 6 | adult | IgG3 | 26, 27 | 0.90 | 0.10 | 0.08 | 1.05 | 0.60 | 0.05 | 0.03 | 0.05 | 0.04 | 0.43 | 0.01 | 0.03 | 0.05 | 0.45 | 0.02 | 0.04 |
| BAR 15 | 14 | IgG3 | 26, 27 | 0.78 | 0.06 | 0.07 | 0.47 | 0.36 | 0.01 | 0.01 | 0.02 | 0.40 | 0.00 | 0.00 | 0.00 | 0.34 | 0.52 | 0.04 | 0.07 |
| C.A. | adult | IgG3 | 28, 24 | 0.86 | 0.07 | 0.07 | 0.96 | 0.67 | 0.05 | 0.03 | 0.33 | 0.03 | 0.61 | 0.03 | 0.04 | 0.17 | 0.61 | 0.05 | 0.06 |
| BON | adult | IgG4 | unpublished[e] | 1.45 | 0.08 | 0.21 | 0.00 | 0.25 | 0.04 | 0.01 | 0.01 | 0.40 | 0.46 | 0.01 | 0.01 | 0.13 | 0.46 | 0.01 | 0.00 |
| GRI | adult | IgG4 | unpublished[e] | 1.67 | 0.14 | 0.09 | 0.00 | 0.20 | 0.10 | 0.07 | 0.01 | 0.27 | 0.67 | 0.04 | 0.05 | 0.03 | 0.86 | 0.03 | 0.02 |
| PRE | adult | IgG4 | unpublished[e] | 1.90 | 0.07 | 0.09 | 0.01 | 0.55 | 0.19 | 0.17 | 0.01 | 0.29 | 0.78 | 0.00 | 0.04 | 0.20 | 0.91 | 0.02 | 0.01 |
| TOM | adult | IgG4 | unpublished[e] | 1.96 | 0.02 | 0.08 | 0.00 | 0.54 | 0.10 | 0.36 | 0.01 | 0.34 | 0.86 | 0.01 | 0.08 | 0.02 | 0.83 | 0.04 | 0.02 |

[a] Results are given as net absorbance after 20 minutes.

[b] Below detection level of the respective subclass (IgG1; 0.1 G/L, IgG2; 0.2 G/l, IgG3; 0.02 G/L and IgG4 0.01 G/L).

[c] Given as original reference and in addition the reference for the data on the presence of the corresponding heavy chain constant region gene on Southern blots.

[d] B. M. J. Zegers.

[e] M. DeMarchi and A. O. Carbonara.

[f] Not tested.

$IgG_1$ antibodies against carbohydrate antigens (3, 4) with a gradual shift toward $IgG_2$ as the immune system matures. The rather low levels of specific $IgG_2$ antibodies in both children may therefore be quite a normal finding. It is evident, however, that the lack of $IgG_1$ antibodies against polysaccharides is not, to any major degree, compensated for by marked increases in specific $IgG_3$ or $IgG_4$ levels. Our findings also strengthen the idea that although an ordered $IgG_1$, $IgA_1$, $IgG_4$ switch pattern of antibodies against antigens such as alphatoxin (35) is usually seen, their appearance is not necessarily sequential and $IgG_4$ responses may indeed be high in spite of $IgG_1$ deficiency.

The pattern of antibodies against polysaccharide antigens in the sera from $IgG_2$-deficient individuals turned out to be rather complex. Previous studies have suggested that most anti-carbohydrate antibodies are absent or present in markedly reduced amounts in these individuals (36–39). Exceptions to the rule have however previously been noted (29) and were again recognized in this study. Although the factors which determine the levels of specific antibodies are unclear to us at the moment, it may seem as though donors with a *selective* lack of $IgG_2$ (usually the deficiency is combined with a lack of $IgG_4$ and IgA) may have retained levels of anti-polysaccharide antibodies. It is apparent though, that our initial subdivision of individuals with or without deletion of the $\gamma_2$ constant region gene as a cause of $IgG_2$ deficiency (based on the former having normal total levels of anti-polysaccharide antibodies restricted to the $IgG_1$ and/or $IgG_3$ subclasses) may not be entirely correct and that the regulation of responses against polysaccharides is more complex than previously anticipated. Further studies on the $V_H$ usage may therefore be required to clarify this issue.

A relative lack of $IgG_3$ appears to be rather common (28), whereas a total deficiency is seldom observed (26, 28). The former disorder is suggested to be allotype linked in Swedes (40) but these particular Gm markers have not been found in the latter cases (which are of Lebanese origin). The antibody pattern in these individuals does not seem to be markedly perturbed, with a few as yet unexplained minor exceptions. These data thus support our previous findings on the relatively normal anti-viral antibody pattern in $IgG_3$-deficient individuals (41). It is noteworthy that specific $IgG_3$ antibodies are in fact demonstrated, suggesting the presence or minute levels of the "lacking" subclass (detection level being on the order of 0.1 ng/ml), implying a regulatory nature of the defect, as suggested also by the presence of the $C_\gamma 3$ gene in the BAR family (27).

It is becoming increasingly clear that protein antigens induce $IgG_3$ *or* $IgG_4$ in addition to the dominating subclass $IgG_1$ (for references see (42)). Outer membrane protein from *H. influenzae* is an antigen of the former category and the high levels of $IgG_4$ antibodies in an $IgG_3$-deficient individual is therefore somewhat surprising but is again indicative of the flexibility of the immune system.

$IgG_4$ or $IgA_2$ deficiency, disorders affecting the antibody subclasses with the lowest mean concentrations, do not to any major degree perturb the predicted IgG subclass responses. In view of the large fraction of individuals normally found to be lacking $IgG_4$ (<0.01 g/liter) this is scarsely surprising. Since the $IgG_4$ subclass also contains antibodies with a rather low mean affinity (5), its relevance in biological systems is questionable.

Taken together, our data indicate a high degree of complexity in the regulation of the immunoglobulin subclass pattern of specific antibodies, and in various forms of subclass deficiency (on what is believed to be a regulatory basis) multiple guidelines which govern the normal immune system are overruled and compensatory mechanisms evidently emerge in response to environmental pressure.

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