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Immunoglobulin subclass distribution of human anti-carbohydrate antibodies: aberrant pattern in IgA-deficient donors

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Summary. The IgG and IgA subclass distribution of anti-carbohydrate antibodies in normal and immunodeficient donors was investigated. In normal donors, the specific anti-dextran antibodies were mainly of the IgG2 and IgA2 subclass, although substantial amounts of antibodies could also be of the IgG1 subclass. In children, IgG1 was the predominant subclass expressed. An aberrant IgG subclass distribution pattern of specific antibodies occurred in some IgA-deficient donors, with preferential expression of IgG1 and IgG3 anti-dextran antibodies.

INTRODUCTION

Human IgG is composed of four structurally distinct subclasses with different biological properties (for review see Shakib & Stanworth, 1980a). Serum levels of each subclass appear to be independently regulated during ontogeny: adult levels of IgG1 and IgG3 are reached at an early age, whereas IgG2 and IgG4 may not reach adult levels until adolescence (Oxelius, 1979). Human IgA is divided into two antigenically different subclasses, IgA1 and IgA2, but the biological properties of these two subclasses is still largely unknown.

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Previous studies in man have suggested a marked subclass restriction of specific antibodies where protein antigens mainly gave rise to IgG1 and, to a lesser degree, IgG3 and IgG4 antibodies (Shakib & Stanworth, 1980b; Hammarström et al. 1984a; Hammarström, Persson & Smith, 1984c). Anti-carbohydrate antibodies, on the other hand, are usually restricted to the IgG2 subclass in adult donors (for review see Hammarström & Smith, 1983). Similar subclass restriction of immune responses to polysaccharide antigens have previously been described in experimental animal systems (Perlmutter *et al.*, 1978; der Balian *et al.*, 1980). In children, however, antibodies to carbohydrate antigens such as pneumococcal capsular polysaccharides and teichoic acid from *Staphylococcus aureus* are mainly of the IgG1 subclass (Freijd *et al.*, 1984; Hammarström *et al.*, 1984a).

In this paper, the normal IgG and IgA subclass distribution of anti-carbohydrate antibodies is described. In IgA-deficient donors, an aberrant subclass distribution of anti-carbohydrate antibodies was seen, suggesting a regulatory defect more fundamental than a mere lack of IgA.

MATERIALS AND METHODS

Sera

Serum was collected from normal healthy adults and children, and stored at −70° until used. Serum

samples from 369 adults and 23 children (below 3 years of age) which were submitted to our department for screening of autoantibodies (anti-nuclear antibodies) were also tested for anti-dextran antibodies. Some of the high-titred anti-dextran antisera (used for reference purposes), were gifts from Dr W. Richter (Pharmacia, Uppsala, Sweden). Selected sera from patients with known immunoglobulin class or subclass deficiencies (serum levels of the respective class or subclass $<0{\cdot}02$ g/l) and their families were also collected.

Antigens

Dextran B512 (α(1–6) linked glucosyl residues), 500,000 MW, was purchased from Pharmacia Fine Chemicals. Dextran B1355 (α(1–6) and α (1–3) linked glycosyl residues) was a gift from Dr C. Moreno (The Wellcome Research Laboratories, Beckenham, Kent, U.K.). Pneumococcal capsular polysaccharide serotypes 6A, 19F and 23 was obtained from the R. I. T. Division of Smith, French & Kline, Rixensart, Belgium. Teichoic acid from *S. aureus* was a gift from Dr R. Möllby (State Bacteriological Laboratories, Stockholm, Sweden).

Immunoglobulin quantification

Immunoglobulin class levels were measured in commercial immunodiffusion plates (Behringwerke, Marburg, West Germany). Immunoglobulin subclass levels were determined in immunodiffusion assays utilizing polyclonal rabbit antisera (kindly performed by Dr V. Oxelius, Dept. of Pediatrics, Lunds Hospital, Sweden) and, in the case of IgG2, re-checked using a monoclonal anti-IgG2 antibody in an immunodiffusion assay according to the method supplied by the manufacturer (Seward Laboratories, London, U.K.) (clone GOM1-BAM 10).

ELISA

All antigens were coated on polystyrene microtitre plates at a concentration of 10 μg/ml. For screening of specific antibodies, sera were diluted 1:100 in phosphate-buffered saline (containing 0·05% Tween 20) and incubated overnight on antigen-coated plates. Thereafter, a 1:1000 dilution of alkaline-phosphatase-conjugated rabbit anti-human IgG (γ-chain specific) or IgA (α-chain specific) (DAKO Immunoglobulins, Copenhagen, Denmark) was added. After an additional 4 hr of incubation, the substrate was added after repeated washings and absorbance measured after 10 min. An absorbance value exceeding five times the background levels was considered a positive sample.

The subclass distribution of specific antibodies was determined as described previously in detail (Hammarström *et al.*, 1984a, b, c). Briefly, after incubation of the serum samples (diluted 1:100 in phosphate-buffered saline with 0·05% Tween 20) overnight on antigen-coated plates, commercially available monoclonal antibodies against the various human IgG (Seward Laboratories, London, U.K.) or IgA (Becton Dickinson, Sunnyvale, CA)-subclasses, purified on an ion-exchange column (FPLC) (Pharmacia Fine Chemicals) were added in optimal concentrations (anti-IgG1, BAM 15 diluted 1:3200; anti-IgG2, clones BAM 10 and BAM 14 diluted 1:400, anti-IgG3, clone Bam 08, diluted 1:8000; anti-IgG4, clone BAM 16 diluted 1:800, anti-IgA1, code 5100 diluted 1:150; and anti-IgA2, code 5110 diluted 1:150). After 4 hr of incubation at room temperature, the plates were washed and a 1:2000 dilution of rabbit anti-mouse Ig (DAKO Immunoglobulins) was added. After an additional 4-hr incubation, the plates were washed and a 1:1000 dilution of alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma Chemical Company, St Louis, MO.) was added and the plates were again incubated overnight. After additional washes, disodium *p*-nitrophenyl phosphate, 1 mg/ml (Sigma Chemical Company) in 10% diethanolamine buffer was added and the plates were incubated for 20–30 min. Absorbance was measured at 405 nm using a Titertek multiscan (Elflab OY, Helsinki, Finland). Concentrations were estimated from a standard of pooled myeloma of the respective subclass run in parallel ELISA, as described previously in detail (Persson, Hammarström & Smith, 1985). The immunoglobulin concentrations given in Tables 1–4 have been corrected for the efficiency of coating of the myeloma proteins. The myeloma proteins were obtained from Dr F. Skvaril (WHO, Bern, Switzerland).

RESULTS

Subclass distribution of anti-carbohydrate antibodies in normal donors

In healthy adult blood donors, low levels of IgG1, IgG2 and IgA1 anti-dextran B512 (Table 1) and B1355 (data not shown) antibodies were normally found. When sera from 369 adults, originally submitted to the laboratory for determination of autoantibodies, were

Table 1. Subclass distribution of anti-dextran B512 antibodies*

Antibody level	n‡	Specific Ig (mg/l)†					
		IgG1	IgG2	IgG3	IgG4	IgA1	IgA2
Normal	20	18 (8–27)	16 (0–54)	1 (0–2)	2 (0–5)	30 (28–42)	13 (0–49)
High§	20	53 (25–70)	36 (10–63)	0 (0–1)	2 (0–5)	47 (35–61)	73 (52–145)

* Antibody levels were measured in sera from normal adult blood donors and from high titred (anti-dextran) sera obtained from the screening of the samples submitted for measurement of anti-nuclear antibodies.
† Results are expressed as arithmetic mean (range).
‡ Number of donors.
§ For definition, see Materials and Methods.

screened for high levels of anti-dextran antibodies, 20 sera, positive for IgG and/or IgA, were found. These sera, where no relation to ANA titres was seen (data not shown), contained increased amounts of IgG1, IgG2 and IgA2 anti-dextran antibodies (Table 1). In sera from 23 children (below 3 years of age), only low levels of antibodies, restricted to the IgG1 subclass, were found. In three IgG2-deficient children studied (below 3 years of age), no specific anti-dextran antibodies of any subclass were found.

Antibodies against *S. aureus* teichoic acid and *Staphylococcus pneumoniae* capsular polysaccharides have previously been shown to be mainly of the IgG2 subclass in normal adult donors (Freijd *et al.*, 1984; Hammarström *et al.*, 1984a, b). However, no clear preference for any of the IgA subclasses could be seen (Table 2). When the normal dominance of IgA1 in serum is taken into account, proportional amounts of IgA1 and IgA2 anti-teichoic acid antibodies were found both in donors with low IgA anti-teichoic acid antibody levels (Table 2) and in donors with increased levels of IgA anti-teichoic acid antibodies (mean levels in 12 donors: IgA1 83·2 mg/l, IgA2 29·2 mg/l).

Table 2. Subclass distribution of anti-carbohydrate antibodies*

Antigen	n‡	Specific Ig (mg/l)†					
		IgG1	IgG2	IgG3	IgG4	IgA1	IgA2
Teichoic acid§	25	52	62	3	16	25	16
PPS¶ type 6A	22	2	20	3	1	20	7
PPS type 19F	22	3	17	4	1	39	7
PPS type 23	10	2	15	2	1	23	8

* Normal adult blood donors.
† Results are expressed as arithmetic mean.
‡ Number of donors.
§ Polyribitol phosphate teichoic acid (type Aβ) from *Staphylococcus aureus*.
¶ PPS, pneumococcal capsular polysaccharide.

Subclass distribution of anti-carbohydrate antibodies in IgA-deficient donors

Eighty adult donors with selective IgA deficiency were tested for anti-dextran B512 antibodies; 14 IgG-positive samples were found. In six of these donors, an unusual IgG subclass distribution pattern of the anti-dextran B512 antibodies was seen. In these sera, a marked increase in the amounts of IgG1 and IgG3 anti-dextran B512 antibodies was seen (Table 3), whereas IgG2 anti-dextran B512 antibodies were virtually absent, in spite of normal total IgG2 serum

Table 3. Subclass distribution of anti-dextran B512 in selected IgA-deficient donors

Donor	Serum Ig (g/l)*				Specific Ig (mg/l)			
	IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4
1	13·26	6·66	0·99	0·74	24	1	50	0†
2	6·19	2·33	0·35	<0·01	80	2	0	0
3	10·88	3·66	1·24	<0·01	21	0	30	0
4	13·26	6·86	0·91	<0·01	40	1	6	0
5	6·12	2·43	1·53	0·16	48	1	0	0
6	8·16	3·33	1·81	<0·01	29	0	20	0

* Normal adult levels IgG1, 7·55 (median) (4·22–12·92) (range), IgG2, 3·80 (1·17–7·47); IgG3, 0·73 (0·41–1·29); IgG4, 0·55 (<0·01–2·91) (Oxelius, 1979).
† Less than 1 mg/l.

Table 4. Subclass distribution of anti-pneumococcal capsular polysaccharide in selected IgA-deficient donors

	PPS* 6A				PPS 19F				PPS 23			
Donor†	IgG1‡	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4
1	0§	25	0	0	11	50	0	0	10	20	0	0
2	0	9	0	0	5	3	0	0	12	3	0	0
3	31	29	0	0	24	15	0	0	37	20	0	0
4	33	24	0	0	19	7	0	0	36	19	1	0
5	0	29	0	0	7	12	0	0	6	27	0	0
6	0	31	3	0	7	12	6	0	9	26	14	0

* Pneumococcal capsular polysaccharide.
† Same donors as in Table 3.
‡ Specific antibodies (mg/l).
§ Less than 1 mg/l.

levels. However, these donors all showed a normal subclass distribution of anti-teichoic acid antibodies (data not shown) and, with a few exceptions, the subclass distribution of anti-pneumococcal capsular polysaccharides was also normal (Table 4). However, IgG1 was slightly more predominant than expected in Donors 3 and 4, and IgG3 in Donor 6. Two HLA-identical normal adult sibs of one of the IgA-deficient donors with aberrant subclass distribution of anti-pneumococcal capsular polysaccharide antibodies were found to have a normal pattern with a predominance of IgG2 antibodies (data not shown).

DISCUSSION

Antibodies against dextran B512 (α(1–6)) were originally described as being mainly or even exclusively of the IgG2 subclass in adult human donors (Yount *et al.*, 1968). However, in certain individuals, quite substantial amounts were of the IgG1 subclass (Yount *et al.*, 1968), and anti-dextran antibodies may, in fact, be found in all IgG subclasses (Kraft *et al.*, 1982), a finding confirmed in this paper. Similar observations have previously been published on mice (Ivars *et al.*, 1983; Moreno & Esdaile, 1983). In children, who infrequently express anti-dextran antibodies (Hattevig *et al.*, 1983), IgG antibodies were found to be exclusively of the IgG1 subclass. This pattern has previously also been found in the response to other polysaccharides in children (Hammarström *et al.*, 1984a, b; Freijd *et al.*, 1984).

Knowledge of the subclass distribution of antigen-specific IgA antibodies is still very limited. In a previous series of experiments antibodies against *S. aureus* alpha-toxin, a protein antigen, were shown to be of the IgA1 subclass (Hammarström *et al.*, 1984c). In this paper, antibodies against dextran were found to be mainly of the IgA2 subclass. However, certain protein antigens such as cytomegalovirus nuclear antigen (Linde *et al.*, 1983), and selected carbohydrate antigens such as teichoic acid and possibly also capsular polysaccharide from *S. pneumoniae* (Table 2), may induce IgA antibodies of both subclasses. Previous studies have suggested a distinct subclass distribution pattern of anti-protein antibodies where specific IgG1, IgA1 and IgG4 antibodies develop sequentially (Hammarström *et al.*, 1984c). The simultaneous presence of IgG1, IgG2, IgG4, IgA1 and IgA2 anti-teichoic acid antibodies may suggest a mixed response against two separate epitopes on the same antigen triggering different B-cell clones. A similar situation where antibodies against different epitopes on the same antigen are of differing idiotypes, light chain class or even heavy chain subclass has previously been described both in man (Yount *et al.*, 1968) and in experimental animals (Bona *et al.*, 1979; Sarvas *et al.*, 1983a). It is possible that the two patterns, IgG1-IgA1-IgG4 and IgG2-IgA2, reflect the potential of two different subsets of B cells, responding to either T-cell dependent or T-cell independent stimuli. This is supported by *in vitro* studies on mitogen-induced polyclonal responses where pokeweed mitogen, a T-cell dependent activator, will mainly induce IgG1 antibodies; whereas lipopolysaccharide, a T-cell independent activator, will stimulate IgG2-producing cells

(Andersson *et al.*, 1981; Morell *et al.*, 1981; Cooper *et al.*, 1983; Walker, Johnsson & MacLennan, 1983). In experimental animal systems, two separate subsets of anti-dextran antibody-producing cells exist, which appear to express distinct repertoires as reflected by differing antibody affinity (Sarvas *et al.*, 1983b) and light chain class (Ward, Kearney & Köhler, 1981) of the secreted antibodies.

The amounts of specific antibodies in the tested serum samples were determined by comparing the absorbance values with those from a microplate coated with various amounts of pooled myeloma proteins of the respective subclasses (Persson *et al.*, 1985). Since the myeloma proteins differ with regard to the extent to which they bind to plastic, values have been corrected for coating efficiency. Due to the different affinity of the monoclonal anti-subclass regents used, resulting in a varying lower limit detection level for the different subclasses (Persson *et al.*, 1985), low levels of specific antibodies may remain undetected. In addition, low affinity antibodies may easily be removed during the extensive washing procedures employed. Furthermore, high affinity antibodies may interfere with the binding of low affinity antibodies and possibly also displace them from the antigen, thus resulting in an underestimation of bound antibody. The test must, therefore, be regarded as semiquantitative, at best (Seppälä *et al.*, 1984). However, since the main findings in this paper relate to the differing patterns of subclass reactivity rather than differencs in absolute amounts of specific antibodies, we feel that the use of the present method is justified.

'Selective' IgA deficiency is frequently associated with a lack of IgG2 (Oxelius *et al.*, 1981). However, even in IgA-deficient donors with normal serum levels of IgG2, specific anti-dextran antibodies of the IgG2 subclass appear to be missing in selected donors (Table 3), even though other IgG2 anti-carbohydrate antibodies are present (Table 4). In mice, a sequential maturation of various anti-carbohydrate antibodies is suggested on the basis of previously published work (Howard & Hale, 1976; Klinman *et al.*, 1976; Fernandez & Möller, 1978; Bona *et al.*, 1979; Rivier, Trefts & Kagnoff, 1983), and lack of maturation of the appropriate IgG2 anti-dextran B512 antibodies in certain IgA-deficient individuals therefore appears plausible. Lack of other specific antibodies in 'selective' IgA deficiency has recently been reported (Cooper *et al.*, 1983; de Graeff *et al.*, 1983; French & Harrison, 1984). Taken together, these data may suggest a maturational defect in IgA-deficient donors more fundamental than a mere lack of IgA.

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