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QUANTIFICATION OF IgG SUBCLASSES IN SERA OF NORMAL ADULTS AND HEALTHY CHILDREN BETWEEN 4 AND 12 YEARS OF AGE

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SUMMARY

The concentration of the four subclasses of IgG was determined in sera of normal adults and healthy children between 4 and 12 years of age, using the radial immunodiffusion technique.

A relation between the concentration of IgG subclasses and Gm type was studied in adults. No influence of Gm type on IgG1 concentration could be shown, except that the group of Gm(fb) individuals had a higher level than the others. The mean concentration of IgG2 was higher in sera positive for Gm(n) than in those lacking this genetic marker. High IgG3 concentrations corresponded to the presence of Gm(b). No clearcut evidence was obtained for a relation between IgG4 concentration and Gm factors, although in general Gm(n) positive individuals had higher and Gm (zag) positive individuals lower concentrations of this subclass in their serum.

Quantification of IgG subclasses in sera from healthy children of different ages revealed that the amount of IgG2 rises slowly with age, having not yet reached the adult level at the age of 12 years. This also holds for IgG4, although in a lesser degree. No significant differences from the adult level were found for the concentrations of IgG1 and IgG3.

INTRODUCTION

Quantification of the level of immunoglobulins in serum is one of the parameters used to evaluate the immunological competence of an individual. To be able to draw conclusions from the value of a certain immunoglobulin class or subclass found in the serum of a particular patient, the normal range has to be known.

The concentration of the different immunoglobulin classes in serum varies widely. It is further known that young children have lower immunoglobulin levels than adults, and

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that the adult level of the various classes is reached at different ages (Stoop *et al.*, 1969; Allansmith *et al.*, 1968; Uffelman, Engelhard & Jolliff, 1970; Collins-Williams *et al.*, 1967; Geny *et al.*, 1974; Berg & Johansson, 1969; Stiehm & Fudenberg, 1966; Zegers *et al.*, 1975).

Concerning the levels of IgG subclasses only a few studies have been performed so far. Morell *et al.* (1972b) described the results of the determination of all four subclasses in 108 male adults. Serum concentrations of IgG3 in adults were determined by Yount, Kunkel & Litwin (1967) and those of IgG2 by van der Giessen *et al.* (1973). From these studies it became clear that a relation exists between levels of certain subclasses and the presence of particular Gm markers.

The development of the serum concentration of the various IgG subclasses in normal infants from birth to 2 years of age was reported by Morell *et al.* (1972a).

The purpose of the present investigation was to obtain more information about the relation between serum levels of IgG subclasses and Gm type and to examine whether in healthy children this level is related to age.

MATERIALS AND METHODS

Sera

The series of adult sera consisted of 107 samples obtained from laboratory personnel whose Gm phenotype was known, fifty-four of these samples had been used in a previous study on the relationship between concentration of IgG2 in serum and the presence of the Gm(n) marker (van der Giessen *et al.*, 1973). Gm typing was carried out according to van Loghem and de Lange (1970). In addition 141 serum samples from healthy children ranging in age from 4 up to and including 12 years, divided into nine 1 year age groups, were studied. Each of the nine age groups comprised eight girls and eight boys, but for three groups which lacked one sample. Sixteen sera from eight women and eight men were included as control in the latter series. All samples of the second collection of sera had previously been used in a study on the quantification of the various immunoglobulin classes (Stoop *et al.*, 1969; Zegers *et al.*, 1975).

Antisera

Antisera specific for the heavy chains of the four human IgG subclasses were obtained as described in detail by van der Giessen, de Lange and van der Lee (1974). The following antisera were used for quantification of the subclasses: two rabbit anti-γ1, two monkey anti-γ2, one rabbit anti-γ3 and one monkey anti-γ4.

Anti-IgG was produced in rabbit by immunization with pool IgG obtained from Cohn-fraction II by anion-exchange chromatography. The immunization schedule was the same as that used for the anti-subclass antisera. Anti-L chain antibodies were removed by absorption with purified IgM.

Quantification of IgG subclasses

IgG subclass concentrations were determined by radial immunodiffusion according to Mancini, Carbonara & Heremans (1965), with a slight modification, and the results expressed in percentages of a standard serum containing 115 i.u. of IgG per millilitre (van der Giessen *et al.*, 1973).

In an attempt to determine the absolute quantities of the four subclasses of IgG in the standard serum, twelve IgG1, twelve IgG2, six IgG3, and nine IgG4 myeloma proteins,

purified as described before (van der Giessen *et al.*, 1973), were used as calibrators. In addition pool IgG and several isolated myeloma proteins of the four subclasses, chosen at random, were used to determine the total amount of IgG. Protein concentrations were calculated from the optical density at 280 nm using an extinction coefficient of 14 for a 1% IgG solution.

Statistical analysis

The Wilcoxon test was used, whenever statistical analyses were performed.

RESULTS

Standard sera

The absolute quantity of each of the different IgG subclasses in the standard serum was calculated taking the mean of the values obtained from the standard curves of single paraproteins of the respective subclasses. Standard deviations did not exceed 10% of the mean for all subclasses. The total amount of IgG was determined directly and calculated by summation of the obtained quantities of the four subclasses. The two values thus obtained agreed very well with the value expressed in i.u. (see Table 1). IgG subclass concentrations of the W.H.O. standard serum 67/97 were determined by comparison with our standard serum. The results show that the levels of IgG1 and IgG2 are rather low (Table 1).

TABLE 1. Concentrations of IgG and IgG subclasses (in mg/ml) in standard serum containing 115 i.u. of IgG and in W.H.O. standard serum 67/97 containing 96 i.u. of IgG

	IgG1	IgG2	IgG3	IGg4	IgG calculated	IgG determined
CLB standard	5·9	3·0	0·6	0·5	10·0	10·2
W.H.O. standard	4·8	2·2	0·55	0·55	8·1	n.d.

n.d. = Not determined.

Sera of adults

The results of the quantification of IgG subclass concentrations in the serum of normal adults are presented in Tables 2 and 3. The concentrations of IgG4 varied widely, confirming the findings of Morell *et al.* (1972b).

The frequency distributions of the concentrations of all four subclasses were skewed to the right. However, after log transformation of the values the frequency distributions did not differ significantly from a normal one, as was shown by the χ^2 test for goodness of fit.

Table 2 shows that the serum concentration of IgG subclasses in male and female adults differed slightly. The largest differences were found in the concentrations of IgG2 and IgG4: while women had a higher mean concentration of IgG2, men showed a higher level of IgG4. When, however, statistical analysis was carried out, using the Wilcoxon test, these differences were not found to be statistically significant.

TABLE 2. IgG subclass concentrations in sera from normal male and female adults, expressed in percentages of the standard serum

Number	IgG1 mean ± s.d. (range)	IgG2 mean ± s.d. (range)	IgG3 mean ± s.d. (range)	IgG4 mean ± s.d. (range)
55 ♂	118 ± 21 (79 – 164)	120 ± 38 (63 – 128)	115 ± 46 (32 – 238)	127 ± 85 (19 – 407)
52 ♀	123 ± 36 (78 – 255)	132 ± 38 (69 – 230)	113 ± 59 (29 – 244)	108 ± 69 (11 – 325)
107 (♂ + ♀)	120 ± 29 (78 – 255)	126 ± 38 (63 – 230)	114 ± 52 (29 – 244)	118 ± 77 (11 – 407)
Mean (mg/ml)	7·1	3·8	0·65	0·6

TABLE 3. IgG subclass concentrations in sera from normal adults of known Gm phenotype, expressed in percentages of the standard serum

Probable Gm genotype	Number	IgG1 mean ± s.d. (range)	IgG2 mean ± s.d. (range)	IgG3 mean ± s.d. (range)	IgG4 mean ± s.d. (range)
fnb/fnb	19	118 ± 27 (81 – 181)	152 ± 42 (77 – 218)	142 ± 50 (79 – 238)	113 ± 66 (19 – 300)
fnb/fb	27	118 ± 24 (79 – 170)	130 ± 34 (79 – 201)	126 ± 41 (46 – 204)	145 ± 85 (30 – 359)
fb/fb	11	148 ± 50 (103 – 255)	121 ± 29 (70 – 169)	135 ± 61 (81 – 223)	110 ± 64 (39 – 253)
fnb/ag	25	118 ± 21 (79 – 155)	127 ± 41 (77 – 230)	106 ± 42 (47 – 244)	122 ± 91 (16 – 407)
fb/ag	12	115 ± 29 (78 – 164)	111 ± 31 (72 – 161)	105 ± 60 (40 – 237)	94 ± 74 (14 – 247)
ag/ag	13	120 ± 27 (84 – 169)	93 ± 20 (63 – 133)	49 ± 18 (29 – 93)	90 ± 52 (11 – 168)
All sera	107	120 ± 29 (78 – 255)	126 ± 38 (63 – 230)	114 ± 52 (29 – 244)	118 ± 77 (11– 407)

In Table 3 it is shown that there is a relation between the concentrations of IgG subclasses and certain Gm markers or haplotypes. The results concerning IgG2 confirm our earlier finding of a connection between high IgG2 levels and presence of Gm(n). The difference in serum concentrations found in n+ individuals, both homozygous and heterozygous, and n− individuals was statistically highly significant: $P<0{\cdot}001$. With respect to IgG3 it was found that an extreme low level of IgG3 corresponded with absence of Gm(b). Here again the difference in serum concentrations found in b+ and b− individuals was highly significant: $P<10^{-5}$. The results of IgG4 quantification were somewhat ambiguous. While most of the low values were found in the groups of individuals having the Gm(zag) haplotype,

TABLE 4. IgG subclass concentrations in sera from 141 children between 4 and 12 years of age and in sixteen adult sera, expressed in percentages of the standard serum

Age (years)	Boys				Girls			
	IgG1 mean ± s.d. (range)	IgG2 mean ± s.d. (range)	IgG3 mean ± s.d. (range)	IgG4 mean ± s.d. (range)	IgG1 mean ± s.d. (range)	IgG2 mean ± s.d. (range)	IgG3 mean ± s.d. (range)	IgG4 mean ± s.d. (range)
4	108 ± 29 (67 – 145)	50 ± 7 (43 – 62)	80 ± 23 (43 – 117)	87 ± 55 (20 – 175)	111 ± 48 (59 – 197)	40 ± 14 (23 – 68)	85 ± 69 (26 – 234)	56 ± 39 (< 5 – 127)
5	106 ± 26 (74 – 149)	47 ± 15 (30 – 77)	66 ± 33 (19 – 126)	72 ± 62 (30 – 195)	105 ± 29 (57 – 143)	47 ± 15 (32 – 70)	95 ± 38 (32 – 139)	52 ± 39 (16 – 115)
6	117 ± 18 (95 – 145)	48 ± 17 (20 – 74)	94 ± 36 (52 – 148)	106 ± 87 (< 5 – 245)	121 ± 36 (71 – 162)	45 ± 23 (20 – 92)	87 ± 27 (45 – 115)	45 ± 41 (< 5 – 125)
7	113 ± 48 (63 – 218)	51 ± 20 (22 – 84)	85 ± 45 (19 – 176)	92 ± 65 (< 5 – 157)	127 ± 24 (97 – 160)	61 ± 19 (37 – 95)	91 ± 43 (26 – 178)	102 ± 61 (34 – 240)
8	108 ± 20 (86 – 147)	54 ± 12 (39 – 77)	69 ± 22 (41 – 104)	72 ± 79 (< 5 – 245)	120 ± 26 (80 – 162)	75 ± 36 (39 – 141)	117 ± 49 (70 – 220)	90 ± 70 (19 – 210)
9	96 ± 16 (76 – 113)	61 ± 17 (30 – 87)	94 ± 29 (47 – 133)	71 ± 55 (< 5 – 152)	150 ± 25 (120 – 189)	79 ± 15 (61 – 101)	114 ± 74 (41 – 252)	105 ± 105 (44 – 335)
10	142 ± 67 (71 – 277)	65 ± 22 (28 – 99)	82 ± 32 (36 – 151)	94 ± 76 (< 5 – 220)	113 ± 53 (92 – 132)	62 ± 24 (28 – 101)	82 ± 41 (18 – 126)	119 ± 107 (< 5 – 345)
11	111 ± 22 (84 – 160)	86 ± 20 (59 – 122)	104 ± 47 (67 – 212)	79 ± 41 (31 – 157)	147 ± 32 (101 – 185)	70 ± 14 (50 – 92)	98 ± 31 (67 – 151)	67 ± 50 (22 – 177)
12	103 ± 30 (59 – 149)	70 ± 25 (28 – 102)	107 ± 41 (56 – 191)	135 ± 116 (26 – 335)	107 ± 23 (69 – 139)	75 ± 24 (42 – 109)	122 ± 42 (63 – 202)	135 ± 82 (21 – 280)
Adults	109 ± 22 (80 – 149)	100 ± 24 (50 – 127)	102 ± 44 (37 – 162)	181 ± 110 (24 – 315)	113 ± 16 (88 – 139)	123 ± 30 (94 – 189)	117 ± 26 (85 – 155)	114 ± 101 (37 – 295)

most of the high values were found in the groups possessing Gm(n). However, neither the difference between groups positive and negative for the haplotype Gm(zag) nor that between groups positive and negative for Gm(n) were statistically significant, the *P* values being 0·07 and 0·06 respectively. As to IgG1 no differences in serum levels were observed, but for the group of the homozygous Gm(fb) individuals. Comparison of the levels found in this group with those found in the groups of heterozygous Gm(zafgb) and the homozygous Gm(zag) individuals gave differences which were not statistically significant, the *P* values being 0·06 and 0·07 respectively. Compared with the remaining groups the differences were statistically significant: *P* values between 0·02 and 0·03.

Sera of children

The results of subclass quantifications in the different age groups, boys and girls separately, are given in Table 4. The *P* values of the differences between the levels of each age group and the adult level are shown in Table 5. No systematic influence of sex on the concentrations of the different subclasses could be shown. With respect to age, however, levels differing from the adult level were found in several cases. In these cases the concentrations of subclasses were always lower in children than in adults, except two groups of girls, i.e. the 9- and 11-year-old groups, which showed levels of IgG1 that were significantly higher than that found in female adults. The greatest difference from the adult level was found for the IgG2 serum concentration which was significantly lower in all the different age groups except one (11-year-old boys). The concentration slowly increased with age, having not yet reached the adult level at the age of 12 years. IgG3 subclass concentrations found in some groups were slightly lower than that in adults. With regard to IgG4 the following was found: eleven out of the 141 children (7·8%), mainly from the younger age groups, had undetectable IgG4 (< 5% of the standard serum) and the mean concentrations in children in general were lower than in adults. However, these differences were not statistically significant in most cases. Those that were, were found almost exclusively in the groups of boys. This might be attributed to the fact that the mean concentration in the group of male controls was rather high. As in adults the levels of IgG4 in children varied widely.

TABLE 5. *P* values of the differences between IgG subclass concentrations in the separate age groups and those in the adults

Age (years)	Boys				Girls			
	IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4
4	—	< 0·01	—	0·08	—	< 0·01	0·01	—
5	—	< 0·01	0·08	0·02	—	< 0·01	—	0·08
6	—	< 0·01	—	0·06	—	< 0·01	0·05	—
7	—	< 0·01	—	0·02	—	< 0·01	0·05	—
8	—	< 0·01	0·06	0·02	—	< 0·01	—	—
9	0·08	< 0·01	—	0·02	< 0·01*	< 0·01	—	—
10	—	0·01	—	—	—	< 0·01	0·06	—
11	—	0·10	—	0·03	0·02*	< 0·01	—	—
12	—	0·02	—	—	—	< 0·01	—	—

* Concentration in children higher than in adults.

Investigators who are interested in the log-normal ranges can be provided with data on request.

DISCUSSION

The calculated mean values of the different IgG subclasses in adults correspond well with those obtained by Morell *et al.* (1972b), except for IgG4, the value of which was higher in our study.

The relation found between IgG subclass serum concentrations and Gm type suggests a genetical influence on the synthetic rate of IgG subclasses. As to IgG1 no such influence could be demonstrated. This is in contrast to the findings of Litwin & Balaban (1972), who found a slightly higher concentration in individuals positive for Gm(f) than in subjects lacking this marker. Whether the high concentration of IgG1 present in the group with the Gm(fb) phenotype is of real significance or that it must be considered as an accidental finding has to be further investigated. It is of interest in this respect that Morell *et al.* (1972b) also found the highest IgG1 concentration in this group.

The finding of high IgG2 concentrations in individuals positive for Gm(n) confirms our previous results obtained with an antiserum different from that used in the present study (van der Giessen *et al.*, 1973). A very low level of IgG3 was found in individuals homozygous for the haplotype Gm(zag). The values found in individuals heterozygous for the IgG3 markers were somewhat lower than those found in persons homozygous for Gm(b). A similar correlation was found by Yount *et al.* (1967) and Morell *et al.* (1972b). This indicates a positive effect of the Gm(b) gene on the concentration of IgG3 in serum.

A relation of IgG4 levels with a Gm marker specific for IgG4 could not be established, since the allotypes known for this subclass, 4a and 4b (Kunkel *et al.*, 1970), cannot be determined in whole serum. However, from the finding of a linkage between markers of different subclasses, called Gm haplotypes (Natvig & Kunkel, 1968; Natvig *et al.*, 1968) and that of a linkage even between markers of different immunoglobulin classes, i.e. Am and Gm markers (Kunkel *et al.*, 1969; van Loghem, Natvig & Matsumoto, 1970), it can be expected that the presence of the IgG4 non-marker 4b, which is linked to the haplotype Gm(fnb) (Natvig and Kunkel, 1973), might exert a positive effect on the IgG4 serum concentration, paralelling the presence of the Gm(n) marker. Although the findings of Steinberg *et al.* (1973) and Morell *et al.* (1972b) did show a connection between Gm(n) and IgG4 level, such a relation could not be deduced from our results.

An interesting feature of our study is, that individuals homozygous for the haplotype Gm(zag) show shortage of all IgG subclasses as compared to other people. If the quantity of immunoglobulins is important for the immunological competence a correlation between Gm types and certain diseases might be expected. As far as we know such a correlation has not yet been found. Consequently, one must assume that the finding that a normal individual with a certain Gm type has a lower rate of IgG subclass synthesis than individuals with another Gm type does not necessarily mean that he is unable to increase this synthesis and produce specific antibodies upon an antigenic stimulus at the same rate as the others. The results obtained in a study on antibody formation against flagellin (Wells, Fudenberg and Mackay, 1971) confirm this and point to an even better response of Gm(zag) positive individuals. Another possibility is that a decreased concentration of IgG is compensated by a slight increase of other immunoglobulins.

The results of Morell *et al.* (1972a) on quantification of IgG subclasses in infants have shown that the adult level of IgG1 and IgG3 is reached before the age of 1 year, while the concentration of IgG2 and IgG4 is still low at the age of 2 years. From the present study it is

apparent that there is a slow increase in IgG2 and IgG4 with age, which indicates a slow maturation of the ability to synthesize these subclasses, similarly to IgA (Allansmith *et al.*, 1968; Collins-Williams *et al.*, 1967; Stiehm and Fudenberg, 1966; Stoop *et al.*, 1969; Uffelman *et al.*, 1970). The slow maturation of the ability to synthesize IgG2 and IgG4 might be explained by the possibility that antigens that particularly stimulate the production of IgG2 and IgG4 antibodies occur less frequently.

The finding of a low serum concentration of IgG2 is interesting in view of the results of subclass determinations of immunoglobulin bound to B lymphocytes in neonates as described by Frøland & Natvig (1972a). The percentage of cells bearing IgG2 was much higher than that of cells bearing the other subclasses. Therefore, it seems likely that it is not the capacity to synthesize this particular subclass that matures slowly, but the differentiation into immunoglobulin-secreting plasma cells. However, the finding of Frøland & Natvig (1972b) that adults also have a preponderance of IgG2-bearing cells may indicate a special biological function of this subclass.

The above mentioned findings would indicate that IgG2 resembles IgD, which is also found in a high percentage in B lymphocytes in adults as well as in neonates (Rowe *et al.*, 1973a; van Boxel *et al.*, 1972), while the serum concentration is low or undetectable. These findings prompted Rowe *et al.* (1973b) to suggest that IgD may have a special receptor function. Whether IgG2 resembles IgD in this respect remains to be investigated.

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