Clin. exp. Immunol. (1979) 36, 112–116.

Quantitative and qualitative investigations of serum IgG subclasses in immunodeficiency diseases

VIVI-ANNE OXELIUS *Department of Pediatrics, University Hospital, S-221 85 Lund, Sweden*

(*Accepted for publication* 28 *September* 1978)

SUMMARY

Determinations of IgG subclasses were made by electroimmunoassay and crossed immunoelectrophoresis, and Gm markers were typed in sera from seventeen patients with well-defined immunodeficiency diseases. Certain IgG subclass and Gm patterns were recognized in various diseases: IgG2 deficiency and homozygosity of Gm(4,5) in the cartilage-hair-hypoplasia syndrome, in the ataxia telangiectasia syndrome and in selective IgG subclass deficiency; and IgG3 deficiency and homozygosity of Gm(1,-5) in the Wiskott–Aldrich syndrome. The findings suggest a common structural or regulator gene defect in some immunodeficiency diseases. In IgA deficiencies, the levels of IgG1 were raised. In patients with IgG subclass deficiencies there was sometimes a compensatory increase of the remaining IgG subclasses, with a preponderance of IgG1 and IgG3. The increased IgG1 showed restricted heterogeneity with only an increase of the electrophoretically cathodal part. This part contained both kappa and lambda chains. IgG subclass deficiency indicates treatment with gammaglobulin even if the serum levels of IgG are normal or increased.

INTRODUCTION

Human IgG contains four subclasses based on antigenic differences in the polypeptide heavy chains (Grey & Kunkel, 1964; Terry & Fahey, 1964). The approximate percentages of the IgG subclasses of normal adult IgG are IgG1, 66%; IgG2, 23%; IgG3, 7%; and IgG4, 4% (Yount, Kunkel & Litwin, 1967). Individual Gm genetic markers are found in molecules of only one subclass (Yount *et al.*, 1968).

Structural or regulator gene abnormalities might be expected to result in individuals lacking the capacity to synthesize specific types of heavy chains and manifest themselves as hypogammaglobulinaemic. This would also imply disproportionate levels of IgG subclasses since heavy chains are synthesized at separate, although closely linked, loci (Natvig, Kunkel & Gedde-Dahl, 1967). In addition to their genetic implications, IgG subclass imbalances are of importance because of the variable distribution of biological properties of antibodies among such subclasses and because certain antibody populations may be markedly restricted or limited to a single IgG subclass (Yount *et al.*, 1968). Several instances of alterations in IgG subclass concentrations have been reported (Terry, 1968; Schur *et al.*, 1970; Yount *et al.*, 1970; Yount, 1975; Morell *et al.*, 1975; Oxelius, 1974).

This paper concerns IgG subclass levels in patients with well-defined immunodeficiency diseases. The IgG subclass levels were correlated with some Gm markers. Serum electrophoresis had revealed restricted heterogeneity of IgG and it was decided to examine the distribution of IgG subclasses in the electrophoretic field.

MATERIALS AND METHODS

Sera from seventeen patients with well-defined immunodeficiency diseases, classified according to the WHO classification of primary immunodeficiency syndromes (Fudenberg *et al.*, 1970) were examined. The patients are presented in Table 1. Sera

Correspondence: Dr Vivi-Anne Oxelius, Department of Pediatrics, University Hospital, S-221 85 Lund, Sweden.

0099-9104/79/0040-0112$02.00

from the patients were frozen and kept at −20°C until use. Only specimens obtained before, or more than 3 weeks after, injection of gammaglobulin were accepted.

IgG subclasses. These were studied with electroimmunoassay (Laurell, 1966; 1972), as described in earlier investigations (Oxelius, 1978).

Immunoglobulin classes. These were quantified by electroimmunoassay (Laurell, 1966; 1972) or by single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965).

Gm determinations. These were made according to Grubb (1956) using a microtitre adaptation (Borel, Pryce & Allen, 1967).

Restricted heterogeneity of IgG. This was investigated by crossed immunoelectrophoresis (Laurell, 1965) with IgG subclass antisera and light chain antisera.

TABLE 1. Patients with immunodeficiency states. Some clinical and laboratory data

Name	Age (years)	Sex	Diagnosis	Affected relatives	On gammaglobulin therapy	IgA (g/l)	IgM (g/l)	IgD (g/l)
B.M.	16	M	X-linked hypogammaglobulinaemia	+	+	< 0·01	< 0.01	< 0·01
J.M.	4	M	(Bruton)	+	+	< 0·01	0·02	< 0·01
J.N.	2	M	Common variable immunodeficiency	−	−	0·02	0·54	< 0·01
M.J.	4	M		−	−	< 0·01	0·25	< 0·01
C.P.	8	F	Selective IgA deficiency	−	−	< 0·01	0·57	< 0·01
J.B.	9	M		−	−	< 0·01	0·69	< 0·01
E.F.	11	F		−	−	0·02	1·80	< 0·01
Y.W.	16	F	Selective IgG subclass deficiency	+*	−	0·63	0·95	< 0·01
R.W.	9	M		+*	−	0·51	0·57	< 0·01
U.R.	6	M	ID with short limb dwarfism	−	−	3·20	1·50	< 0·01
K.C.	10	F	ID with ataxia telangiectasia	−	−	0·03	1·79	< 0·01
R.J.	8	M	ID with thrombocytopenia and	+	−	7·37	0·39	0·06
S.P.	13	M	eczema (Wiskott–Aldrich)	−	−	4·75	0·68	0·14
S.N.	2	M	Chronic candidiasis	−	−	0·97	0·86	< 0·01
A.H.	6	M	Nezelof syndrome	+*	−	0·06	1·54	< 0·01
I.H.	1	F		+*	−	0·27	0·63	< 0·01
M.O.	3/12	M	Severe combined immunodeficiency	−	+	< 0·01	< 0·01	< 0·01

* Siblings.

RESULTS

The results obtained with sera from the patients with the various immunodeficiencies are summarized in Table 2.

Congenital X-linked hypogammaglobulinaemia (Bruton)

The two patients investigated showed low levels of IgG1 (Oxelius, 1979), low or normal IgG2 and, with the methods used, no IgG3 or IgG4.

Common variable immunodeficiency

The IgG subclass pattern was the same as in X-linked hypogammaglobulinaemia.

Selective IgA deficiency

The three patients investigated had associated disorders, such as rheumatoid arthritis, hepatitis and eosinophilic granuloma. In all of them IgG1 was increased. The other IgG subclasses were within normal limits. Electrophoretic analyses of sera showed a marked increase of IgG in the cathodal gamma region. This was due to an increase of IgG1 with both kappa and lambda chains, which was shown by crossed immunoelectrophoresis. Anodal IgG1 was normal.

TABLE 2. IgG subclass levels and Gm factors in immunodeficiencies

Name	Age (years)	IgG1 (g/l)	IgG2 (g/l)	IgG3 (g/l)	IgG4 (g/l)	IgG* (g/l)	Gm	Restricted heterogeneity of IgG
B.M.	16	0·09	0·09	< 0·01	< 0·01	0·11	1,4,5	—
J.M.	4	1·49	1·10	< 0·01	< 0·01	2·59	n.d.	—
J.N.	2	1·16	0·57	< 0·01	< 0·01	1·73	1,4,5	—
M.J.	4	0·70	0·40	0·09	< 0·01	1·19	n.d.	—
C.P.	8	9·52	3·33	0·98	1·20	15·03	n.d.	+
J.B.	9	22·44	2·06	0·75	0·20	25·45	n.d.	+
E.F.	11	15·50	3·33	0·95	0·79	20·57	n.d.	+
Y.W.	16	5·44	0·29	0·62	< 0·01	6·35	4,5	+
R.W.	9	7·48	0·32	0·95	< 0·01	8·75	4,5	+
U.R.	6	14·42	< 0·01	2·80	< 0·01	17·22	4,5	+
K.C.	10	8·50	< 0·01	0·85	< 0·01	9·35	4,5	+
R.J.	8	10·47	1·03	0·42	< 0·01	11·92	1,-5	+
S.P.	13	5·24	3·43	0·15	< 0·01	8·82	1,-5	+
S.N.	2	7·68	0·97	0·34	< 0·01	8·99	n.d.	+
A.H.	6	0·88	0·33	0·07	0·17	1·45	1,4,5	−
I.H.	1	5·78	1·10	0·28	< 0·01	7·16	1,4,5	+
M.O.	3/12	1·42	0·29	< 0·01	< 0·01	1·71	1,4,5	−
Normal range	3–5 m	1·43–3·94	0·23–1·47	0·04–1·00	< 0·01–0·14			
	6–8 m	1·90–3·88	0·37–0·60	0·12–0·62	< 0·01			
	9 m–2 yr	2·86–6·80	0·30–3·27	0·13–0·82	< 0·01–0·65			
	3–4 yr	3·81–8·84	0·70–4·43	0·17–0·90	< 0·01–1·16			
	5–6 yr	2·92–8·16	0·83–5·13	0·08–1·11	< 0·01–1·21			
	7–8 yr	4·22–8·02	1·13–4·80	0·15–1·33	< 0·01–0·84			
	9–10 yr	4·56–9·38	1·63–5·13	0·26–1·13	< 0·01–1·21			
	11–12 yr	4·56–9·52	1·47–4·93	0·12–1·79	< 0·01–1·68			
	13–14 yr	3·47–9·93	1·40–4·40	0·23–1·17	< 0·01–0·83			
	Adults	4·22–12·92	1·17–7·47	0·41–1·29	< 0·01–2·91			

* IgG1 + IgG2 + IgG3 + IgG4.

Immunodeficiency with short-limb dwarfism

One patient with the cartilage-hair-hypoplasia syndrome was investigated. The level of IgG was high. No IgG2 or IgG4 could be demonstrated. IgG1 and IgG3 were increased as was IgA. IgG was substantially increased in the cathodal gamma region of the electrophoretic pattern. Crossed immunoelectrophoresis with IgG subclass specific antisera showed that the increased cathodal IgG contained IgG1, IgG3, kappa and lambda chains. Anodal IgG1 was normal. The Gm factors were Gm (4,5).

Immunodeficiency with ataxia telangiectasia

Serum from one patient was investigated. IgG2 and IgG4 were deficient. IgG1 and IgG3 were within normal ranges. There was a restricted heterogeneity of IgG with an increase of cathodal IgG consisting of IgG1, containing kappa and lambda chains. Gm genetic markers were Gm(4,5). This patient also showed IgA deficiency.

Immunodeficiency with thrombocytopenia and eczema (Wiskott–Aldrich)

In the two patients, IgG3 was low or within the low normal range. No IgG4 was detected. In the serum of one of the patients IgG was of restricted heterogeneity, due to a cathodal increase of IgG1 containing both kappa and lambda chains.

Chronic mucocutaneous candidiasis

Increased IgG1, normal IgG2, and IgG3 and non-detectable IgG4 were seen in the one patient investigated.

Nezelof syndrome

The two patients studied were siblings. Differences in their Ig pattern were obvious. One of them had a low IgG level, low levels of all the IgG subclasses and high IgM, while the other had normal levels of IgG1, IgG2 and IgG3. The latter showed restricted heterogeneity of the cathodal IgG. This IgG portion contained IgG1, IgG3, kappa and lambda chains. Both siblings were heterozygous Gm (1,4,5).

X-linked severe combined immunodeficiency

Serum of one patient showed proportionate low levels of all IgG subclasses and the Gm markers were Gm(1,4,5).

DISCUSSION

IgG4 deficiency was found in all the patients except those with IgA deficiency. IgG4 deficiency is seen in about one out of every four normal adults and more often in children (Oxelius, 1979).

IgG3 deficiency was found in the two patients with the Wiskott–Aldrich syndrome. They also lacked IgG4. Gm factors were Gm(1,-5). This is in agreement with the findings of Yount *et al.* (1967) that low IgG3 levels are associated with homozygosity of Gm(−5). Yount (1975) also showed that patients with the Wiskott–Aldrich syndrome had low levels of IgG3.

IgG2 deficiency, as seen in the cartilage-hair-hypoplasia syndrome and in ataxia telangiectasia, has also been described in the members of a family without neurological or skeletal symptoms (Oxelius, 1974). These patients with IgG2 deficiency also lacked IgG4. It should be noted that these patients were homozygous Gm(4,5). This means that IgG2 deficiency may result when both heavy chain cistrons are expressed in the same way with Gm(4,5) allelles. There is now evidence that the order of the IgG heavy chain cistrons may be IgG4-IgG2-IgG3-IgG1 (Natvig *et al.*, 1967). In the above-mentioned patients there was either a weak expression, or a deletion, of the IgG4–IgG2 locus. Similar IgG subclass and Gm patterns found in many immunodeficiency disorders are evidence of a common disturbance of the structural or regulator gene.

In the serum of patients with IgG2 and IgG4 deficiencies, the IgG1 and IgG3 levels were normal or increased, possibly reflecting an effort to compensate for an insufficient antibody response to antigenic stimulation. The normal or high levels of IgG1 and IgG3 resulted in a normal or raised total IgG level. IgG subclass deficiency must therefore also be suspected in patients with normal or high IgG. Yount (1975), who examined ten patients with ataxia telangiectasia, found no imbalance of IgG subclasses. Deficiency of Gm(23) in ataxia telangiectasia had been reported by Rivat *et al.* (1969). Taken together these findings suggest that two forms of ataxia telangiectasia may exist.

IgG1, IgG2, IgG3 and IgG4 deficiencies were seen in congenital X-linked agammaglobulinaemia, common variable immunodeficiency, Nezelof syndrome and severe combined immunodeficiency, and all these patients were heterozygous Gm(1,4,5). However, normal levels of IgG2 were seen in some of the patients with congenital X-linked hypogammaglobulinaemia, common variable immunodeficiency and Nezelof syndrome. It is also of interest that the two siblings with the Nezelof syndrome clearly differed from each other in IgG subclass pattern.

In IgA deficiency and chronic mucocutaneous candidiasis, there was a preponderance of IgG1 with normal levels of the other IgG subclasses. Increases in IgG1 may possibly compensate for IgA deficiency.

Cumulative evidence from the literature together with the data obtained in this study strongly suggest that restricted heterogeneity of IgG may often be observed in the serum of patients with immunodeficiency diseases and may reflect IgG subclass imbalances. Lack of synthesis of one IgG subclass may be compensated for by an increased synthesis of other IgG subclasses. We could show that there was a

polyclonal increase of the cathodal IgG1 and of the IgG3, whereas the anodal part of IgG1 was normal. These changes could not be explained by allelic exclusion. It is known that some antigens give rise to antibodies of mainly one IgG subclass but, generally, antigens give rise to antibodies of all IgG subclasses (Yount *et al.*, 1968). No specific antibodies can be demonstrated in immunodeficiency diseases, despite high levels of at least some IgG subclasses. The possibility of abnormal, non-functioning antibodies in the IgG subclass populations deserves further attention.

Determination of serum IgG subclasses is useful in the diagnosis of immunodeficiency diseases. Furthermore, such analyses may provide information about the underlying mechanisms responsible for some of these diseases. IgG subclass deficiency should always be suspected when there is a restricted heterogeneity of Ig.

I thank Mrs Ann-Margreth Carlsson for skilful technical assistance.

This work was supported by grants from Alfred Österlund's Foundation, Crownprincess Lovisa's Foundation and the Medical Faculty of Lund.

REFERENCES

BOREL, H., PRYCE, S. & ALLEN, F.H. (1967) Gm typing with microtitre plates. *Vox Sang.* (*Basel*), **12**, 319.

FUDENBERG, H.H., GOOD, R.A., HITZIG, W., KUNKEL, H.G. ROITT, I.M., ROSEN, F.S., ROWE, D.S., SELIGMAN, M. & SOOTHILL, J.R. (1970) Classification of the primary immune deficiencies: WHO recommendation. *New Engl. J. Med.* **283**, 655.

GREY, H.M. & KUNKEL, H.G. (1964) H chain subgroups of myeloma proteins and normal 7S γ-globulin. *J. exp. Med.* **120**, 253.

GRUBB, R. (1956) Agglutination of erythrocytes coated with 'incomplete' anti-Rh by certain rheumatoid arthritic sera and some other sera. The existence of human serum groups. *Acta path. microbiol. scand.* **39**, 195.

LAURELL, C.-B. (1965) Antigen–antibody crossed electrophoresis. *Analyt. Biochem.* **10**, 358.

LAURELL, C.-B. (1966) Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Analyt. Biochem.* **15**, 45.

LAURELL, C.-B. (1972) Electroimmunoassay. *Scand. J. clin. Lab. Invest.* **29**, suppl. 124, 21.

MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.

MORELL, A., SKVARIL, F., RADL, J., DOOREN, L.J. & BARANDUN, S. (1975) IgG subclass abnormalities in primary immunodeficiency diseases. *Immunodeficiency in Man and Animals* (ed. by D. Bergsma, R.A. Good and J. Finstad), Original Article Series on Birth Defects. Vol. XI. p. 108. Sinauer Associates Inc., Massachusetts.

NATVIG, J.B., KUNKEL, H.G. & GEDDE-DAHL, T. (1967) Genetic studies of the heavy chain subgroups of G globulin. Recombination between the closely linked cistrons. *Gammaglobulins. Structure and control of biosynthesis. Nobel Symposium* (ed. by J. Killander), Vol. 3. p. 313. Almqvist & Wiksell, Stockholm.

OXELIUS, V. (1974) Chronic infections in a family with hereditary deficiency of IgG2 and IgG4. *Clin. exp. Immunol.* **17**, 19.

OXELIUS, V. (1978) Crossed immunoelectrophoresis and electroimmunoassay of human IgG subclasses. *Acta path. microbiol. scand.* Sect. C. **86**, 109.

OXELIUS, V. (1979) IgG subclass levels in infancy and childhood. *Acta Paediatr. Scand.* **68**, 23.

RIVAT, L., ROPARTZ, C., BURTIN, P. & KARITZKY, D. (1969) Abnormalities in the synthesis of some subclasses of γG in a family with two cases of ataxia teleangiectasia. *Vox Sang.* (*Basel*), **17**, 5.

SCHUR, P.H., BOREL, H., GELFAND, E.W., ALPER, C.A. & ROSEN, F.S. (1970) Selective gamma-G globulin deficiencies in patients with recurrent pyogenic infections. *New Engl. J. Med.* **283**, 631.

TERRY, W.D. (1968) Variations in the subclasses of IgG. *Immunologic Deficiency Diseases in Man. Birth Defects* (ed. by R.A. Good and D. Bergsma), p. 357. The National Foundation March of Dimes, New York.

TERRY, W.D. & FAHEY, J.L. (1964) Subclasses of human γ_2-globulin based on differences in the heavy polypeptide chains. *Science*, **146**, 400.

YOUNT, W.J., KUNKEL, H.G. & LITWIN, S.D. (1967) Studies of the Vi (γ_{2c}) subgroup of γ-globulin. A relationship between concentration and genetic type among normal individuals. *J. exp. Med.* **125**, 177.

YOUNT, W.J., DORNER, M.M., KUNKEL, H.G. & KABAT, E.A. (1968) Studies on human antibodies. VI. Selective variations in subgroup composition and genetic markers. *J. exp. Med.* **127**, 633.

YOUNT, W.J., HONG, R., SELIGMAN, M., GOOD, R.A. & KUNKEL, H.G. (1970) Imbalances of gammaglobulin subgroups and gene defects in patients with primary hypogammaglobulinemia. *J. clin. Invest.* **49**, 1957.

YOUNT, W.J. (1975) Imbalances of IgG subclasses and gene defects in patients with primary hypogammaglobulinemia. *Immunodeficiency in Man and Animals* (ed. by D. Bergsma, R.A. Good and J. Finstad), Original Article Series on Birth Defects, Vol. XI, p. 99. Sinauer Associates Inc., Massachusetts.