Clin. exp. Immunol. (1976) **25**, 117–121.

The subclasses of human IgG antibodies against tetanus toxoid

MARIJKE VAN DER GIESSEN & OSCARINA GROENEBOER-KEMPERS *Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands*

(*Received* 5 *January* 1976)

SUMMARY

The subclass of IgG antibodies against tetanus present in the serum of thirty-five human individuals, who received an injection with tetanus toxoid, was determined. Six successive serum samples were obtained from twenty-five normal individuals (laboratory personnel) 0, 3, 7, 14, 28 days and 2–3 months after the injection with tetanus toxoid had been given. Another ten serum samples were obtained from ten persons with a positive IgE–RAST, taken 2 weeks after the injection. Antibodies were determined with a quantitative immunofluorescence method known as the defined antigen substrate spheres (DASS) system. The normal individuals in whose serum a clearly positive IgG binding was found (nineteen) showed activity in all four subclasses. The binding activity in all individuals reached a maximum between 2 and 4 weeks after the injection. The antibody activity in the serum of four individuals whose serum gave weak IgG binding was confined to IgG1. Two individuals did not show any IgG binding activity at all. In the ten persons with a positive IgE–RAST and three of the normal individuals, who also had a positive IgE–RAST, the distribution of the antibodies over the subclasses was the same as in the others.

INTRODUCTION

It is commonly known that animals produce IgM antibodies in the primary immune response and predominantly IgG antibodies in the secondary immune response. The same switch from the formation of IgM antibodies to that of IgG antibodies seems to occur in humans (Bandilla, McDuffie & Gleich, 1969; Curtis & Hersh, 1972; Rowley, Wistar & Mackay, 1972; Peacock, Jones & Gough, 1973). Four subclasses of human IgG can be distinguished which differ in physicochemical and biological properties (see review by Schur, 1972). The distribution of antibodies among the different subclasses appears to be variable. Some antigens elicit antibodies of limited heterogeneity, i.e. belonging to only one subclass; examples are dextran, which evokes the formation of IgG2 antibodies almost exclusively (Yount *et al.*, 1968), clotting factor VIII, against which only IgG4 antibodies are formed (Anderson & Terry, 1968; Robboy *et al.*, 1970), and grass pollen, which elicits predominantly IgG4 antibodies (van der Giessen *et al.*, 1967 Antibodies against red cells have been found to be primarily IgG1 and IgG3 (Natvig & Kunkel, 1973), but also IgG2 and IgG4 (Frame, Mollison & Terry, 1970; Abramson & Schur, 1972; Morell, Skvaril & Rufener, 1973; Engelfriet *et al.*, 1976). Antinuclear antibodies have been found in all four subclasses although individual differences in the distribution between the subclasses have been described (Tojo, Friou & Spiegelberg, 1970; Schur, Monroe & Rothfield, 1972; Wiik & Munthe, 1972; Puritz *et al.*, 1973). Antibodies against protein antigens, such as diphtheria toxoid, tetanus toxoid and thyroglobulin appear to be formed in quantities which parallel the relative amount of the subclasses of IgG in normal serum (Hay & Torrigiani, 1973; Spiegelberg, 1974).

However, no detailed studies have been reported about possible individual differences in subclass distribution, and the sequence in which antibodies of the different subclasses are synthesized, as a result of a booster injection.

Correspondence: Dr Marijke van der Giessen, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, P.O. Box 9190, Amsterdam, The Netherlands

In this paper we report the results of an investigation into the distribution over the subclasses of IgG of antibodies present in six successive serum samples of twenty-five normal individuals, who received an injection with tetanus toxoid. A further ten serum samples obtained from persons with a positive IgE-RAST were also studied.

MATERIALS AND METHODS

Serum samples. Sera were obtained from twenty-five normal individuals (laboratory personnel) who were injected intramuscularly with 5 Lf $AlPO_4$ adsorbed tetanus toxoid (RIV). Most of them knew they had been immunized previously, but some were not sure about this. Serum samples were obtained on day 0, 3, 7, 14, 28 and 2–3 months after the injection. The same sera were used in a study on the IgE response to tetanus toxoid (Aalberse, Hoorweg & Reerink-Brongers, paper in preparation). A positive control serum was obtained from an actively immunized military conscript. Ten serum samples were obtained from IgE-RAST-positive persons 2 weeks after a tetanus injection had been given.

Antisera. The anti-IgG subclass sera used were the same as applied before (van der Giessen, *et al.*, 1976). Anti-human IgG and fluorescein-conjugated horse anti-rabbit immunoglobulin (Ig) were the same as used by Capel (1974).

Coupling to Sepharose. The activation of Sepharose 4B (Pharmacia) with CNBr (Koch-Light), the coupling of tetanus toxoid to Sepharose (1 ml containing 100 Lf to 1 ml packed Sepharose), the inactivation of the Sepharose with ethanolamine (Merck) and the washing procedure have been previously described (van der Giessen *et al.*, 1976).

Antibodies to tetanus toxoid. The detection of IgG anti-tetanus antibodies and the determination of their subclass was performed with the defined antigen substrate spheres (DASS) system in the same way as described for anti-grass pollen antibodies (van der Giessen *et al.*, 1976). A 500-μl serum sample diluted 1 in 100 was incubated with 100 μl (2×10^3 beads) antigen-coupled Sepharose, containing 1 Lf. Further incubations and washing procedures were the same as previously described (van der Giessen *et al.*, 1976). The intensity of fluorescence was measured and the results were expressed in arbitrary units as the relative fluorescence intensity (RFI) as described by Capel (1974), after correction for the aspecific binding of the antisera to the tetanus-Sepharose beads.

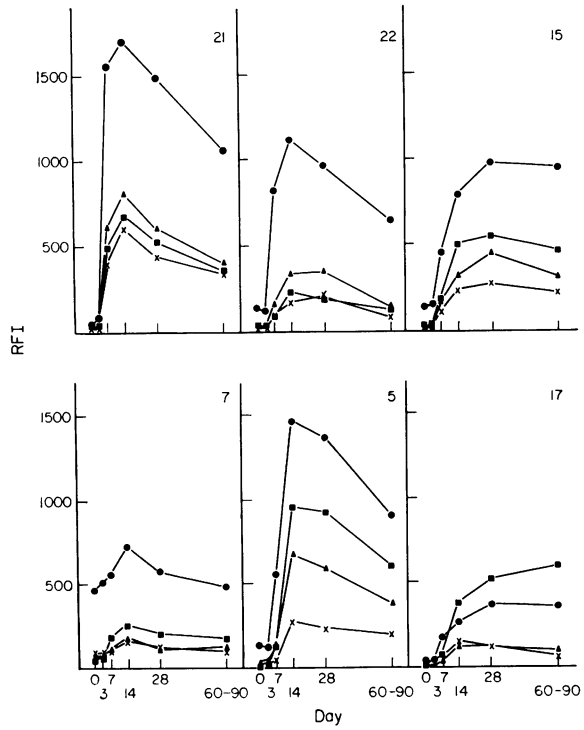

FIG. 1. Characteristic patterns of tetanus toxoid binding of the different IgG subclasses. Results are shown on the binding activity (in RFI) of the four IgG subclasses in six successive serum samples obtained from six normal individuals: (●) IgG1; (×) IgG2; (▲) IgG3; (■) IgG4.

RESULTS

When the antibody activity in IgG and the subclasses of IgG were determined in the successive serum samples of the normal individuals, the following results were obtained. In nineteen persons with a clearly positive IgG binding (RFI>450) antibody activity was demonstrated in all four subclasses. All subclasses showed identical patterns: a negative binding (sometimes a weak IgG1 binding) in the first two samples, then an increasing binding activity with a maximum reached in the fourth or fifth sample. Characteristic patterns of six individuals are shown in Fig. 1. The four subjects in whose serum only weak IgG binding was demonstrated, gave positive results with anti-IgG1 while no detectable fluorescence was seen with the other three subclass antisera. The two subjects with negative results in the IgG test also gave negative results in the tests with subclass antisera. The binding activity which was present in some of the first serum samples was mainly due to IgG1. The specificity of the binding was confirmed by complete inhibition after pre-incubation of the serum sample with 0·5 μl of soluble tetanus toxoid. The mean values for all the successive serum samples are shown in Fig. 2 and Table 1. It is clear that the

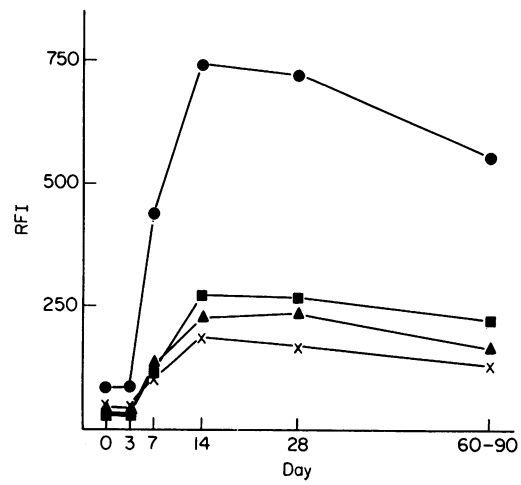

FIG. 2. Mean values are shown for the binding activity (in RFI) of the four IgG subclasses in six successive serum samples obtained from twenty-five normal individuals: (●) IgG1; (×) IgG2; (▲) IgG3; (■) IgG4.

TABLE 1. Tetanus toxoid binding in sera of normal and allergic persons detected with the DASS system (in RFI)

Serum obtained from:	Day	IgG1	IgG2	IgG3	IgG4
25 Normal persons	0	82 (0–460)	44 (0–90)	27 (0–89)	29 (12–48)
	3	84 (0–516)	39 (0–90)	24 (0–81)	30 (14–62)
	7	435 (21–1560)	101 (0–392)	128 (6–609)	113 (20–498)
	14	740 (25–1800)	186 (11–611)	227 (0–807)	271 (22–959)
	28	722 (19–1790)	168 (8–489)	236 (0–968)	267 (18–932)
	60–90	549 (13–1575)	128 (4–453)	163 (0–747)	220 (16–605)
10 Allergic persons	14	473 (62–941)	156 (60–440)	242 (128–372)	291 (60–759)

The results are expressed as the mean levels and observed range of the various subclasses.

antibody distribution over the various subclasses in general parallels the relative amount of the subclasses in normal serum, with the exception of the antibody activity in IgG2, the relative amount of which is lower than in normal serum.

However, some individual differences were found. In three cases a relatively high IgG4 binding was detected, which approached the values obtained for the IgG1 binding (see Fig. 1, nos 5 and 17).

When the subclass of the IgG antibodies present in serum samples of IgE–RAST-positive persons, obtained two weeks after a tetanus injection had been given, was determined, the same distribution over the subclasses was found as in the normal individuals (Table 1).

DISCUSSION

Our finding that anti-tetanus antibodies belong to all four subclasses of IgG and that they are synthesized in quantities that parallel the relative amounts of the subclasses in normal serum, agrees with data mentioned by Spiegelberg (1974) in his review on immunoglobulins. The relatively low binding activity of IgG2 compared to that of IgG3 and IgG4 may have been caused by a somewhat lower sensitivity of the IgG2 detection system (van der Giessen *et al.*, 1976). In our studies some individual differences were shown (Fig. 1). This was also found by Hay & Torrigiani (1973) and by Pinedo & Capel (unpublished observations) when studying antibodies against thyroglobulin.

Furthermore, we observed no difference between antibodies of the various subclasses as to the time of appearance or disappearance, at least not in the period studied, i.e. from the time of injection until 3 months afterwards, although it is known that the turnover rate of the different subclasses of IgG is not the same (Spiegelberg, 1974). It is possible that the two individuals whose serum did not show any IgG binding activity and the four individuals with weak antibody activity had not previously been immunized. However, it must be borne in mind that results obtained with the mouse protection test (Cohen *et al.*, 1971) show that even in individuals who are properly vaccinated for tetanus there may be no antibody response: only 80–90% become positive. In our study only IgG1 antibodies were found in these weakly positive serum samples. It cannot be decided at present whether this means that IgG1 antibodies are the first to be formed as a result of an antigenic stimulation, or whether these antibodies are the only ones that reach a concentration above the threshold of detection.

There was one individual (see Figs 1 and 7) with a rather high starting level of IgG1 antibodies which did not increase very much as a result of the injection. This high antibody activity could not be explained by a recent booster injection. Furthermore, a serum sample taken 10 months later still contained the same antibody activity. This finding indicates that this person continued to produce antibodies in spite of the disappearance of the antigen, or that the antigen was catabolized more slowly than in the average person.

The results obtained in the allergic patients and in three of the normal subjects, who considered themselves to be weakly allergic and indeed had a positive IgE–RAST (two with grass pollen and the third with house dust), show that these individuals, just like non-allergic people, have the capacity to synthesize antibodies of all four subclasses of IgG when injected with tetanus toxoid. In contrast, when grass pollen allergic patients are actively immunized with an aqueous extract of grass pollen as immunotherapy, they predominantly make IgG4 antibodies (van der Giessen *et al.*, 1976). This finding suggests that the antigen may influence the immune response and may determine the class and subclass of the immunoglobulins that are produced. Which property or properties of the antigen are responsible for a restricted antibody response remains an as yet unsolved but intriguing problem.

The authors wish to express their gratitude to Miss M. van der Veer and the National Institute of Health (RIV) at Bilthoven, Netherlands, for their generous supply of purified tetanus toxoid.

REFERENCES

ABRAMSON, N. & SCHUR, P.H. (1972) The IgG subclasses of red cell antibodies and relationship to monocyte binding. *Blood*, **40**, 500.

ANDERSON, J.R. & TERRY, W.D. (1968) Gamma G4 globulin antibody causing inhibition of clotting factor VIII. *Nature (Lond.)*, **217**, 174.

BANDILLA, K.K., MCDUFFIE, F.C. & GLEICH, G.J. (1969) Immunoglobulin classes of antibodies produced in the primary and secondary response in man. *Clin. exp. Immunol.* **5**, 627.

CAPEL, P.J.A. (1974) A quantitative immunofluorescence method based on the covalent coupling of protein to Sepharose beads. *J. immunol. Meth.* **5**, 165.

COHEN, H., HAGENAARS, A.M., BIJKERK, H. & LEUSSINK, A.B. (1971) Vergelijking van de werkzaamheid van een aantal geadsorbeerde tetanus toxoiden. *Ber. RIV*, Utrecht 1971, p. 169.

CURTIS, J.E. & HERSH, E.M. (1972) The human secondary immune-response to keyhole limpet haemocyanin. *Clin. exp. Immunol.* **10**, 171.

FRAME, M., MOLLISON, P.L. & TERRY, W.D. (1970) Anti-Rh activity of human γG4 proteins. *Nature (Lond.)*, **225**, 641.

GIESSEN, M. VAN DER, HOMAN, W.L., KERNEBEEK, G. VAN, AALBERSE, R.C. & DIEGES, P.H. Subclass typing of IgG antibodies formed by grass pollen allergic patients during immunotherapy. *Int. Arch. Allergy appl. Immunol.* (In press.)

HAY, F.C. & TORRIGIANI, G. (1973) The distribution of anti-thyroglobulin antibodies in the immunoglobulin G subclasses. *Clin. exp. Immunol.* **15**, 517.

MORELL, A., SKVARIL, F. & RUFENER, J.L. (1973) Characterization of Rh antibodies formed after incompatible pregnancies and after repeated booster injections. *Vox Sang. (Basel)*, **24**, 323.

NATVIG, J.B. & KUNKEL, H.G. (1973) Human immunoglobulins: classes, subclasses, genetic variants, and idiotypes. *Advanc. Immunol.* **16**, 1.

PEACOCK, D.B., JONES, J.V. & GOUGH, M. (1973). The immune response to φX174 in man. I. Primary and secondary antibody production in normal adults. *Clin. exp. Immunol.* **13**, 497.

PURITZ, E.M., YOUNT, W.J., NEWELL, M. & UTSINGER, P.D. (1973) Immunoglobulin classes and IgG subclasses of human antinuclear antibodies. A correlation of complement fixation and the nephritis of systemic lupus erythematosus. *Clin. Immunol. Immunopath.* **2**, 98.

ROBBOY, S.J., LEWIS, E.J., SCHUR, P.H. & COLMAN, R.W. (1970) Circulating anticoagulants to factor VIII. Immunochemical studies and clinical response to factor VIII concentrates. *Amer. J. Med.* **49**, 742.

ROWLEY, M.J., WISTAR, R. & MACKAY, J.R. (1972) Measurement of antibody-producing capacity in man. V. Immunoglobulin classes of antibodies to flagellin. *Immunology*, **22**, 475.

SCHUR, P.H. (1972) Human gamma-G subclasses. *Progr. clin. Immunol.* **1**, 71.

SCHUR, P.H., MONROE, M. & ROTHFIELD, N. (1972) The γG subclasses of antinuclear and antinucleic acid antibodies. *Arthr. Rheum.* **15**, 174.

SPIEGELBERG, H.L. (1974) Biological activities of immunoglobulins of different classes and subclasses. *Advanc. Immunol.* **19**, 259.

TOJO, T., FRIOU, G.J. & SPIEGELBERG, H.L. (1970) Immunoglobulin G subclass of human antinuclear antibodies. *Clin. exp. Immunol.* **6**, 145.

WIIK, A. & MUNTHE, E. (1972) Restrictions among heavy and light chain determinants of granulocyte-specific antinuclear factors. *Immunology*, **23**, 53.

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.