HUMAN TETANUS ANTIBODIES: ISOLATION AND CHARACTERIZATION WITH SPECIAL REFERENCE TO THE IgG SUBCLASSES

S. CARREL, A. MORELL, F. SKVARIL and S. BARANDUN

Swiss Center for Clinical Cancer Research, Tiefenau, 3004 Bern, Switzerland

Received 27 October 1971

1. Introduction

Since human tetanus antitoxins are commercially produced for passive protection [1], it was of interest to study the qualitative and quantitative distribution of specific antibodies in several individual sera. Preliminary tests showed that in human hyperimmune sera, antibodies are mainly concentrated in the γ-globulin fraction [2].

Human individual tetanus antibodies were isolated by means of a specific polyacrylamide-immunoadsorbent [3, 4]. The activity of the isolated antibodies was determined *in vivo* by the mouse protection test [5] and *in vitro* by the Ramon flocculation test [6]. The isolated antitoxins were identified as belonging essentially to the IgG class of immunoglobulins. As human IgG consists of 4 distinct groups of molecules, known as the subclasses of IgG, and referred to as G1, G2, G3 and G4 [7–11], the IgG subclasses of isolated tetanus antibody from several individuals were investigated. Distributions of IgG subclasses of isolated tetanus antibodies were compared with IgG subclasses in the corresponding whole sera.

2. Experimental

Human tetanus antibodies from 6 individual hyperimmunized donors (kindly given by the Swiss Serum and Vaccine Institute, Bern) with antibody titers in the range from 8–33 IU were isolated by means of a specific immunoadsorbent. The immunoadsorbent was based on the mechanical entrapment of antigens into a lattice of a highly cross-linked macroporous synthetic polyacrylamide gel, as previously reported by Carrel et al. [3, 4].

In our experiments 7 ml of a monomer solution containing 6 g acrylamide and 2 g bis-acrylamide per 100 ml water were mixed with 1 ml of highly purified tetanus toxoid containing 2100 LF and polymerized with ammonium peroxydisulphate and riboflavin as catalyst under light activation for 1 hr at 27°. Since oxygen inhibits polymerization, nitrogen was first passed through the monomer solution. The block of polymerized antigen gel was mechanically dispersed by forcing it through a needle with an inner diameter of 0.8 mm. The gel particles were repeatedly washed with 0.1 M Tris-NaCl buffer pH 7.5 until no more toxoid remained in the supernatant. The extent of insolubilization was about 92% of the amount of antigen originally added, as determined by entrapment of tritium-labeled toxoid.

A chromatographic column was packed with the toxoid-containing gel particles and connected to a UV absorptiometer and the adsorption was recorded at 280 nm. After application of the serum samples on top of the antigen-containing column, the column was washed with 0.1 M Tris-NaCl buffer pH 7.5 until an absorbance of less than 0.01 was recorded in the effluent. The antibodies bound to the immunoadsorbent were eluted with 0.2 M glycine-HCl buffer pH 2.3. After elution of the antibodies the columns were re-equilibrated with 0.1 M Tris-NaCl buffer pH 7.5 and used again.

The pH of the eluted antibodies was adjusted to neutrality by buffer exchange on Sephadex G-25 against 0.1 M Tris-NaCl buffer pH 7.5 and concentrated by pressure dialysis. The specificity of the isolated antibodies was tested *in vivo* by comparison with a standard sample of antitoxin with the L_+-technique in mice [5], or *in vitro* by the Ramon flocculation test [6]. The extent of adsorption was also tested *in*

vivo by collecting all the non-bound ballast proteins (washes) and reconcentrating them up to the original protein level.

The IgG subclass distribution was tested by means of a radioimmuno-assay [12]. Four different immunoadsorbents were prepared by coupling the globulin fractions of specific anti-subclass antisera to bromoacetylcellulose. The binding of ^{125}I-labeled G1, G2, G3 and G4 myeloma proteins to these immunoadsorbents was inhibited quantitatively by isolated IgG antibodies. With this technique it was possible to detect 0.05 μg of G1, G2, G3 and G4 per ml. The presence of antibodies of the IgA and IgM classes in the isolated antitetanus antibodies was tested by the double diffusion technique.

3. Results

Antibodies from 6 individual donors were isolated. Except for donor D, all antibodies were of the IgG class. The IgG subclass distribution of the respective individual antibodies is summarized in table 1. In 5 donors (A, B, C, D, E and F) the 4 IgG subclasses were present in the isolated antibodies in a percental distribution corresponding nearly to the subclass distribution in the original individual sera, as shown in table 2. Donors B and C (table 1) had a relatively high percentage of G4, but they also showed an increased G4 in the whole serum. Donor D had tetanus antibodies of the IgM class only, while in the original serum all IgG subclasses were present in a normal distribution. The *in vitro* and *in vivo* tests of the isolated antibodies showed that they still had a protective activity, but the antibody titer had decreased in the range of 50% (table 1) compared

Table 1
IgG subclass distribution in isolated tetanus antibodies.

Donor	Antibody titer (IU/ml)	Total* IgG (μg/ml)	G1 (μg/ml)	G1 (%)	G2 (μg/ml)	G2 (%)	G3 (μg/ml)	G3 (%)	G4 (μg/ml)	G4 (%)
A	5	22	13.0	58	7.0	32	1.2	5	1.2	5
B	16	64	43.0	67	8.0	12.5	1.6	2.5	11.5	18
C	9	55	43.0	78	9.0	16.5	1.6	3	1.5	2.5
D**	4	–	–	–	–	–	–	–	–	–
E	5	30	16.0	53.5	6.0	20	1.1	4	7	22.5
F	10	25	16.0	64	5.0	20	1.7	7	2.2	9

* Sum of the 4 subclasses.
** Donor D had IgM type antibodies only.

Table 2
IgG subclass distribution in whole serum.

Donor	Antibody titer (IU/ml)	Total* IgG (mg/ml)	G1 (mg/ml)	G1 (%)	G2 (mg/ml)	G2 (%)	G3 (mg/ml)	G3 (%)	G4 (mg/ml)	G4 (%)
A	8	8.0	4.2	52.5	3.1	39	0.4	5.0	0.3	3.5
B	33	7.5	4.2	56	2.3	30.5	0.4	5.3	0.6	8.0
C	17	11.3	7.4	65.5	3.1	27.5	0.5	4.4	0.3	2.6
D	8	8.6	5.0	58	2.8	32.5	0.6	7	0.2	2.5
E	8	9.1	4.6	50.5	3.2	35	0.6	6.5	0.7	8.0
F	18	5.6	3.1	55.5	1.7	30	0.7	12.5	0.1	2.0

* Sum of the 4 subclasses.

with the titers in the original serum samples (table 2) during the isolation and concentration processes. In each serum sample, between 0.2 and 1.5 IU could be detected in the ballast proteins (washes).

4. Discussion

A discussion concerning the problems of immune specificity is rendered extremely difficult because of the great number of different cell clones which participate in the antibody production and which are all able to embed the same antibody specificity into different classes of the antibody globulins as γG, γA and γM immunoglobulins [13, 14]. In our experiments, however, only the anti-tetanus toxoid activity of the IgG class of immunoglobulins was investigated. For this reason, the antibodies were isolated from serum of hyperimmunized donors. Considering the IgG subclass distribution in the isolated antibody fractions, all 4 subclasses were present in a percental distribution corresponding to the distribution of IgG subclasses in the original sera: G1 carrying the major part of the antibody activity, followed by G2, G3 and G4.

Previous studies on isolated human tetanus antibodies revealed a relative homogeneity when compared with the whole γ-globulin of the donor [15]. Yount et al. [16] found that anti-tetanus toxoid antibodies do consist primarily of the major IgG1-subgroup. In contrast to these studies, our results demonstrate a clear heterogeneity of the anti-tetanus IgG antibodies. The reason for this discrepancy may be due to technical differences: we could accept that the methods applied by those investigators [15, 16] were less sensitive than the radioimmuno-assay used in our study.

If we consider the antigenic heterogeneity of the tetanus toxoid used for the immunization, the heterogeneous antibody response is not surprising.

Acknowledgements

This work was supported by the Swiss National Foundation for Scientific Research. We thank Dr. R. Heiz (Swiss Serum and Vaccine Institute, Bern) and Mrs. M. Meier for their assistance.

References

[1] E.A. Kabat, New. Engl. J. Med. 269 (1963) 247.
[2] J. Ungar, in: Proc. of the International Conference on Tetanus, ed. L. Eckmann (H. Huber, Bern and Stuttgart, 1966) p. 409.
[3] S. Carrel, H. Gerber and S. Barandun, Nature 221 (1969) 385.
[4] S. Carrel and S. Barandun, Immunochemistry 8 (1971) 39.
[5] H. Schmidt, Die Praxis der Auswertung von Toxinen und Antitoxinen (G. Fischer-Verlag, Jena, 1931).
[6] G. Ramon, Compt. Rend. Soc. Biol. 86 (1922) 661.
[7] Bull. W.H.O. 35 (1966) 953.
[8] W.D. Terry and J.L. Fahey, Science 146 (1964) 400.
[9] H.M. Grey and H.G. Kunkel, J. Expt. Med. 120 (1964) 253.
[10] B. Frangione, C. Milstein and J.R.L. Pink, Nature 221 (1969) 145.
[11] J.W. Prahl, Biochem. J. 105 (1967) 1019.
[12] D. Mann, H. Granger and J.L. Fahey, J. Immunol. 102 (1969) 618.
[13] J.F. Heremans, J.P. Vaerman and C. Vaerman, J. Immunol. 91 (1963) 11.
[14] E.C. Franklin, Prog. Allergy 8 (1964) 58.
[15] M. Mannik and H.G. Kunkel, J. Exp. Med. 118 (1963) 817.
[16] W.J. Yount, M.M. Dorner, H.G. Kunkel and E.A. Kabat, J. Exp. Med. 127 (1968) 633.