

IgG4: a possible mediator of anaphylaxis in a haemophiliac patient

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(Received 19 January 1979; accepted for publication 20 February 1979)

Summary

Immunological investigations have been carried out on a haemophiliac patient who had developed severe anaphylaxis to bovine factor VIII administration. Results have shown that the development of anaphylaxis in this patient was associated with a sudden increase in total and specific IgG4 antibodies. The significance of this observation is discussed in relation to current evidence suggesting the involvement of IgG4 antibodies in some anaphylactic conditions.

Introduction

Haemophilia is a sex-linked, recessive coagulation disorder in which the biological activity of factor VIII is reduced because the factor VIII molecule, though immunologically detectable, is functionally impaired (Ingram, 1976). Patients with haemophilia are, therefore, treated with factor VIII concentrates such as cryoprecipitates (factor VIII rich fibrinogen 'sludge' which remains after slowly thawing frozen plasma) and anti-haemophilic globulins (AHG) of animal sources. Antibodies to coagulation factor VIII develop in about 5–10% of patients with classical haemophilia treated with infusion of factor VIII (Bird, 1975; Ingram, 1976). These circulating anti-factor VIII antibodies were also reported to be present in post-partum women, patients with diseases believed to be of immunologic nature and in otherwise healthy elderly persons (Robboy *et al.*, 1970). This so called 'inhibitor of factor VIII activity' was shown to be mostly of the IgG class (Bidwell, Denson & Dike, 1966; Feinstein, Rapaport & Chong, 1969) and in only two reported cases was the inhibitor of the IgA (Glueck & Hong, 1965) and IgM (Castaldi & Penny, 1970) classes respectively. It was further shown that permanent disappearance of such inhibitors could be achieved as a result of immunosuppressive therapy (Hultin *et al.*, 1976).

The present paper reports results of an immunological investigation carried out on

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a haemophilic patient who developed severe anaphylactic reaction to bovine anti-haemophilic globulin administration.

Materials and methods

(a) Serum samples

Serial serum samples were obtained from a 46-year-old haemophilic patient (admitted to the General Hospital, Birmingham, suffering from a fractured thigh with a haematoma) before and after developing a severe anaphylactic reaction to bovine AHG administration. Samples were kept in the frozen state until required for testing.

In order to avoid between batch variation of the assay methods analyses of the serial serum samples were performed in one batch.

(b) Bovine AHG

This is a dried anti-haemophilic globulin factor VIII containing 360 units of AHG. Each unit is equivalent to 1 ml of normal plasma (Speywood Lab. Ltd., Nottingham, NG 13 8DR).

(c) Measurement of total IgG and IgG subclass levels

Serum total IgG and IgG subclass levels were determined using the radial immunodiffusion method described previously (Shakib *et al.*, 1975).

(d) Measurement of IgE levels

Serum IgE levels were determined using the radioimmunosorbent technique (RIST) described by McLaughlan & Stanworth (1975).

(e) Assay of specific anti-factor VIII antibodies

The method employed for measuring specific anti-factor VIII activity within IgM, IgG and IgG subclasses is a modification of the solid phase radioimmunoassay previously employed for measuring anti-gammaglobulin activity within IgG subclasses (Shakib & Stanworth, 1978).

(e1) *Coating of tubes with factor VIII.* One ml. volumes of the bovine factor VIII solution (40 i.u./100 ml PBS) were incubated in 2.5 ml plastic tubes (Luckham Ltd., Victoria Gardens, Sussex) for 1 hr at room temperature and overnight at 4°C. The factor VIII solution was then discarded and the tubes washed three times with PBS. Free sites (i.e. sites on the tube surface not occupied by factor VIII material) were blocked by incubating 2 ml of Lister human serum albumin (HSA) solution (10 g/l in PBS) in the tubes for 2 hr at room temperature. The HSA solution was then discarded, tubes washed three times with PBS, stoppered and stored at 4°C until used for testing.

(e2) *Preparation of the radiolabelled antisera.* The method used for labelling antisera specific for IgM, IgG and individual IgG subclasses, with ¹²⁵I, has previously been described (Shakib and Stanworth, 1978).

(e3) *Detection of factor VIII specific antibodies.* To each factor VIII coated plastic tube 450 µl of 10 g/l of HSA/PBS solution was added as a diluent, followed by 50 µl of the test serum sample. The tubes were then incubated for 1 hr at 37°C and for 0.5 hr at 4°C. After the incubation period the solutions were discarded and the tubes washed three times with cold PBS. One ml. of 10 g/l HSA/PBS solution containing 5 µl of the labelled antibody solution was added to each tube and the tubes incubated for 2 hr at

37°C and for 0.5 hr at 4°C. The radioactive solutions were then discarded and the tubes washed three times with cold PBS. The tubes were drained free of any liquid and counted in a gamma-ray counter (Tracer lab gamma/guard 150) for 50 sec. All samples were assayed in duplicate.

Because of the lack of a standard anti-factor VIII antiserum, anti-factor VIII activity in the haemophiliac's sera was expressed as the ratio of the test serum counts/50 sec to the counts/50 sec of a normal serum sample; the ratio being called anti-factor VIII activity (AF-VIII A) index.

(e4) *Non-specific adsorption of antisera.* The non-specific adsorption of the radioactively labelled antisera onto the plastic tube surface was assessed by incubating, as described under section (e3) one ml of 10 g/l HSA/PBS solution containing 5 µl of the appropriate labelled antibody solution in factor VIII coated tubes which were not treated with the patient's sera. The adsorption of each of anti-IgM, anti-IgG and anti-IgG subclass specific antisera onto the tubes surface was found to be negligible unless the tubes were first treated with a test serum sample.

(e5) *Non-specific adsorption of test serum antibodies.* Non-specific adsorption of test serum antibodies onto the plastic tube surface was assessed by treating HSA, but not factor VIII, coated tubes with a test serum sample then followed by treatment with ¹²⁵I-labelled antiserum as described under section (e3). The binding of serum IgM, IgG and each of IgG subclasses, as indicated by the adsorption of their respective specific ¹²⁵I-labelled antibodies, to the HSA coated tube surface was negligible.

(e6) *Specificity of the antisera.* The ability of the radioactively labelled antisera to react only with their specific immunoglobulin class or subclass was fully assessed elsewhere (Shakib and Stanworth, 1978).

(f) *Passive cutaneous anaphylactic testing in baboon*

The haemophiliac serum samples, taken before and after the patient had developed the anaphylactic reaction to bovine AHG administration, were tested for their capacity to sensitise monkey skin and subsequently cause histamine release upon challenge with bovine AHG (i.e. factor VIII). A reference reaginic serum (diluted 1/5) from a Timothy pollen sensitive individual, and a normal serum (diluted 1/2) were also included in the test as positive and negative controls respectively. An aliquot of each serum sample was heated for 1 hr at 56°C (under these conditions IgE, but not IgG, loses its skin sensitising activity).

Sites on the shaved abdomen of a normal baboon were injected (i.d.) 2 hr and 24 hr prior to antigen challenge with 0.2 ml of heated or unheated serum specimens; all tests being performed in duplicate. At the end of the sensitization period, 2 ml of 2% Evans blue mixed with 0.3 ml of Timothy pollen solution was given i.v. followed by a second i.v. injection of 20 ml of bovine AHG solution (i.e. one ampoule containing 360 i.u. of factor VIII) and the appearance of any blueing reaction was recorded.

Results

The patient under study was a haemophiliac who was admitted (day 0) to the hospital suffering from a fractured thigh with a haematoma. The patient was initially treated with human and porcine factor VIII preparations until day 51 when he started to bleed into the thigh again. This was then treated, for the first time in 8 years, with bovine factor VIII preparation. The patient showed a very severe anaphylactic reaction within 30 min after the administration of the bovine factor VIII.

Total IgE, IgG and IgG subclass levels were found to be normal in the serum samples taken before the patient developed the anaphylactic reaction to bovine AHG treatment, except possibly for IgG2 which was slightly raised on day 42 (Table 1). On day 53 (i.e. 2 days after the anaphylactic reaction) serum levels of total IgG, IgG1 and IgG3 were approximately double the pre-reaction levels. The most striking elevation was that of IgG4 which increased from 0.04 g/l, 9 days before the bovine AHG administration, to 4 g/l (i.e. 100-fold increase) 2 days after the anaphylactic reaction. Levels of IgE and IgG2 remained as they were before the reaction. Analyses performed on serial, post-reaction, serum samples showed that the high levels of total IgG, IgG1, and IgG4 persisted for several months until the patient died.

Table 1. Total IgE, IgG and IgG subclass levels measured in the sera (pre- and post-reaction) of a 46-year-old haemophiliac patient who developed anaphylactic reaction to bovine anti-haemophilic globulin (AHG) administration. ND = not detected. Figures between brackets represent the normal ranges for serum IgE and IgG subclasses as determined previously (McLaughlan & Stanworth, 1975; Shakib *et al.*, 1975)

Day	IgE μ /ml	Total IgG g/l	IgG1 g/l	IgG2 g/l	IgG3 g/l	IgG4 g/l
6	ND	14.5	9.6	3.7	0.32	0.03
42	55	15.2	6.5	5.5	0.29	0.04
51 (reaction to bovine AHG)	ND	ND	ND	ND	ND	ND
53	50	30.0	12.4	4.0	0.70	4.00
54	19	32.0	12.5	4.8	0.69	4.00
55	76	22.0	13.0	5.3	0.67	3.80
58	ND	20.7	12.5	4.3	0.34	3.70
62	ND	22.6	11.8	5.0	0.36	3.60
71	ND	24.8	12.2	5.2	0.32	3.30
75	ND	24.0	11.6	4.5	0.40	4.20
93	ND	25.5	11.9	5.0	0.42	5.60
144	ND	27.5	12.0	5.9	0.44	4.50
	(8-1660)	(7-18.5)	(3.7-12.3)	(0.46-4.3)	(0.15-2.45)	(0.015-0.185)

Using a radioimmunoassay system, antibodies specific for the bovine factor VIII proteins were measured within IgM, total IgG and IgG subclasses in the haemophiliac patient's sera (pre- and post-reaction samples). Results, which were expressed as anti-factor VIII activity (AF-VIIIa) index (obtained by taking the ratio of the counts/50 sec of the test sample to that of a normal serum specimen), are presented in Table 2.

In the pre-reaction serum sample levels of anti-bovine factor VIII antibodies (measured as radioactive counts/50 sec) of the IgM and IgG classes were found to be comparable to those found in a single normal serum sample. But analysis of the serum sample taken two days after the anaphylactic reaction showed a marked elevation in IgG anti-bovine factor VIII antibodies. Such antibody activities were found to be mainly of the IgG4 and, to a lesser extent, of the IgG3 subclasses. Subsequent analysis of serum samples taken 3 and 4 days after the anaphylactic reaction showed a

Table 2. Specific anti-bovine factor VIII antibodies measured in the sera (pre- and post-reaction) of a 46-year-old haemophiliac patient who developed anaphylactic reaction to bovine anti-haemophilic globulin (AHG) administration. ND = not determined. AF-VIII A = anti-factor VIII activity index (obtained by taking the ratio of the counts/50 sec of the test sample to that of a normal serum)

		AF-VIII A index					
Day	IgM	Total IgG	IgG1	IgG2	IgG3	IgG4	
42	1.03	0.96	0.89	0.99	0.94	1.07	
51 (reaction to bovine AHG)	ND	ND	ND	ND	ND	ND	
53	1.04	1.64	0.89	0.87	1.09	2.45	
54	1.13	1.82	0.81	0.79	1.30	2.55	
55	1.03	2.20	0.92	0.95	1.50	2.56	

persistent increase in these IgG4 and IgG3 anti-bovine factor VIII antibodies. Levels of IgM, IgG1 and IgG2 anti-factor VIII antibodies remained unchanged in the post-reaction serum samples.

Discussion

The use of a specific antibody assay system had demonstrated that anti-bovine factor VIII antibodies detected in the present haemophiliac patient are predominantly of the IgG4 and to a lesser extent, of the IgG3 subclasses. These findings are consistent with those of Andersen & Terry (1968) and Robboy *et al.* (1970) who reported, on the basis of IgG subclass specific absorption procedures, that antibodies to partially purified factor VIII protein were highly restricted to the IgG4 subclass in one case (Andersen & Terry, 1968) and to the IgG3 and IgG4 subclasses in one and four other cases respectively (Robboy *et al.*, 1970). It is not certain, however, whether the antibody activity found in the serum of the present patient is directed against the factor VIII protein or against the contaminating fibrinogen which represents the bulk (about 80%) of the factor VIII preparation used for the patient's treatment and for coating the tubes in the assay method. The development of antibodies (with severe anaphylaxis in the hypersensitised cases) to fibrinogen following replacement therapy in patients with congenital afibrinogenemia has been reported (De Vries *et al.*, 1961).

Nevertheless, the present haemophiliac patient represents the first instance where a severe anaphylactic reaction to bovine-AHG administration was shown to be associated with a significant increase in serum IgG4, both total (100-fold increase) and specific. IgE can hardly be implicated in the generation of the anaphylactic symptoms in this patient, since the measurement of its total levels (although not altogether adequate) in the post-reaction serum samples did not show any noticeable increase over the pre-reaction concentration which was well within the normal range. The increase (about 2 fold) of serum IgG1 levels following the bovine-AHG administration seems to be a non-specific one, since no anti-bovine factor VIII antibody activity was demonstrable in this subclass.

The ability of the patients sera (pre- and post-reaction samples injected 2 hr and 24 hr prior to antigen challenge) to mediate histamine release in monkey skin upon challenge with bovine factor VIII (one ampoule containing 360 i.u. of factor VIII) was tested. The results were negative, possibly due to the unavailability of sufficient antigen, i.e. factor VIII material, to initiate a response. Or alternatively a substantial amount of the antigen could have been neutralized by high concentration of non-skin sensitising antibodies at the injection site.

In the present patient the development of severe anaphylaxis in association with a sudden increase in total and specific IgG4 is interesting in view of some indirect evidence suggesting that short-term sensitising IgG antibodies are representative of the IgG4 subclass. Thus it has been shown (Stanworth & Smith, 1973; Vijay & Perelmutter, 1977) that a human myeloma protein of the IgG4 subclass, in contrast to myeloma proteins of the other three IgG subclasses, is capable of blocking PCA reactions in baboons mediated by human reaginic antibodies of the IgE class. Furthermore, six out of twenty four hay fever patients who were hyposensitised with a mixed pollen-tyrosine adsorbate were shown (McLaughlan & Stanworth, 1974) to mount a serum IgG anti-pollen antibody response that was restricted to the IgG4 subclass. This observation has been confirmed by other investigators who had observed that antibodies to castor allergen in certain castor bean sensitive individuals (Devey & Panzani, 1975) and to grass pollen in a relatively high proportion of grass pollen allergic patients (Van Der Giessen *et al.*, 1976; Devey, Wilson & Wheeler, 1976) were predominantly of the IgG4 subclass. It is also of interest in this connection that we have previously reported grossly elevated levels of IgG4 in a relatively high proportion of patients with diseases in which immediate-type hypersensitivity disorders are implicated, namely cystic fibrosis (Shakib *et al.*, 1976) and atopic dermatitis (Shakib *et al.*, 1977).

Our observations and those of others would, therefore, seem to indicate strongly the possible involvement of IgG4 antibodies in some human anaphylactic disorders. Confirmation of this, however, should await further tests to demonstrate such IgG4 anaphylactic antibodies in a monkey PCA system. In this connection it is interesting to know that Lakin and his associates (Lakin *et al.*, 1978) have recently reported the development of anaphylaxis to protamine sulphate mediated by IgG antibodies which could passively sensitise monkey skin for the anaphylactic release of histamine.

Acknowledgments

We are very grateful to Dr A. Pollock and Dr A. Lewis of the General Hospital, Birmingham for allowing us to study their interesting haemophiliac patient.

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