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Screening of blood donors for IgA deficiency: a study of the donor population of south-west England

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SUMMARY Altogether 29 745 English blood donors were screened for IgA deficiency by double diffusion analysis; 57 had apparent absence of IgA, a frequency of 1:522. Further examination by the more sensitive haemagglutination inhibition assay revealed 34 samples having no detectable IgA, a frequency of 1:875. All donors negative by double diffusion analysis were tested for the presence of antibodies to IgA. Six class specific anti IgA antibodies and four anti IgA antibodies of limited specificity were detected. Three of these had the specificity anti $\alpha 2$ and one anti A2m(2). The 34 IgA deficient donors detected provide a source of IgA deficient blood for transfusion to patients with anti IgA antibodies.

Selective IgA deficiency is the most common immunoglobulin deficiency. It has been shown to be associated with numerous disorders including ataxia telangiectasia, recurrent respiratory tract infection, coeliac disease, and many autoimmune diseases (Hobbs, 1968; Ammann and Hong, 1971; Bergström *et al.*, 1973). Selective IgA deficiency has also been found in apparently healthy blood donors with a frequency ranging from 1:300 to 1:3000 (Johansson *et al.*, 1968; Natvig *et al.*, 1971; Frommel *et al.*, 1973; Koistinen, 1975; Vyas *et al.*, 1975).

The relatively high frequency of this major immunoglobulin deficiency in turn gives rise to problems in transfusion practice. Individuals with IgA deficiency may have antibodies to IgA (Vyas and Fudenberg, 1971). These antibodies, usually known as 'class specific' anti IgA antibodies because they react with all IgA proteins tested, can be the cause of serious anaphylactic transfusion reactions (Vyas *et al.*, 1968b; Schmidt *et al.*, 1969; Bjerrum and Jersild, 1971; Leikola *et al.*, 1973; Pineda and Taswell, 1975). In addition, some patients with apparently normal levels of IgA can produce antibodies of limited specificity, reacting with some but not all IgA proteins. These antibodies may also cause transfusion reactions (Vyas *et al.*, 1968b; Pineda and Taswell, 1975).

In cases of transfusion reaction due to anti IgA antibodies it is important to provide compatible blood or plasma derived from IgA deficient donors. The aim of this study was to establish a panel of IgA deficient blood donors to meet this need.

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Material and methods

SCREENING FOR IGA DEFICIENCY

Serum samples from 29 745 volunteer blood donors were tested by double diffusion analysis in a medium containing 1% agarose (BDH, Poole, UK), in phosphate buffered saline (PBS) pH 7·3 (Dulbecco 'A', Oxoid, Basingstoke, UK) using a monospecific anti-IgA serum. The anti-IgA serum was prepared by immunising rabbits with a pool of four purified monoclonal IgA proteins. The antiserum was rendered specific for IgA by absorption with a pool of purified monoclonal IgG and IgM proteins. The lowest level of IgA detected in this double diffusion system was defined as 40 μg IgA/ml serum using a standard serum (Hoechst Pharmaceuticals, Hounslow, UK). Those sera failing to give a line of precipitation in the double diffusion screening test were further examined in a haemagglutination inhibition system using five haemagglutinating doses of a human class specific anti-IgA serum (JM) and human group O red cells coated with IgAl protein (Vyas and Fudenberg, 1971). The lowest level of IgA detected in the haemagglutination inhibition system was 0·5 μg IgA/ml serum. Only sera which failed to show any inhibition of the agglutinator were classified as IgA deficient (that is, less than 0·5 μg IgA/ml).

DETECTION OF ANTIBODIES TO IGA, IGM, AND IGG

Antibodies to IgA were detected by passive haemagglutination assay using a panel of six IgA myeloma proteins (Table 1) by the chromic chloride method of Gold and Fudenberg (1967). Optimal conditions for

Table 1 *Protein coats used in passive haemagglutination tests*

Proteins[1]	*Immunoglobulin class*	*'H' chain allotype*	*'L' chain type*
Hanks	IgA	—	Lambda
Watts	IgA	—	Kappa
Yeates	IgA	—	Kappa
Herbert	IgA	—	Kappa
Bruton	IgA	A2m (1)	Kappa
Schnell	IgA	A2m (2)	Lambda
Bennett	IgM	—	Kappa
Box	IgM	—	Kappa
Crumpler	IgM	—	Kappa
Turl	IgM	—	Lambda
Anti-D GD-78[2]	IgG	Glm (1, 2, 17)	—
Anti-D GD-59	IgG	Glm (1, 2)	—
Anti-D GD-97	IgG	Glm (1, 17)	—
Anti-D GD-99	IgG	Glm (3)	—
Anti-D GD-110	IgG	G3m (5, 10, 11, 13, 14)	—
Anti-D GD-68	IgG	G3m (5, 10, 11, 14)	—
Anti-D GD-93	IgG Kappa	Km (1, 2)	Kappa

[1]IgA proteins purified by method of Fine and Steinbuch (1970); IgM proteins purified by method of Johnson (1970).
[2]Gm and Km coating sera supplied by Miss D. M. Brazier, Blood Group Reference Laboratory, Gatliffe Road, London, UK.

Table 2 *Results of testing volunteer blood donors for the absence of IgA by double diffusion and haemagglutination inhibition assays*

	No. of samples	*Frequency*
Total samples tested	29 745	—
IgA deficient by double diffusion (<40 μg IgA/ml)	57	1:522
IgA deficient by haemagglutination inhibition (<0·5 μg IgA/ml)	34	1:875
Very low level IgA (0·5-40 μg IgA/ml)	23	1:1293

the coupling of the proteins were determined by the chequerboard method. The IgA coated cells were used at a concentration of 1% in PBS containing 2·5 mg/100 ml poly-vinylpyrolidone (PBS-PVP buffer) in V-shaped microtitre plates by the method of Vyas *et al.* (1968a). Sera were screened for antibodies at initial dilutions of 1/4 and 1/8 in PBS-PVP buffer. Antibodies detected in the screening tests were titrated and confirmed by specific inhibition tests using purified paraproteins as described by Vyas and Fudenberg (1971). Antibodies to IgM were detected by similar methods using a panel of four purified IgM paraproteins. Antibodies to IgG (Gm antibodies) were detected by incubating test serum at a dilution of 1/4 in saline with 2% saline suspensions of group O CDē/cDE cells coated with incomplete anti-D (selected for their ability to coat certain Gm and Km antigens) for two hours at 20°C (Table 1).

Results

Of 29 745 serum samples from volunteer blood donors tested by the double diffusion precipitation method, 57 were found to have less than 40 μg IgA/ml serum (a frequency of 1:522). The 57 samples were further examined for the presence of IgA by the more sensitive haemagglutination inhibition method; 23 samples contained detectable IgA by this method. The remaining 34 samples had less than 0·5 μg IgA/ml serum and were denoted IgA deficient for the purposes of this study (Table 2).

The 34 IgA deficient sera (<0·5 μg IgA/ml) and those sera with very low level IgA (0·5-40 μg IgA/ml) were examined for the presence of antibodies to IgA, IgM, and IgG by the passive haemagglutination method (see Methods) and the results are presented in Table 3. Antibodies specific for IgA but reacting with all six IgA coats (class specific) were found in six serum samples. Three samples contained antibodies specific for the IgA2 subclass of IgA (anti α2 antibodies) and one serum contained an antibody specific for the allotype A2m(2). Four samples contained anti IgM of limited specificity reacting with some but not all IgM coats. One sample contained an anti-Gm antibody specific for the Glm(1) allotype. In no case were anti IgA antibodies found in the 23 sera having very low levels of IgA (0·5-40 μg/ml).

Discussion

The frequency of IgA deficiency has been the subject of study by a number of workers (Bachmann, 1968; Frommel *et al.*, 1973; Hobbs, 1968; Johansson *et al.*, 1968; Natvig *et al.*, 1971; Koistinen, 1975; Vyas *et al.*, 1975) and reported values vary from 1:300 to 1:3000. This variation, although reflecting differences

Table 3 *Antibodies found in serum samples containing less than 40 μg IgA/ml*

	No. tested	*Class specific anti IgA low titre (<256)*	*Class specific anti IgA high titre (>256)*	*Anti IgA of limited specificity*	*Anti IgM of limited specificity*	*Anti Gm/Km antibodies*
IgA deficient (<0·5 μg IgA/ml)	34	2	4	4	3	1
Very low level IgA (0·5-40 μg IgA/ml)	23	0	0	0	1	0

in the populations studied, is to some extent due to the different techniques employed to define IgA deficiency (immunodiffusion, passive haemagglutination, radioimmunoassay). The results presented here for blood donors in England (frequency 1:875 using passive haemagglutination inhibition) are in broad agreement with data obtained by other workers using the same methods; in America, Vyas *et al.* (1975) found a frequency of 1:886 from a series of 73 569 blood donors, and in Finland Koistinen (1975) found 1:507 from 64 588 donors. The more sensitive method of radioimmunoassay has not been employed in this study, but from the data of Koistinen (1975) it would be expected that some of the donors defined as IgA deficient in this report would be found to have very low levels of IgA by this method.

The presence of specific anti IgA antibodies in the serum of IgA deficient blood donors is well known. In this study six of 34 (18%) IgA deficient donors were found to have class specific anti IgA antibodies, a finding in broad agreement with that of other workers (Koistinen and Sarna, 1975; Vyas *et al.*, 1975). In disease states associated with IgA deficiency the frequency of such antibodies is much higher, that is, 40-44% (Cassidy *et al.*, 1969; Vyas *et al.*, 1969; Ammann and Hong, 1971; Nadorp *et al.*, 1973), and this may be related to the higher incidence of blood transfusion in such patients.

An interesting finding of this study has been the presence of anti IgA antibodies of limited specificity in four of the 34 IgA deficient donors. The presence of antibodies of limited specificity in IgA deficient individuals has not been widely reported (van Loghem *et al.*, 1973; Nadorp *et al.*, 1973).

Human antibodies to IgM are known to occur in normal donor populations (Leikola *et al.*, 1971; Wells *et al.*, 1973); as yet no clinical problems have been related to their presence and their significance remains unclear. The antibodies found in this study are of low titre (1/4-1/16) and so are unlikely to cause problems in transfusion.

The 34 IgA deficient donors detected form the basis of a panel of IgA deficient donors providing blood for transfusion to patients with anti IgA antibodies. Similar donor panels are also available in America, Canda, Denmark, and Finland (Vyas and Perkins, 1976).

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