Distribution of immunoglobulin G subclasses in anti-A and anti-B sera

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SUMMARY Sera from 117 immunologically normal subjects, who had been selected for the presence of high titre ABO system antibody on routine screening, were further evaluated for the presence of IgG and its subclasses IgG₁, IgG₂, IgG₃, and IgG₄ using an indirect antihuman globulin technique.

Subjects of all ABO groups had the capacity to produce IgG antibodies within each subclass, but those of group O produced the broadest spectrum of IgG subclasses and greatest strength of reactions.

From the age of 6 months normal subjects show "naturally occurring" antibody to non-self ABO antigens. These antibodies are predominantly of IgM class, but under some circumstances, particularly in subjects of group O, large amounts of "immune" IgG antibody may be produced.¹ This is reflected in phenomena such as ABO haemolytic disease of the newborn.

To define more clearly the nature of the "immune occurring" antibody sera from immunologically normal subjects of various ABO groups, who had been found to contain high titre antibody on routine screening, were analysed for the presence of IgG subclasses IgG₁, IgG₂, IgG₃, and IgG₄.

Material and methods

One hundred and seventeen subjects were selected for the presence of high titre antibodies to ABO system antigens: 107 were normal blood donors, and 10 were immunologically normal patients requiring cross match before surgery. Forty three subjects were group A, 38 subjects group B, and 36 group O. Serum samples from blood donors of groups A, B, and O, known to have high titres of anti-A or anti-B, were selected at random and until testing were stored at -70°C. All other samples were tested within two days of collection.

NEUTRALISATION OF IGM ANTI-A OR ANTI-B

One volume (400 µl) of each serum sample was mixed with an equal volume of Dithiothreitol (DTT:20 mM in saline) and left to stand at room temperature (20-25°C) for 45 minutes. The sera were then tested for the presence of IgG anti-A or anti-B agglutinability using a tube technique; (two volumes of

serum treated with DTT mixed with one volume of 3% group A₁ or B red cells incubated at room temperature for 15 minutes, followed by a spin of 1500 rpm for 30 seconds). The samples were then inspected macroscopically and microscopically for agglutination. Any treated serum showing the presence of agglutinating IgG anti-A or anti-B was noted. No attempt was made to study the IgG subclasses of these sera as this would have entailed further dilution. Ignoring these sera ensured that when comparing the reaction strengths of the incomplete IgG subclasses of anti-A or anti-B in groups A, B, and O, the comparison was made at a serum dilution of 1:2.

INCOMPLETE IGA ANTI-B OR ANTI-B SUBCLASSES IgG subclass specific antihuman-globulin reagents (AHG) were obtained from Organon Teknika Ltd. Each serum sample was tested for the presence of incomplete IgG anti-A or anti B subclasses using each of the four subclass specific AHG reagents by a low ionic strength saline (LISS) indirect antihuman-globulin technique. Two volumes of serum treated with DTT were thoroughly mixed with one volume of 3% group A₁ or B red cells and two volumes of LISS reagent (LISS additive: Ortho Diagnostic Systems). Incubation of 15 minutes at 37°C took place, after which the cells were washed four times in physiological saline using a Sorvall II Coombs washer (Ortho Diagnostic Systems). Two volumes of the appropriate IgG subclass specific AHG reagent was added, mixed, and spun for 30 seconds at 1500 rpm. Each indirect subclass specific antihuman-globulin (AGH) test was inspected macroscopically and microscopically. The reaction strengths for each subclass within a treated serum sample were recorded by means of the scheme used in the National External Quality Assessment Scheme (NEQAS).

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Table 1 Characteristics of sera in which high titre antibodies were detected on screening

	Blood group of subjects			
	A (anti-B)	B (anti-A)	O (anti-B)	O (anti-A)
No in whom no IgG remained after neutralisation	7	6	1	2
No of sera (containing IgG) that could be evaluated	36	32	26	27
No of sera containing IgG agglutinins	0	0	3	7
Total No of subjects in each group	43	38	30	36

Table 2 No of IgG subgroups produced within sera containing IgG antibody (figures in parentheses are numbers %)

No of IgG subclasses	Group A subjects with anti-B	Group B subjects with anti-A	Group O subjects with anti-B	Group O subjects with anti-A
1	14 (38.8)	12 (37.5)	0 (0)	2 (7.4)
2	9 (25)	12 (37.5)	4 (15.3)	4 (14.8)
3	8 (22.2)	4 (12.5)	8 (30.6)	8 (29.6)
4	5 (13.8)	4 (12.5)	14 (53.8)	13 (48.2)
Total No of sera that could be evaluated	36	32	26	27

Table 3 No of subjects within each ABO group with particular IgG subclasses (figures in parentheses are numbers %)

Subclasses produced	Group A with anti-B	Subject group B with anti-A	Group O subjects with anti-B	Group O subjects with anti-A
IgG ₁	25 (69.0)	18 (54.2)	23 (87.4)	25 (92.5)
IgG ₂	25 (69.0)	20 (62.5)	24 (91.8)	25 (92.5)
IgG ₃	20 (55.2)	19 (58.9)	23 (87.4)	20 (74.0)
IgG ₄	5 (13.8)	7 (21.7)	19 (72.2)	15 (55.5)

Results

Table 1 shows the total number of subjects with high titre antibody compared with the number that could be evaluated for IgG subclasses after measures to neutralise IgM. No IgG remained after neutralisation in seven subjects from group A and six from group B. In only one group O serum was all the anti-B present neutralised and in only two was all the anti-A neutralised.

Numbers of subjects in whom no IgG remained after neutralisation were small for all groups, suggesting that all ABO groups are capable of an IgG response to non-self ABO antigens. In some group O cases agglutinating IgG anti-A or B remained after incubation with the neutralising agent. Such sera were not evaluated further.

THE RANGE OF IgG RESPONSE

Table 2 shows the spectrum of IgG shown by subjects of different ABO groups. Although the numbers within each group were small, there was a striking difference between patterns of IgG subgroups of anti-A and anti-B shown by group O sera compared with

those of groups B or A, respectively.

Subjects from group O were more likely to produce a wide spectrum response entailing three or more IgG subclasses than were subjects from groups A or B. Conversely, those from groups A and B tended to produce a smaller range of IgG subclasses. Around 50% of group O subjects produced all four IgG subclasses compared with only 12% of subjects from groups A or B. Around 38% of group A and B subjects produced only one IgG subclass of IgG anti-B or anti-A, respectively; in contrast, no group O subject produced less than two IgG components of IgG anti-B, and only two (7.4%) produced a single subclass in IgG anti-A.

THE NATURE OF THE IgG SUBCLASSES

Table 3 shows the number of subjects in each blood group showing the presence of the various IgG subclasses. The capacity to produce any particular subclass of IgG was not limited by blood group. IgG₄ immunoglobulin, however, was present in fewer subjects of groups A and B than in those of group O.

IgG₁ alone was produced by six group A subjects and four from group B for anti-B and anti-A,

Table 4 Presence of relatively high strength reactions (three-four plus) in indirect AHG tests, using antihuman IgG₁, G₂, G₃, G₄ in subjects of different ABO groups

	Group A anti-B		Group B anti-A		Group O anti-B		Group O anti-A	
No of sera	IgG ₁ 1	IgG ₃ 0	IgG ₁ 0	IgG ₃ 0	IgG ₁ 6	IgG ₃ 1	IgG ₁ 8	IgG ₃ 1
No of sera	IgG ₂ 0	IgG ₄ 0	IgG ₂ 1	IgG ₄ 1	IgG ₂ 4	IgG ₄ 0	IgG ₂ 9	IgG ₄ 2

respectively. Only one group O subject showed the presence of IgG₁ in isolation, and this was for the anti-A component alone.

IgG₂ was produced in isolation by six group A subjects (anti-B) and five group B subjects (anti-A). One group O subject showed isolated IgG₂ anti-A alone.

IgG₃ appeared alone in two group A subjects and three group B subjects, but was never an isolated finding in group O subjects either for anti-A or anti-B.

In no subject in which IgG₄ occurred did it appear alone: in almost all examples it appeared with IgG₁, IgG₂, and IgG₃. In three cases (representing all of the ABO groups) it occurred only with IgG₂. Conversely, in only three instances was IgG₄ found in the absence of IgG₂.

THE STRENGTH OF THE IgG RESPONSE

The sera of group O subjects showed a greater than "3" (NEQAS) response for IgG₃ or IgG₁—in eight cases for anti-A and in six cases for anti-B. For subjects of groups A and B the IgG₃ and IgG₁ components were relatively weak ("1-2" reaction), and only one subject (of group A) produced a "4" reaction with IgG₁ antiserum alone. Overall, IgG₁ was more often found in strong reactions than IgG₃.

The sera of group O subjects was also more commonly found to give strong reactions ("4" or greater) by indirect AHG, using anti-IgG₂ and anti-IgG₄ reagents. Thus no group A subject showed a greater than "3" response to either IgG₂ or IgG₄, and only one group B subject showed a strong IgG₂ reaction, and one a strong IgG₄ reaction.

Four group O subjects produced a strong reaction with the IgG₂ reagent within the anti-B fraction, and nine group O subjects showed a strong IgG₂ reaction within the anti-A fraction. Within the anti-B fraction no group O subject showed a strong reaction with the IgG₄ reagent, but two subjects gave strong reactions with IgG₄ reagent, within the anti-A fraction.

Table 4 shows the relative strength of sera of different ABO groups in indirect AHG tests using anti-IgG₁, anti-IgG₂, anti-IgG₃, and anti-IgG₄.

Discussion

It has long been accepted that subjects of group O produce anti-A and anti-B of a greater destructive

potential than do those of group B and group A. Group O blood donors are more often found to possess haemolytic anti-A and anti-B in their serum, and babies with ABO haemolytic disease of the newborn are almost exclusively born to mothers of group O.²⁻⁴

After deliberate immunisation with appropriate glycoprotein (probably T cell dependent) antigen Contreras *et al*⁵ showed that the IgG response is greater in group O subjects than in those from groups A or B. They also noted that even before immunisation the presence of some IgG antibody could be shown in all subjects, although at a lower titre in those of groups A or B. IgG subclasses were not analysed.

Our study shows that among normal subjects with a high titre antibody to ABO system antigens most show some IgG activity: this is not restricted to subjects of group O. It is possible for subjects of any ABO group to show the appropriate antibody within any, or all of, the four subclasses of IgG. Our results, however, suggest that subjects of group O produce a wider spectrum of response and a greater strength of antibody within each subclass. Although only a few subjects in this study produced strong reactions within IgG₁ and IgG₃ subclasses, they were all group O with the exception of one subject. The Fc portions of IgG₁ and IgG₃ confer the ability to cross the placenta, adhere to macrophages, and fix complement, all of which are lacking in IgG₂ and IgG₄: they may help to explain the greater destructive potential of immune anti-A and anti-B noted in group O subjects.

Recently, it has been suggested that subjects of group O are able to produce IgG antibodies to non-self ABO system antigens more readily than those of group A or B because there is a greater antigenic disparity between group O and groups A/B than that between group A and group B. This was postulated to allow a greater ability to provide T cell help during the response to T dependent (glycoprotein) forms of ABO system antigens in subjects of group O.⁶ T cell help is known to allow responding B cells to change from IgM production to IgG, giving a less restricted response. Responses to T independent antigens (for example, environmental ABO antigen) are known to be more restricted, often to IgM alone, or to limited IgG subgroups—predominantly IgG₂ and IgG₄.⁷

The results of our study suggest that T cell help during response to non-self ABO antigen is not restricted to subjects of group O, but that the efficiency of T cell help is greater in these subjects, permitting a broader stronger response. The cellular interactions entailed⁶ will require in vitro analysis.

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