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A monoclonal IgMκ from a blood group B individual with specificity for α-galactosyl epitopes on partially hydrolyzed blood group B substance [+]

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ABSTRACT

We have characterized a human monoclonal IgMκ, designated IgM^{DON}, from a blood group B individual. IgM^{DON} is specific for α-galactosyl residues on blood group B substance; its fine specificity as defined by hemagglutination, quantitative precipitin, and inhibition ELISA assays was for the defucosylated terminal Gal(α1-3)Gal epitope. Gal(α1-3)Gal epitopes are also found on a variety of normal and pathogenic intestinal bacteria, and polyclonal IgG antibodies with the same specificity are found in the serum of nearly all normal individuals. The specificity of IgM^{DON} was also quite similar to that of a human antiserum, serum 262, obtained by immunizing an individual with blood group B substance that had been subjected to mild acid hydrolysis (BP1). The possible ways whereby IgM^{DON} might have arisen are discussed.

INTRODUCTION

Human serum monoclonal macroglobulins with specificity for various red blood cell antigenic determinants have been well described. Most have specificity for glycoproteins and glycolipids present on all erythrocytes[1–4]. Human monoclonal macroglobulins specific for ABO blood group determinants are much less common. Antibodies to I and i determinants found in the interior of blood group A, B, H, Le^a, and Le^b substances have been reported and may also arise in mycoplasma infections[1]. A monoclonal, IgM^{WOO}, with specificity for a non-I, non-i core determinant of the blood group A, B, H glycoprotein structures in a patient with bronchogenic carcinoma, was reported by this laboratory in 1982 (ref 5) and further evaluated by Picard et al.[6]. The antibody was associated with an unusually

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favorable clinical course, raising the possibility that it was recognizing altered blood group determinants on the tumor cells. In 1972, Rochant et al. reported an IgMλ which could agglutinate type A_1 red blood cells (including the patient's own) in a patient of A_1 blood type who had reticulosarcoma[7]. This again suggested that plasma cell dyscrasias might in certain circumstances arise in response to antigens on tumor cells and that in some cases these antigens might be blood group substances.

We have now characterized a human monoclonal IgMκ, IgM^{DON}, from an individual of blood group B with specificity for the terminal α-galactosyl epitope revealed after mild acid hydrolysis of blood group B substances, BP1. The antibody also binds to a lesser degree to blood group A substances subjected to mild acid hydrolysis. It does not appear to cause hemolysis. It is remarkably similar in specificity to a human antiserum described by this laboratory in 1959 (ref 8) which was made by an A_1B individual immunized with BP1 fraction. The specificity of IgM^{DON} is also closely related to that of naturally occurring polyclonal IgG antibodies found in human sera that bind Gal(α1-3)Gal[9–12]. These antibodies bind defucosylated blood group B substance[11], as well as the carbohydrate portion of a variety of bacterial lipopolysaccharides[13]. Their physiological significance is not known, but their titers appear to rise in response to certain infections, such as American cutaneous leishmaniasis and Chagas disease[14–17]. In addition, they may bind to senescent erythrocytes (RBCs), and it has been proposed that they could play a role in enhancing the extravascular destruction of these erythrocytes[18,19]. Although the role of antigenic stimuli in macroglobulinemia is not known, it is possible that this antibody represents a response to defucosylated blood group B substance, or to α-galactosyl residues present on intestinal flora, or possibly revealed on senescent RBCs.

MATERIALS AND METHODS

Serum.—The monoclonal IgMκ, IgM^{DON} was identified incidentally in the serum of an 81 year old man by serum electrophoresis and immunofixation. Significant events in his medical history included porcine aortic valve replacement and coronary bypass surgery for aortic stenosis and single vessel coronary artery disease in 1984. After his surgery he had ventricular arrhythmias treated with procainamide and complicated by procainamide induced leukopenia and thrombocytopenia, which responded to withdrawal of procainamide and a short course of corticosteroids. The monoclonal IgM was identified during the course of the evaluation of his hematological abnormalities, but was not thought to be causally related. Between 1985 and 1990 the monoclonal protein was present in the serum at a concentration of 6–11 mg/mL with no associated hematological abnormalities. In November, 1990 the patient became jaundiced and anemic and was found to have a pancreatic carcinoma from which he died several months later.

Blood group substances.—The blood group glycoproteins used in the quantitative precipitin studies have been described previously. The human blood group substances were fractionated from human ovarian cyst fluid and saliva[20–25], and cow, horse, and hog blood group substances from gastric mucosa[26–28]. P1 fractions were prepared from the purified blood group substances by mild acid treatment (pH 1.5–2.0 at 100°C for 2 h), exhaustive dialysis against distilled water, and isolation of the nondialyzable residue by ethanol precipitation or lyophilization. These procedures and the analytical properties of the untreated preparations and their P1 fractions have been described in detail[20].

Inhibitors.—Monosaccharides, glycosides, and oligosaccharides used as inhibitors were as previously described[8,29]. Commercial samples of galactose, lactose, and melibiose were from Eastman Kodak, and galactinol was from the Great Western Sugar Co., Denver, CO. Gal(α1-3)Gal was prepared in this laboratory by Dr. J. Weissman. The A and B trisaccharides were kindly provided by Dr. A. Lundblad.

Hemagglutination.—Hemagglutination tests were carried out at room temperature and at 0°C in microtiter plates, using 25-μL volumes of 2% human type A, B, and O red blood cells and dilutions of the patient's serum. Hemagglutination was also performed using a 10% suspension of glutaraldehyde fixed rabbit RBCs and with rabbit RBCs pretreated with α-galactosidase. Both preparations of rabbit RBCs were kindly provided by Dr. U. Galili.

Immunochemical studies.—Quantitative precipitin assays were carried out as described previously[29]. A sample (35 μL) of a 1:4 dilution of serum was added to varying quantities of blood group substance or polysaccharide, and the total volume was brought to 200 μL with saline. The contents were mixed, kept at 37°C for 1 h, and then at 4°C for one week with daily mixing. The tubes were then centrifuged at 4°C at 2000 rpm, the supernatants were decanted, and the precipitates were washed twice with 0.5 mL of chilled saline. Total nitrogen in the washed precipitates was determined by the ninhydrin assay[23].

Inhibition ELISA assay.—Inhibition ELISAs were carried out as described[29] using Corning polystyrene plates (Corning, New York). The plates were coated with 1 μg of bovine blood group B substance[26], cow 26. The wells were washed with phosphate-buffered saline (PBS)–0.05% Tween, blocked with 1% BSA, and washed again. Varying quantities of the inhibitors and 50 μL of a 1:500 dilution of IgM^{DON} were brought to 100 μL with borate-buffered saline, mixed, and added to the wells. After incubation for 3 h at 37°C, plates were washed and alkaline phosphatase conjugated goat anti-human IgM (Sigma) was added. Plates were developed using *p*-nitrophenyl phosphate. Percentage inhibition was calculated as follows (A = absorbance): % inhibition = $100 \times$ [(standard A − blank A) − (A with inhibitor − blank A)]/(standard A − blank A).

ELISA assays for thyroglobulin binding.—ELISA plates were coated with preparations of either porcine, bovine, or human thyroglobulin at a concentration of 30 μg/mL in carbonate buffer, pH 9.5 provided by Dr. U. Galili. They were

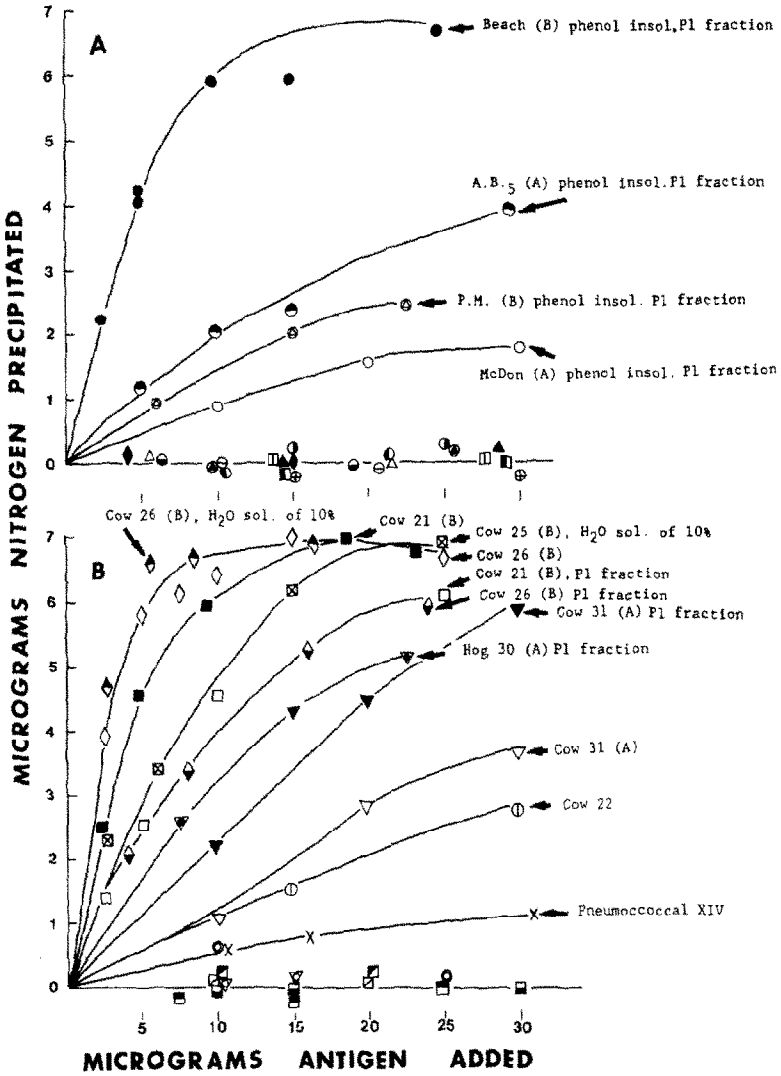

Fig. 1. Quantitative precipitin curves of IgM^{DON} with various antigens. **A**, with human blood group substances: ◧, Beach (B) phenol insol.; ●, Beach (B) P1 fraction; ⊖, Beach (B) phenol insol. first IO_4; ⊕, P.M. (B) phenol insol.; ○, P.M. (B) P1 fraction; ◔, P.M. (B) 10%; ◐, $A.B._5$ (A) phenol insol.; ◓, A.B.5 (A) P1 fraction; ◑, $A.B._5$ 10% ppt.; ◒, McDon (A) phenol insol.; ○, McDon (A) P1 fraction; △, J.S. (H) IO_4BH_4 nondial.; ◆, Morgan (B); ▲, Tij II (BI) phenol insol.; ◫, Tij II (BI) 20% 2X. **B**, with cow, horse, and hog blood group antigens as well as pneumoccocal XIV polysaccharide: ◇, Cow 26 (B); ◇, Cow 26 (B) P1 fraction; ◈, Cow 26 (B) H_2O sol. of 10%; ■, Cow 21 (B) H_2O sol. of phenol insol. 10% ppt.; □, Cow 21 (B) P1 fraction; ⊠, Cow 25 (B) H_2O sol. of 10%; ▽, Cow 31 (A); ▼, Cow 31 (A) P1 fraction: ⊕, Cow 22 (inactive); ⊛, Cow 22 P1 fraction; ▼, Hog 30 (A); ▽, Hog 30 (A) P1 fraction; ◙, Hog 13 (H); ◙, Hog 13 (H) P1 fraction; □, Horse 4 25%; ◩, Horse 4 P1 fraction; ×, pneumococcal XIV polysaccharide.

incubated overnight at 4°C, blocked with 1% BSA, washed with PBS-Tween, and incubated with serial dilutions of serum. The plates were then washed again and incubated with a 1:1000 dilution of alkaline phosphatase-labeled goat anti-human IgM (Sigma). Plates were developed using *p*-nitrophenyl phosphate.

RESULTS

Hemagglutination assays.—At both 37 and 0°C, serum^{DON} agglutinated type A but not type B or O erythrocytes, as would be expected of a type B individual. The IgMκ in this serum was not a cold agglutinin or a cryoglobulin. The serum agglutinated glutaraldehyde fixed rabbit RBCs, which contain abundant α-galactosyl epitopes, at a dilution of 1:3200, but did not agglutinate α-galactosidase-treated rabbit RBCs at all.

Quantitative precipitin reactions.—As shown in Fig. 1A, IgM^{DON} did not react with intact human A, B, BI, or H substances. However, it did react with the acid hydrolyzed P1 fractions of human blood group B substance Beach and to a lesser extent with the P1 fraction of human blood group B substance P.M. The difference in reactivity between the two P1 derivatives probably reflects intrinsic differences between the two starting materials in chemical composition and numbers of determinants per molecule (P.M. is from human saliva, Beach from ovarian cyst fluid). IgM^{DON} also reacted with the P1 derivatives of blood group A substances A.B.$_5$ and McDon. As shown in Fig. 1B, it reacted with intact bovine blood group B substances cow 26, cow 21, and cow 25, and to a lesser degree with bovine A substance, cow 31, and an inactive blood group substance, cow 22. It also reacted strongly with the acid hydrolyzed P1 fractions of cow blood group A (cow 31) and B (cow 21, 26) substances, but not with the P1 fraction of cow 22 (inactive). It did not react with intact hog blood group A (hog 30) or H (hog 13) substances, but it did react with the acid hydrolyzed P1 fraction of hog blood group A substance (hog 30P1). It did not react with either the intact or the P1 fraction of a horse blood group B substance, horse 4. Thus, the specificity appeared to be for a determinant found on both intact and acid hydrolyzed P1 fractions of bovine blood group substances but only on acid hydrolyzed P1 fractions of human blood group A and B and hog blood group A substances. Since the precipitin reactions were done using whole serum it was important to determine that these reactivities were due to the monoclonal IgM and not to other serum immunoglobulins. Supernatants from samples having the maximum nitrogen precipitated were subjected to electrophoresis on cellulose acetate in parallel with the original serum. Reaction with cow 26 completely removed the monoclonal peak as shown in Fig. 2.

The pattern of reactivity was strikingly similar to that of a human antiserum 262 described from this laboratory in 1959. That antiserum was obtained by immunizing an A_1B individual with human BP1 substance[8]. As shown in Table I serum 262, like IgM^{DON}, reacted with both intact and acid-hydrolyzed P1 bovine blood group substances, and acid-hydrolyzed P1 human B substance. Serum 262 however

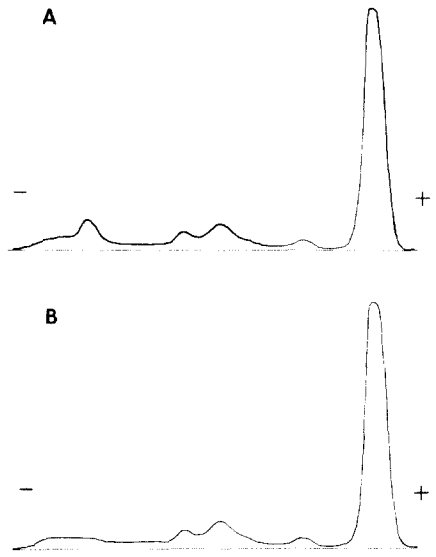

Fig. 2. Electrophoresis of serum DON before (A) and after (B) reaction with blood group substance cow 26.

reacted less well than IgM^{DON} with the acid-hydrolyzed P1 fractions of human and hog blood group A substances.

There were also some interesting similarities and differences between IgM^{DON} and horse anti-type XIV pneumoccocal sera, as shown in Table I. The horse

TABLE I

Comparison of the binding of IgM^{DON}, serum 262, and horse type XIV pneumococcal antiserum to various blood group substances and their acid hydrolyzed P1 fractions

Blood group substance	Reactivity with intact antigens			Reactivity with P1 antigens		
	IgM^{DON}	Serum 262	Ho Anti-XIV	IgM^{DON}	Serum 262	Ho Anti-XIV
Human:						
Beach phenol insol. (B)	−	−	+	+ + + +	+ + + +	+ +
P.M. phenol insol. (B)	−	+	+ +	+ +	+ + + +	+ +
$A.B._5$ phenol insol. (A)	−	−	+ + + [a]	+ +	−	+ + + + [a]
Cow:						
Cow 26 (B)	+ + + +	+ + + +	+ +	+ + +	+ + + +	+ + +
Cow 22 (Inactive)	+ +	+ +	+ + + +	−	+	+ + + +
Cow 31 (A)	+ +	+ + +	−	+ + +	+ + + +	+ + + +
Horse:						
Horse 4 25% (B)	−	+	+ +	−	+ + +	+ +
Hog:						
Hog 30 (A)	−	−	+	+ + +	−	+ + +
Hog 13 (H)	−	+	+	−	+	+ + +

[a] Horse type XIV pneumoccocal antiserum was not tested with human $A.B._5$. The data shown reflect cross-reactivity with several other human A substances[32].

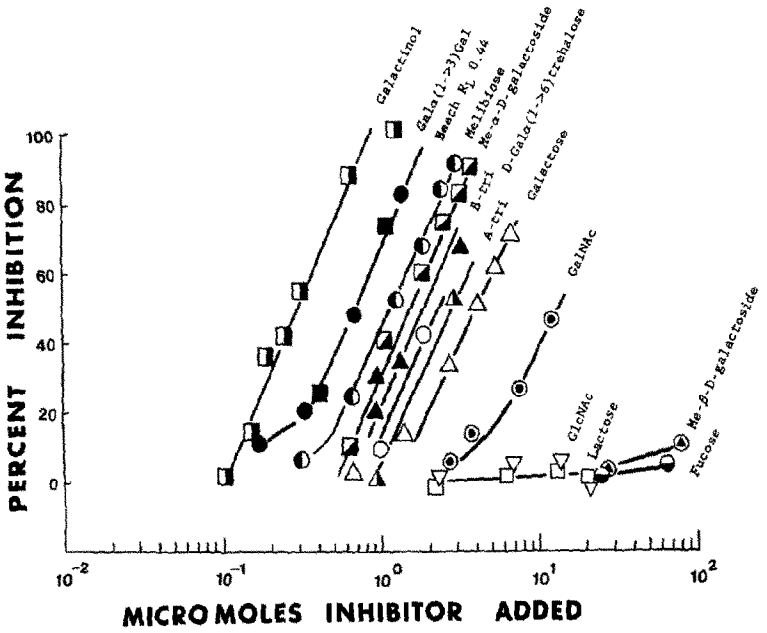

Fig. 3. Inhibition by various oligosaccharides of the binding of IgM^{DON} by blood group substance cow 26. See Table II for key to the symbols and structures of the corresponding oligosaccharides.

antisera have been extensively characterized and their cross-reaction with human, bovine, horse, and hog blood group substances, particularly with the mild acid hydrolyzed P1 derivatives, has been detailed[30–34]. Further studies indicated that the type XIV pneumococcal antisera were probably recognizing terminal β-galactosyl groups exposed by mild acid hydrolysis[32,33]. The sites recognized by the anti-BP1 serum differed, and appeared to consist of terminal α-galactosyl residues[34,35]. IgM^{DON} reacted minimally with type XIV pneumococcal, polysaccharide (Fig. 1B); its pattern of reactivity was much more closely related to that of serum 262 than to that of horse pneumoccocal XIV antisera.

Inhibition ELISAs.—The binding specificity of IgM^{DON} was further defined by inhibition ELISAs in which various oligosaccharides were used as antagonists to its binding to blood group B substance cow 26 (Fig. 3 and Table II). The best antagonist was galactinol[36] [Gal(α1-3)1D-*myo*-inositol] with 50% inhibition obtained 0.3 μmol added. Gal(α1-3)Gal and Beach R_L 0.44, a B-oligosaccharide which retains the fucose group α-linked to the subterminal galactose, were about half as active, 0.7 μmol needed for 50% inhibition. Melibiose and Me α-D-galactoside were less active, with 1.3 and 1.6 μmol, respectively, required for 50% inhibition. The B-trisaccharide, D-Gal(α1-6)trehalose, A-trisaccharide, and galactose gave 50% inhibition at 1.8, 2.2, 2.8, and 4.0 mol, respectively. *N*-Acetylgalactosamine was a much weaker inhibitor with 12 μmol required for 50% inhibition.

TABLE II

Inhibition by various oligosaccharides and related sugars of the binding of IgM^{DON} and serum 262 to BP1 antigens

Symbol	Oligosaccharide	Amount of 50% inhibition (μmol) [a]	
		IgM^{DON}–Cow 26	Serum 262–P.M.(B)P1
◨	Galactinol: Gal(α1-3)1D-*myo*-inositol	0.3	0.2
●	Gal(α1-3)Gal	0.7	0.1
■	Beach R_L 0.44: Gal(α1-3)Gal(1(β1-4)GlcNAc-R 2 ↑ Fucα1	0.7	NA [b]
◐	Melibiose: Gal(α1-6)Glc	1.3	≫ 20
◪	Me α-D-galactoside	1.6	6.0
▲	B-tri: Gal(α1-3)Gal 2 ↑ Fucα1	1.8	NA
○	D-Gal(α1-6)trehalose	2.2	NA
▲	A-tri: GalNAc(α1-3)Gal 2 ↑ Fucα1	2.8	NA
△	Galactose	4.0	12
◉	GalNAc	12.0	≫ 5
▽	GlcNAc	≫ 20	≫ 25
□	Lactose: Gal(β1-4)Glc	≫ 20	≫ 80
◓	Me β-D-galactoside	≫ 80	≫ 20
◒	Fucose	≫ 70	≫ 6

[a] For IgM^{DON} compounds were tested for inhibition of binding to cow 26. Data for Serum 262 show inhibition of binding to the P1 fraction of human blood group B substance P.M. and were reported in refs 8 and 35. However the experiments reported in ref 8 used five times the amount of antiserum as those in ref 35, so that for compounds not retested in ref 35 (galactose, GalNAc, GlcNAc, lactose, Me β-D-galactoside, and fucose), the values from ref 8 were divided by five for the purposes of this table.
[b] NA: oligosaccharide not available at the time of the studies cited.

N-Acetylglucosamine, lactose, Me β-D-galactoside, and fucose all gave less than 10% inhibition at the maximum amounts used.

With several notable exceptions this pattern of inhibition was similar to that obtained with serum 262 (Table II and refs 8 and 35). Serum 262 was also inhibited by α-galactosides, although for serum 262 Gal(α1-3)Gal was a more potent inhibitor than galactinol. As for IgM^{DON}, Me α-D-galactoside and galactose were weaker inhibitors, but in contrast to its behavior with IgM^{DON} melibiose was a very weak inhibitor. As with IgM^{DON}, binding of serum 262 by BP1 was not significantly inhibited by *N*-acetylglucosamine, lactose, Me β-D-galactoside, or fucose. Although the studies with serum 262 were done by addition of oligosaccharide inhibitors to quantitative precipitin assays and the studies on IgM^{DON} were done using ELISAs the two methods yield comparable results when the amounts of antigen and antibody are adjusted according to the quantitative precipitin data. The advantage of ELISAs is that far less serum is needed[37]. The results with serum 262 were interpreted as evidence that terminal nonreducing α-galactosyl

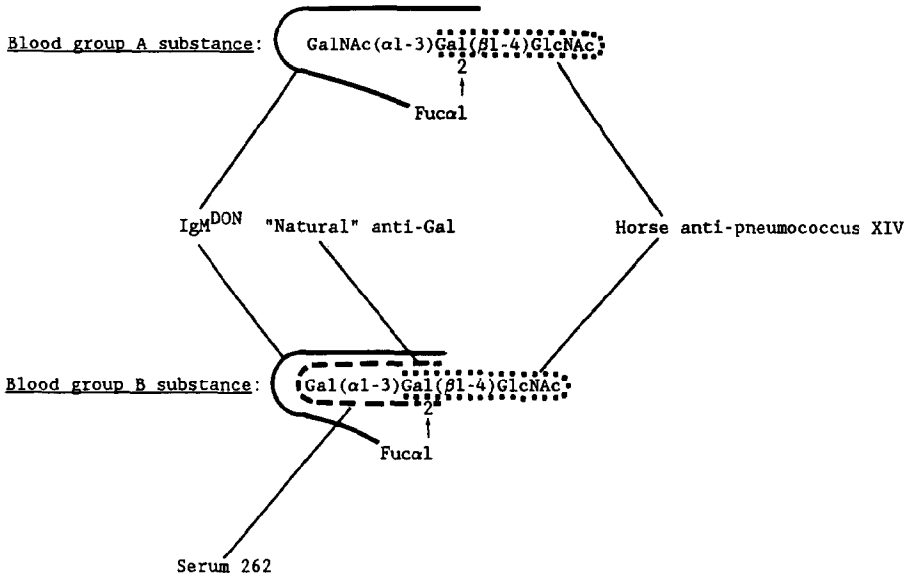

Fig. 4. Probable binding sites of IgM^{DON}, serum 262, and type XIV pneumococcal antisera on blood group A and B substances. Only type 2 structures in which the second sugar (Gal) is linked β1–4 to the third sugar (Glc or GlcNAc) are shown because the type XIV pneumococcal antisera were much more specific for the β1–4 than the $\beta 1 \rightarrow 3$ linkages[22,28].

residues were involved in the BP1 specificity[35]. This was later confirmed with the elucidation of the structure of B antigenic determinants[38–40].

Studies of acid-hydrolyzed and enzyme-treated B oligosaccharides showed that the B determinant consists of Gal(α1-3)Gal, β-linked to GlcNAC either 1–3 in type 1 structures, or 1–4 in type 2, with an L-Fuc linked to the subterminal galactose. The BP1 determinant was revealed with removal of the fucose groups[35]. Both serum 262 and IgM^{DON} appeared to react best with terminal α-galactosyl moieties, while the type XIV antipneumococcal sera recognized the second and third sugars and their β linkage (Fig. 4). In our current studies, IgM^{DON} was remarkably similar to serum 262 except that it also reacted to some extent with the P1 fractions of blood group A determinants in which GalNAc rather than Gal is linked α1–3 to Gal, and also compared to serum 262, it was inhibited better by melibiose, in which galactose is linked α1–6 to glucose. The similarity in reactivity with 262 raises the possibility that BP1-like antigens could under certain circumstances be present in vivo.

ELISAs for thyroglobulin binding.—Thyroglobulins from different species contain varying amounts of Gal(α1-3)Gal(β1-4)GlcNAc, with bovine thyroglobulin containing the most, porcine thyroglobulin containing $\sim$ 50% as much, and human thyroglobulin containing none.[41] In ELISAs, IgM^{DON} bound to bovine thyroglobulin at a dilution of $> 1:500$, but did not bind to porcine or human thyroglobulin.

DISCUSSION

Monoclonal macroglobulins with specificity for blood group I, i, and Pr antigens have been well described[1], and they are sometimes responsible for causing immune hemolytic anemia. Monoclonal antibodies to ABO determinants, however, have been only rarely identified. The reactivity of IgM^{DON} is extremely unusual and its similarity in specificity to that of serum 262 obtained by immunizing an individual of blood type AB with human B P1 blood group substance is striking[8]. It also appears to be closely related to naturally occuring human anti-α-galactosyl IgG[9–12].

Previous studies defining the interactions of A and B antisera and the BP1 antiserum 262, as well as type XIV pneumococcal antisera, with a variety of oligosaccharide inhibitors and blood group derivatives helped to define the structures of blood group A and B substances[8,30–36,38–40]. Those studies revealed that the difference between the anti-B and the anti-BP1 specificities is due to the fucose group linked to the penultimate galactose residue, with the anti-BP1 recognizing the defucosylated moiety. The horse type XIV antipneumococcal sera appeared to recognize a more interior epitope present in the P1 fractions of both A and B blood group substances, consisting of the penultimate galactose linked β1–3 or β1–4 to GlcNAc. In inhibition studies it appeared to react somewhat better with the (β1–4)-linked oligosaccharides[34,42] present in type 2 blood group structures.

IgM^{DON} reacted with intact cow blood group substances, and acid-hydrolyzed human P1 derivatives. As the cow blood group substances differ from the human mainly by virtue of having a lower fucose content, and the acid hydrolysis treatment used to make the P1 derivatives has been shown to remove fucose as well as some other sugar groups[32], IgM^{DON} appeared to recognize defucosylated blood group substances, as did the human BP1 antiserum 262. The inhibition data confirmed the similarity with serum 262. IgM^{DON} differed from serum 262 in that it was also inhibited relatively well by the B trisaccharide and by BR_L 0.44, indicating that in these small oligosaccharides the fucose did not block the interaction of IgM^{DON} with the Gal(α1-3)Gal epitope. IgM^{DON} also differed in that it had some cross reactivity with AP1 blood group substances. Its specificity was, therefore, less dependent on the terminal galactose of the B substances, and probably involved recognition of a region of the terminal GalNAc, present in A substances, not involving the acetamido group (Fig. 4). As IgM^{DON} was monoclonal and serum 262 was polyclonal these minor differences are not surprising.

The specificity of IgM^{DON} was also so similar to that of naturally occurring human IgG antibodies of the anti-α-galactosyl type as to suggest that it was produced from an expanded clone of anti-Gal cells. These anti-Gal antibodies constitute as much as 1% of the circulating IgG in normal healthy adults[9–12], and have been hypothesized to arise from antigenic stimulation by α-galactosyl residues present on normal gut flora[13]. As mentioned earlier, high titers of anti-α-galactosyl antibodies are also present in patients with Chagas disease or American cutaneous

leishmaniasis, and in both cases they appear to have a major role in lysing parasites[14–17]. Inhibition studies using normal sera and those from patients with trypanosoma and leishmania infections demonstrate a pattern of oligosaccharide inhibition strikingly like that of IgM^{DON}, with the antibodies strongly inhibited by Gal(α1-3)Gal, melibiose, Me α-D-galactoside, and the B trisaccharide[9,10,12,18]. Also like the natural anti-Gal's, IgM^{DON} bound to rabbit RBCs with their abundant Gal(α1-3)Gal epitopes, but not to α-galactosidase-treated rabbit RBCs, and it bound to bovine but not human thyroglobulin.

Galili et al.[43] have demonstrated that there is a reciprocal relationship between the presence of anti-Gal's and the expression of Gal(α1-3)Gal epitopes. In nonprimate mammals as well as New World monkeys Gal(α1-3)Gal is present in a wide variety of tissues, but anti-Gal is absent, while Gal(α1-3)Gal epitopes are absent from the tissues and erythrocytes of humans and Old World monkeys and apes, but there is anti-Gal present in the sera. During primate evolution Gal(α1-3)Gal expression was apparently suppressed and antibodies to this sequence were stimulated. Although the stimulus for the development of these antibodies and the suppression of the expression of their epitopes is not known, one hypothesis is that selective pressure could have been exerted by a pathogen expressing Gal(α1-3)Gal, or requiring Gal(α1-3)Gal for binding to host tissues. It has also been suggested by Galili and others that anti-α-galactosyl antibodies could play a role in autoimmune phenomena if α-galactosyl epitopes were to become abnormally expressed on tissues through increased (α1-3)-galactosyltransferase activity[44,45].

Although the role of antigen, if any, in plasma cell dyscrasias remains unknown, this particular activity arising in a blood group B individual is intriguing. IgM^{WOO} (ref 5), and antibodies to I, i, and other more interior blood group determinants have been thought to identify structures on the biosynthetic pathway to the blood group substances and to constitute an autoimmune response to incomplete blood group substances present on normal or neoplastic cells[46]. Since the studies of Watkins (reviewed in ref 40) have shown that the L-Fuc is added to generate H substance prior to the α-linked terminal D-Gal or D-GalNAc, and IgM^{DON} recognises the defucosylated terminal sugars not present in the biosynthetic pathway, it is unlikely that IgM^{DON} represents an autoimmune response to an antigen present during normal blood group B biosynthesis.

It is possible that Gal(α1-3)Gal is revealed during the degradation of blood group B substance or other red-cell antigens. Gut flora bearing α-galactosyl epitopes could also have provided an antigenic stimulus, although we have not tested whether IgM^{DON} binds bacterial antigens. Another possibility is that the patient's porcine aortic valve graft was a source of antigen. Although pigs have not been shown to have blood group B determinants, blood group A and H determinants have been found in porcine aorta[47]. Since the antibody did react with Hog AP1 (but not with intact Hog A), even if the antibody arose in response to the porcine graft one would have to invoke a degradative mechanism by which a defucosylated A substance was generated. Samples of serum prior to the valve

replacement surgery were not available to determine the temporal relation of the valve replacement and the appearance of the monoclonal protein.

Alternatively, the clone of cells producing IgM^{DON} may have arisen from the pool of cells which produce natural anti-Gal. Recent studies have demonstrated that these clones may be relatively frequent[48], so that it may be possible to identify other monoclonal proteins of similar specificity from other individuals with monoclonal gammopathy. Continued efforts to characterize the antigenic specificities of human monoclonal antibodies and their relationship to antibodies which occur in normal immune responses or as part of the "natural" unstimulated repertoire is critical in providing clues as to the nature of the role of antigen in provoking or perpetuating plasma cell dyscrasias.

This specificity and that of the closely related BP1 antiserum 262 do not appear to be pathogenic or to be associated with any detectable autoimmune phenomena. The individual who donated serum 262 has been well over the 30 year period since immunization with BP1 (personal communication). Likewise the anti-Gal present in most normal individuals does not appear to be harmful.

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