

Immunogenetics of a macroglobulin allotype in cattle

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SUMMARY

The paper describes a cattle serum antigen (McA2) located on a macroglobulin molecule which has its isoelectric point at pH 5 and is capable of interacting with the wheat germ lectin and concanavalin A.

The specificity is inherited in a simple Mendelian manner and the gene controlling its synthesis is allelic to the one controlling the synthesis of the McA1 antigen.

1. INTRODUCTION

Several serum antigens (allotypes) have been by now identified in cattle. These include markers of both light and heavy IgG chains (Blakeslee, Butler & Stone, 1971; Blakeslee, Rapacz & Butler, 1971), of macroglobulin (Iannelli, Masina & Zacchi, 1968; Rapacz, Korda & Stone, 1968; Iannelli, 1969) and low-density lipoprotein molecules (Iannelli *et al.* 1977). In addition to these, two more allotypic specificities are known, carried on as yet unidentified classes of molecules: Ec1 (Rapacz *et al.* 1975), which is an antigenic determinant derived from lysed erythrocytes, and wbA1 (Iannelli, 1974), common to both cattle and water buffalo, and detected by allo-immune antibodies against water buffalo serum.

Where determinants carried by macroglobulin molecules are concerned, those already described are Mc1 (Rapacz *et al.* 1968) and McA1 – or as it was initially named Ci(a) (Iannelli *et al.* 1968).

The present paper describes a third macroglobulin allotype (McA2) and its genetic and immunochemical relationship to the above mentioned McA1 allotype.

2. MATERIALS AND METHODS

(a) *Single radial immunodiffusion (SRD)*. Agar plates (12 × 9 cm) were prepared as described elsewhere (Iannelli *et al.* 1977) using, for each plate, 3 ml of antiserum.

(b) *Absorptions*. To test the monospecificity of the antiserum this was absorbed with 30 individual positive sera. The antiserum (1.25 ml) was mixed with individual positive sera (50 μ l of serum from homozygous animals for factor McA2 or 100 μ l of serum from heterozygous ones); the mixture was incubated (at 37 °C for 30 min and overnight at 5 °C and then centrifuged (15 min at 2000 g); the supernatant was tested for the presence of residual antibodies by SRD using 4 × 3 cm plates.

(c) *Immunelectrophoresis* and *Immunelectrofocusing* were performed as described by Iannelli (1978), while *Density Gradient Ultracentrifugation* and

DEAE-Sephadex A50 Chromatography were carried out as described by Kunkel (1960) and by Fahey & Terry (1967) respectively.

(d) *Wheat germ-sepharose affinity chromatography*. Ten ml of gel (Pharmacia, Uppsala, Sweden) were packed into a 0.9 × 10 cm column under a hydrostatic pressure of 30 cm using 0.05 M-Na phosphate buffer, pH 7.0, containing 0.2 M NaCl (starting buffer). The material not bound to the gel was eluted with the starting buffer, whilst the material which adsorbed to the gel was eluted with *N*-acetyl-D-glucosamine (Koch-Light, Colnbrook, England) in the starting buffer (100 mg/ml); 0.5 ml of serum were added to the column and eluted at a flow rate of 4 ml/h.

(e) *Ultragel AcA22 Gel Filtration, Sephadex G-200 Gel Filtration, ConA-Sepharose Affinity Chromatography, Treatment of Sera with Aerosil, Ultracentrifugal Fractionations of Lipoproteins* were performed as described by Iannelli *et al.* (1977).

(f) *Immunization*. It was planned in an attempt to obtain antibodies against a hypothetical gene allelic to *McA1* which synthesizes McA1 (Iannelli *et al.* 1968). Therefore, two Holstein-Friesian cows, both homozygous for the *McA1* gene, were immunized with the macroglobulin preparation obtained from the serum of eight cows of the same breed which lacked the *McA1* gene. Details of the preparation of the macroglobulin fraction of the serum have been given elsewhere (Iannelli *et al.* 1977). Each cow received 5 ml of macroglobulin preparation (8.0 mg of protein/ml) emulsified with an equal volume of complete Freund's adjuvant at 1 month intervals. The immunization was continued for 10 months but both recipients failed to form antibodies. At this stage the macroglobulin preparation was replaced with an equal quantity of pooled serum from the same donors, and the immunization continued at monthly intervals. Antibodies against the specificity McA2 were detected in one of the two recipients 2 weeks after the second injection.

3. RESULTS

(i) *Identification and characterization of the McA2 antigen*

One of the two immunized cows produced an antiserum which reacted with some but not all tested cattle sera. This antiserum was termed anti McA2* (Mc denoting macroglobulin, A the locus and 2 the factor); the antigenic marker detected with it McA2; the gene controlling the synthesis of McA2 was *McA2* and the corresponding locus McA.

Classification of the animals into McA2(+) (those with specificity A2) and McA2(-) (those without) was performed by SRD (Plate 1, fig. 1). Precipitin rings developed after 24–36 h and further incubation did not reveal additional ones. Two distinct kinds of reaction patterns were observed: large sized rings which developed within 24 h and small-sized ones, which took about 36 h to develop fully. Genetic analysis (see next section) showed that the large rings belonged to

* In the text, for brevity, the prefix Mc will be omitted wherever such an omission does not cause ambiguity.

animals homozygous for factor A2 and the small sized ones to the heterozygous ones, thus indicating that the antiserum has dosage effect. The same phenomenon was observed with the antisera for the McA1 (Iannelli *et al.* 1968) and Ec1 (Rapacz *et al.* 1975) factors.

Absorption of anti A2 antibodies with 30 individual positive sera (from homozygous and heterozygous animals in equal number) exhausted the antiserum completely, thus suggesting that it could not be fractionated by absorption (i.e. it was monospecific). Homozygous sera were approximately twice as efficient as heterozygous sera in absorbing a given amount of anti A2 antiserum (see Material and Methods).

In order to estimate the molecular weight of the molecule carrying the A2 determinant, one of the A2(+) sera was subjected to gel filtration through Sephadex G-200. The material with A2 antigenic activity eluted with the exclusion volume.

The serum used in the previous experiment was also chromatographed on Ultrogel AcA22. Since this resin has a higher exclusion limit than G-200, it can give a more accurate estimate of the molecular weight of the A2 antigen and, what is still more important, it can fractionate at least partially the high-molecular-weight components which eluted in the first peak on the Sephadex G-200. Gel filtration on Ultrogel AcA22 indicated that A2 had a lower molecular weight than LdlA1, a cattle low-density lipoprotein allotype (Iannelli *et al.* 1977). This suggested that A2 and LdlA1 did not belong to the same class of molecules. Conclusive evidence that A2 was not carried on either high (HDL) or low (LDL) density lipoprotein molecules was obtained from the following experiments: firstly, precipitation rings did not stain with specific lipid strains (Oil red O, Sudan Black and Sudan IV); secondly, Aerosil, which removes LDL (Iannelli *et al.* 1977), did not cause A2(+) sera to become unreactive; thirdly, the antiserum did not react with either the LDL or HDL fraction of A2(+) serum obtained by ultracentrifugation.

The behaviour of A2 was also studied by means of ion-exchange chromatography on DEAE Sephadex A50 and shown to adsorb onto the resin at pH 8.0 in 0.1 M Tris-HCl and to elute upon addition of 1.0 M-NaCl.

Since both wheat-germ lectin (WGL), specific for *N*-acetyl-D-glucosamine residue (Golstein, Hammerström & Sundblad, 1975), and concanavalin A (ConA), specific for *N*-acetyl-D-glucosamine and mannose residues (Golstein & Iyer, 1966), react with various serum components, it seemed worth investigating the interaction of the A2 antigen with these lectins in a further attempt to outline some of the characteristics of the former. When an A2(+) serum was chromatographed on ConA Sepharose, all antigen activity was recovered in the second peak (i.e. the one eluted upon addition of 1.0 M α -methyl-D-glucoside). When this same peak, concentrated back to its original volume (1 ml) by ultrafiltration (Amicon UM20E membranes), was chromatographed on WGL-Sepharose, the A2 antigen bound to the resin and eluted upon addition of *N*-acetyl-D-glucosamine. These experiments, when taken together, suggest that the molecule on which the antigen resides

contains *N*-acetyl-D-glucosamine residues necessary for interaction with WGL and, possibly, also mannose residues.

To determine the electrophoretic mobility of the antigen, the serum as used in the previous experiments was subjected to immunoelectrophoresis and the resulting pattern is shown in Plate 1, fig. 2. The pI of the component carrying A2 was also determined by immunoelectrofocusing and shown to occur at approximately pH 5.

Finally, density-gradient ultracentrifugation of the A2(+) serum showed that the antigen did move to the bottom of the tube, as might be expected for macroglobulin molecules (Kunkel, 1960).

(ii) Genetic studies

Serum samples from 474 Holstein-Friesian cattle from the same herd were tested by SRD with anti A2 antiserum. Since the purpose of this study was to detect the product of a hypothetical gene allelic to *McA1*, the samples were also tested with anti A1 antiserum. When the results obtained with the two reagents were compared, it immediately became evident that the two factors were controlled by allelic codominant genes: all tested animals, in fact, reacted either with one or with both antisera while the phenotype, characterized by the lack of both factors, was absent.

Table 1. Family segregation of *McA1* and *MCA2*

Parents genotypes*	No. of matings	Offspring genotypes*		
		A1/A1	A1/A2	A2/A2
A1/A1 × A1/A2	2	2 (1)†	0 (1)	0 (0)
A1/A1 × A1/A2	21	10 (10.5)	11 (10.5)	0 (0)
A1/A1 × A2/A2	32	0 (0)	32 (32)	0 (0)
A1/A2 × A1/A2	52	12 (13)	26 (26)	14 (13)
A1/A2 × A2/A2	129	0 (0)	66 (64.5)	63 (64.5)
A2/A2 × A2/A2	72	0 (0)	0 (0)	72 (72)

* The genotypes *McA1/McA1*, *McA1/McA2* and *McA2/McA2* are abbreviated to A1/A1, A1/A2 and A2/A2.

† Figures in parentheses refer to expected number of progeny in each class.

As seen in Table 1, family data concur with the above proposed theory. It was found that heterozygous animals could belong to either sex thereby indicating that the A locus is not sex-linked.

Finally, the frequencies of the genes *McA1* and *McA2*, calculated on the genotype of 308 offspring (Table 1), were 0.297 and 0.703 respectively.

(iii) Presence of *McA1* and *McA2* on the same molecule

Having shown that A1 and A2 are controlled by allelic genes, it seemed worth ascertaining whether heterozygous individuals contain two kinds of molecules (A1 specific and A2 specific) or whether they contain molecules which carry both A1 and A2 determinants. Anti A2 antiserum is not reactive in a double diffusion test. This technique, consequently, could not be used to obtain evidence for the

existence of macromolecules with simple or double specificities. Instead, the precipitation tests with anti A1 or anti A2 antisera were used for this purpose. Addition of either anti A1 or anti A2 IgG preparations (isolated from the corresponding antisera by recycling G-200 gel filtration) to the serum of a heterozygous animal precipitated both A1 and A2 specificities: the serum became negative in both cases. When the same procedure was applied to an artificial mixture of A1 and A2 homozygous sera, only one specificity was found to be carried down in the precipitate.

4. DISCUSSION

Alloimmunization of cattle homozygous for the *McA1* gene with serum from cows which lacked this gene led to the production of an antiserum (anti A2) which identifies the product of *McA2*, i.e. of the gene allelic to *McA1*.

In an attempt to delineate some of the physical characteristics of the protein carrying the determinant, an A2(+) serum was submitted to gel filtration (Sephadex G-200 and Ultrogel AcA22), ion-exchange chromatography (Sephadex DEAE-A50), immunoelectrophoresis, immunoelectrofocusing and ultracentrifugation. As a result of these studies it has been established that A2 is a macroglobulin since it was found in the exclusion volume upon gel filtration through Sephadex G-200 and at the bottom of the tube upon density gradient ultracentrifugation. This macroglobulin has *N*-acetyl-D-glucosamine (see interaction with WGL) and possibly also mannose (see interaction with ConA) residues on its surface and has its isoelectric point at pH 5.

However, no further attempt was made to ascertain whether this marker is located on α_2 macroglobulin or IgM molecules. The immunoelectrophoretic pattern of A2 would appear to suggest that the first alternative (i.e. A2 being located on α_2 macroglobulin molecules) is the correct one: A2 (Plate 1, fig. 2) formed an arc which extended entirely towards the anode; a similar pattern was reported by Berne, Dray & Knight (1970) for rabbit α_2 macroglobulin allotypes. It has been shown that cattle IgM, on the other hand, form a precipitation arc close to the origin and extended on both anodic and cathodic sides of the well (Butler, 1969; Josephson, Mikolajick & Sinha, 1972).

Segregation data (Table 1) leave little doubt that the A2 antigen is inherited as though controlled by an autosomal gene (*McA2*), allelic to *McA1*. The A2(+) animals included many 3- to 20-day-old calves which would appear to suggest that the antigen is fully expressed at birth or very soon after. The fact that maternal transfer of the antigen might have taken place was excluded since the phenotype of 4- to 5-month-old calves was the same as that established at 3–20 days.

In this study it has been shown that both allotypic specificities occur on the same macroglobulin molecule, a result which indicates that allelic genes contribute to the formation of the same molecule. In addition to the Mtz locus in the rabbit (Berne, Dray & Knight, 1972) and the ABO one in man (Morgan, 1960) another locus where allelic exclusion does not take place at the molecular level has thus been identified.

The McA system in cattle (comprising two immunological specificities controlled by allelic genes and sharply differentiating one type of macromolecule from another) offered a valuable opportunity to study whether the isoantigenic difference at the McA locus was associated with differences in the amino acid portion of the molecule – as, for example, in the case of the human Inv(a+) and Inv(a–) specificities (Baglioni *et al.* 1966) – or in the prosthetic groups (carbohydrates) – as in the case of human ABO, Lewis and MN antigens (Watkins, 1966). The latter possibility seemed equally likely *a priori* since the molecules carrying A1 and A2 determinants have been shown to interact with ConA and WGL and, consequently, to contain carbohydrates which are known to be highly antigenic (Giblett, 1969). Results which will be published in detail elsewhere and which have been derived from two independent experimental approaches (digestion of A1 and A2 antigen preparations with specific glycosidases and inhibition tests of anti A1 and anti A2 with simple sugars) showed that one single sugar plays a dominant role in determining the serological specificity of each of the two allotypes: mannose of the antigen structure A1 and glucose of A2.

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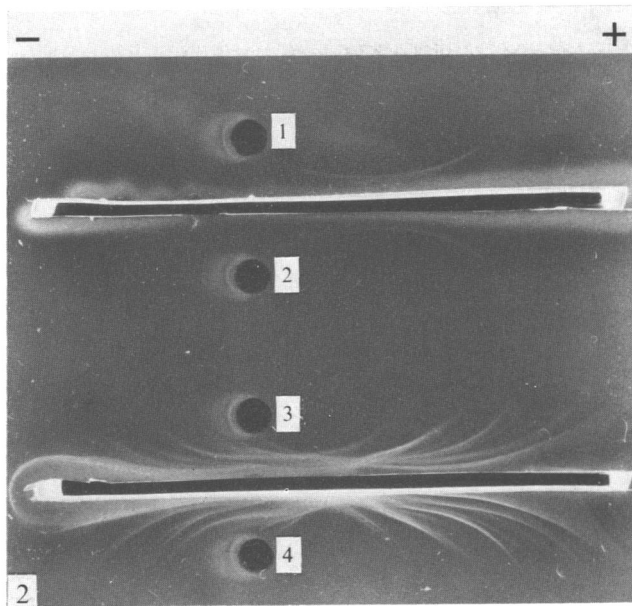
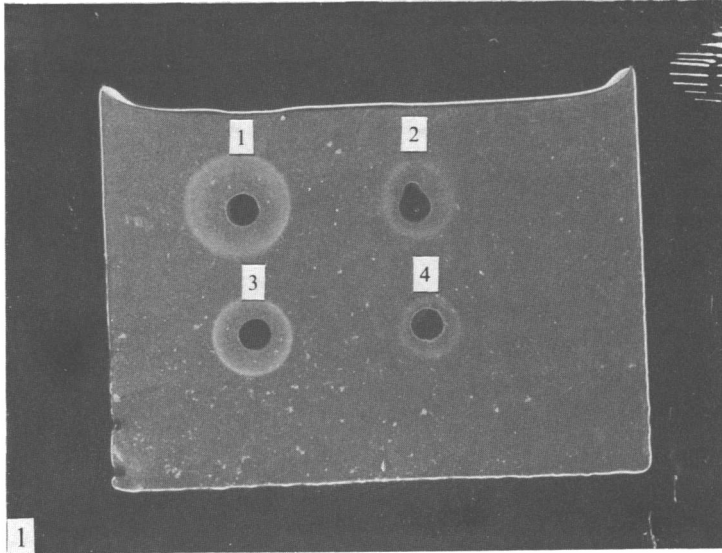


Fig. 1. Single radial diffusion pattern of McA2(+) serum. Plate contained: 4 ml of 3% agarose in glycine buffer pH 7.8; 3 ml of antiserum; 1 ml of glycine buffer pH 7.8. The plate was incubated for 2 days at room temperature. Well no. 1 contains a homozygous sample. Well no. 2 the same sample diluted 50% with a McA2(-) sample. Well no. 3 contains a heterozygous sample. Well no. 4 the same sample diluted 50% with a McA2(-) sample. Note the approximately equal ring size of samples in wells nos. 2 and 3.

Fig. 2. Immunoelectrophoretic pattern of a McA2(+) serum. Electrophoresis was conducted at 2V/cm for 3 h. The plate was incubated at room temperature for 2 days. The anode was on the right. Upper trough contained anti A2 antiserum; lower trough, rabbit antiserum to cattle whole serum. Wells 1, 2 and 3 contained an A2(+) serum and well 4 an A2(-) serum.

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