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Immunogenetics of an antigen identified in both sheep and cattle sera

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SUMMARY

Alloimmunization in sheep revealed an antigen specificity B1 which is also to be found in cattle sera. The molecular weight of the protein carrying B1 was greater in sheep than in cattle, whilst in both cases its isoelectric point was at pH 6·2. The electrophoretic position of the antigen suggests that the molecule carrying B1 might be a β-globulin. Family studies indicated that in both species B1 was inherited in a simple Mendelian manner, as if it were a product of a dominant allele *B1* at an autosomal locus *B*.

1. INTRODUCTION

The analysis of polymorphic serum antigens has at least two significant applications: firstly, that of revealing similarities and differences between populations; secondly, that of investigating gene action at a biochemical level. Detailed studies of this kind have been carried out using the allotypic markers of human (Steinberg, 1967) and rabbit (Feinstein, Gell & Kelus, 1963) serum proteins and also those of a number of other species. Thus, several such antigens have already been identified in both sheep (Rapacz, Hasler & Pope, 1970; Curtain, 1971; Bash & Milgrom, 1972) and cattle (Blakeslee, Butler & Stone, 1971; Rapacz, Korda & Stone, 1968; Iannelli, 1969; Iannelli & De Benedictis, 1978).

Another allotypic form was revealed by alloimmunization in sheep and was also found to be present in cattle. This paper describes its mechanism of inheritance and some physico-chemical properties of the component carrying the allotypic determinant in both species. This work is part of a general project aimed at monitoring the phylogenetic relationships between serum proteins in various species of ruminants, by employing such antigens as markers.

2. MATERIALS AND METHODS

Double diffusion (*DD*), *immunoelectrophoresis* (*IE*), *immunoelectrophocusing* were performed for both sheep and cattle sera as described by Iannelli (1978*a*). Precipitation lines developed fully after an incubation period of 72 h at room temperature.

Sephadex G200 gel filtration, ConcanavalinA Sepharose affinity chromatography were performed as described by Iannelli (1978*b*).

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Absorption tests of antigen activity by Aerosil (Degussa, Abt., W.G.) were performed as described by Iannelli *et al.* (1978).

Absorption tests of sheep alloantibodies by cattle or sheep sera were performed by incubating serum and antiserum in the ratio v/v = 1/5 for 1 h at 37 °C and for 12 h at 4 °C. The samples were then centrifuged at 3000 rev/min for 10 min and the supernatant was checked for residual antibody activity against B1(+) cattle and sheep sera by DD and IE respectively. In a control test the absorbing serum was replaced by phosphate buffer 0·1 M, pH 7·4.

Sheep alloimmunization was performed as described by Iannelli (1978*a*). The animals used as donors or as recipients were obtained by cross breeding (Gentile di Puglia Ile de France × Wüttemberg). Antiserum was checked by DD 7 days after each injection against individual donor sera. Cattle sera were from Holstein Fresian animals. All cattle and sheep sera and sheep alloantiserum were kindly given by Dr Iannelli, Institute of Animal Production, University of Naples.

3. RESULTS

(i) *Identification and characterization of the antigen in sheep and in cattle sera.*

Prolonged alloimmunization of sheep with whole serum resulted in the production of an antiserum which, when checked by DD against sheep sera, identified at least two different specificities (see Plate I, fig. 1); the first, revealed by the antiserum after the fifth injection and named A1, has already been described (Iannelli, 1978*a*); the second was revealed by the antiserum after the tenth injection and was named B1 (*B1* was the gene controlling its synthesis and *B* the locus). Since there was a considerable difference between the electrophoretic mobilities of A1 and B1 (see Plate 1, fig. 2), immunoelectrophoresis was preferred to DD in order to avoid errors in identifying antigen activities in sheep sera. Animals were thus classed by IE as B1(+), those having the B1 specificity, and B1(−), without the B1 specificity; B1 precipitin band developed after 72 h and further incubation did not reveal additional bands.

In order to ascertain possible cross reactions with sera of other related species, sheep alloantiserum was checked by DD against 212 cattle samples; most of the sera reacted, giving a single precipitin band similar to that found for B1 sheep antigen. When 20 B1(+) sera from each species were checked by DD in adjacent wells (to avoid errors A1(−) sheep sera were chosen) in no case were spurs observed, whereas the precipitin lines coalesced indicating a reaction of identity (see Plate 2, fig. 1). Results were confirmed by absorption tests, in which 30 random B1(+) cattle sera were used separately to absorb antiB1 antibodies from sheep alloantiserum. In all cases, after absorption, the antiserum supernatant showed no reaction with cattle sera at DD; immunoelectrophoresis of sheep sera confirmed that cattle sera completely removed antiB1 antibodies from sheep alloantiserum. As expected, the same results followed when the antiserum was absorbed with B1(+) sheep sera: once again, after absorption, the antiserum supernatant did not react with sheep or cattle sera.

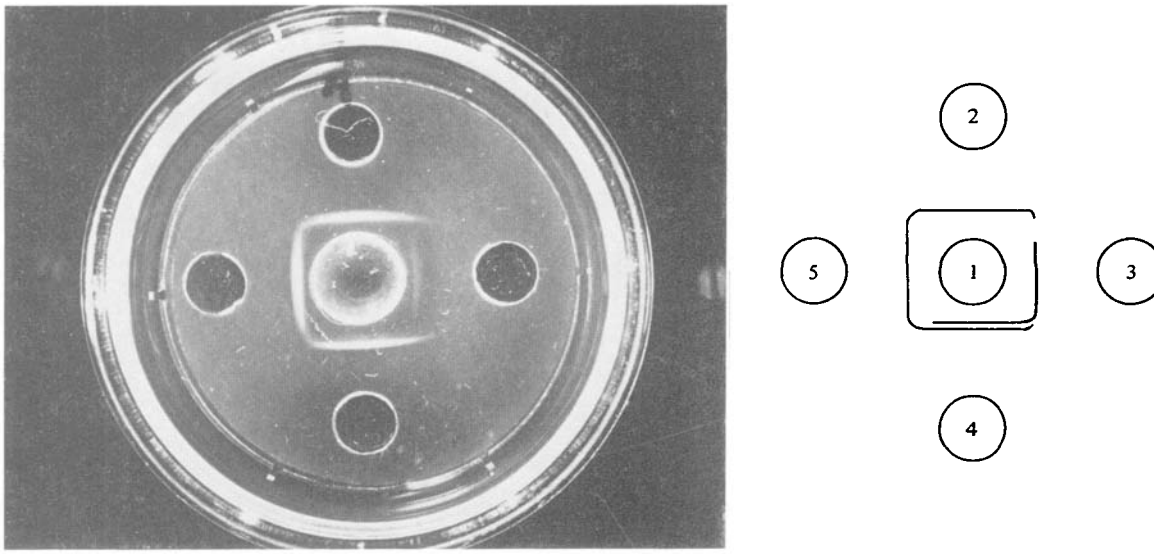

Fig. 1. (*a*) Double diffusion pattern of A_1 and B_1 sheep specificities. Well 1 was filled with 60 μl of alloantiserum; wells 2 and 5 with 40 μl of $A_1(+)$, $B_1(-)$ sera; well 3 with $A_1(-)$, $B_1(+)$ serum; well 4 with $A_1(+)$, $B_1(+)$ serum. (*b*) Diagram of results in (*a*).

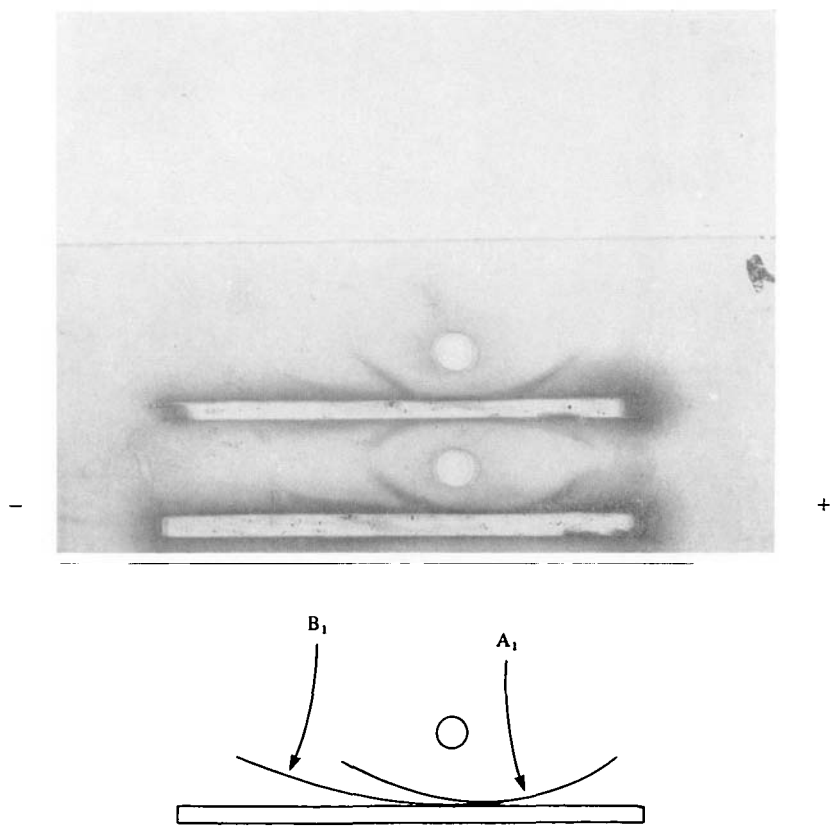

Fig. 2. Immunoelectrophoretic pattern of A_1 (anodic band) and B_1 (cathodic band) antigens. Electrophoresis was conducted at 7 V/cm for 60 min. The anode was on the right. Both troughs contained alloantiserum and both wells contained $A_1(+)$, $B_1(+)$ sera. The plate was stained with Coomassie brilliant blue.

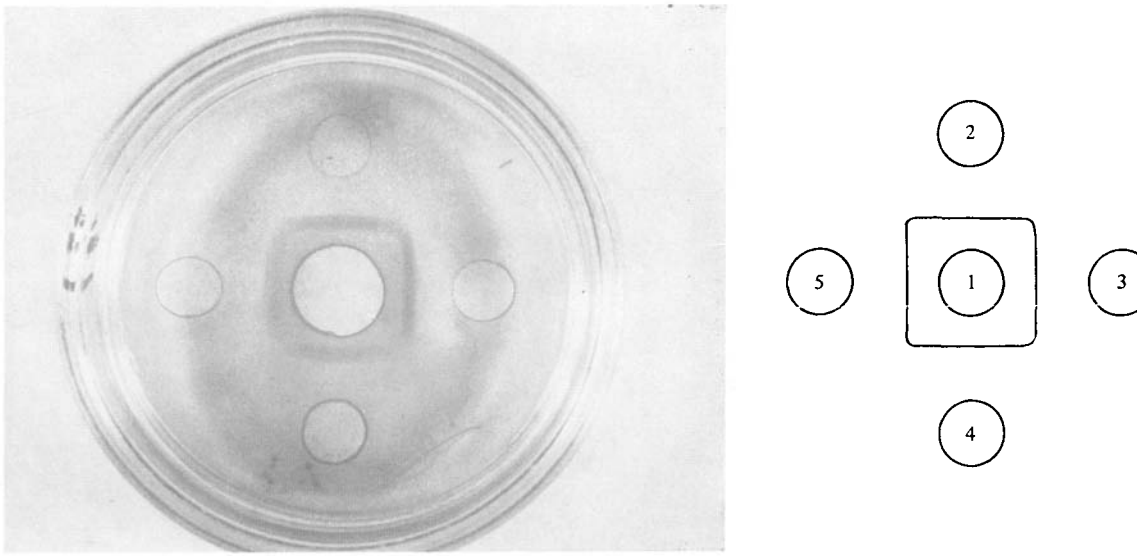

Fig. 1. Double diffusion pattern of B_1 specificity. Well 1 was filled with 60 μl of alloantiserum; wells 2 and 3 with 40 μl of $B_1(+)$ cattle sera; wells 4 and 5 with $A_1(-)$, $B_1(+)$ sheep sera. The plate was stained with Coomassie brilliant blue.

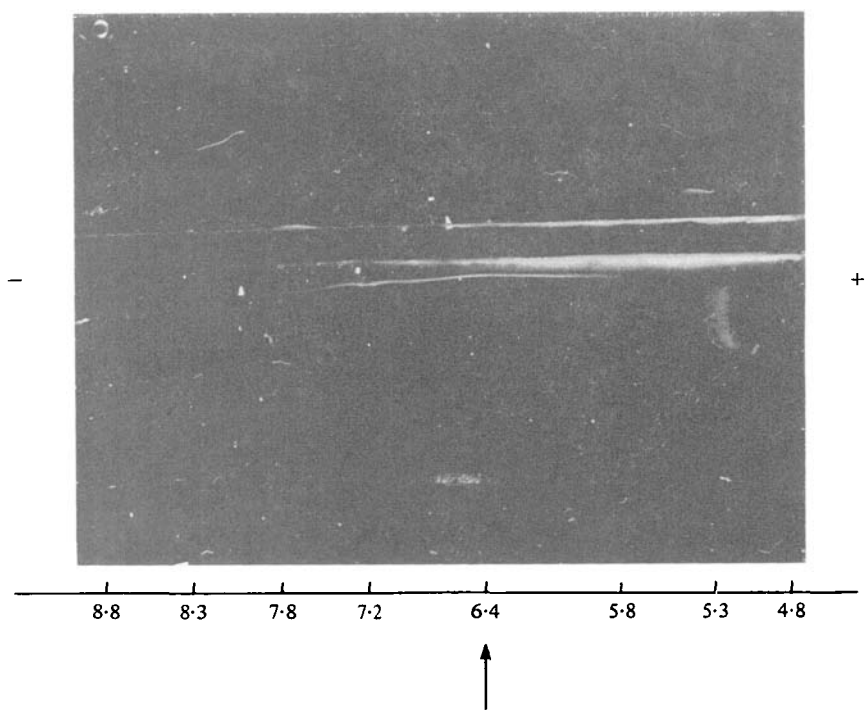

Fig. 2. Analytical immunoelectrophocusing of B1 antigen. Peak II from Sephadex G200 chromatography of sheep serum was concentrated by ultrafiltration to initial serum volume and 15 μl were absorbed on to Whatman 3MM filter paper (5 × 13 mm) and focused for 3 h at 1000 V using carrier ampholytes pH 3·5–9·5 (LKB). After focusing and before the application of antiserum the gel was dipped in glycine buffer pH 8·0 for 30 min. The B1 precipitin arc formed approximately at pH 6·2.

In order to estimate the molecular weight of the molecule carrying the determinant B1 in each species, two B1(+) sera from both cattle and A1(−) sheep were filtered through Sephadex G200 and the respective elution peaks, after concentration to the initial volume (Amicon UM20 membranes), were checked against antiserum by DD: B1 antigen was recovered in the third peak from cattle serum and in the second from sheep.

Table 1. *Inheritance of B_1 antigen in sheep*

Matings	No. of offspring*			χ^2	D.F.
	Total	$B_1(+)$	$B_1(-)$		
+ × +	102	93 (93·1)	9 (8·9)	0·001	1
+ × −	48	34 (33·8)	14 (14·2)	0·004	1
− × −	4	0	4		

* Expected numbers are given in parentheses, and were calculated on the basis of a gene frequency of $B_1 = 0{\cdot}58$.

Immunoelectrophoresis revealed no difference between the electrophoretic mobility of B1 antigen in sheep and cattle; in both cases the precipitin band developed in the same cathodic position. This position suggests that the molecule carrying B1 in both species is a β-globulin. Moreover, when immunoelectrophoresis of B1(+) cattle and sheep sera against antiB1 antiserum and that of human serum against antiβ2-human glycoprotein antiserum (Orion) were both performed on the same slide, the respective precipitin arcs formed in the same position. Analytical immunoelectrophocusing indicated that the molecule carrying B1 had an isoelectric point approximately at pH 6·2, in both sheep and cattle (see Plate 2, fig. 2).

Absorption of B1(+) cattle and sheep sera with Aerosil, which removes lipoprotein (Iannelli *et al.* 1978), did not remove B1 antigen activity, thus indicating that the molecule carrying B1 was not a lipoprotein.

Further, this β-globulin did not react with ConcanavalinA, a protein which interacts with various serum components (Harris & Robson, 1963) carrying non-reducing mannosyl and *N*-acetyl glucosaminyl end-groups (Goldstein & Iyer, 1966). When one B1(+) serum from sheep and one from cattle were chromatographed on ConA-Sepharose, in both cases B1 antigen activity was recovered in the first elution peak, thus suggesting that the molecule carrying the B1 specificity does not contain those residues which specifically interact with this lectin.

(ii) *Genetic data*

Tables 1 and 2 show the results of 154 sheep and 122 cattle matings, involving the B1 specificity. The data are consistent with the hypothesis that the B1 marker is controlled by a dominant allele in both species. The fact that some negative offspring resulted from B1(+) × B1(+) matings suggested that some of the

parents were heterozygous for the marker. In sheep, 4 B1(−) × B1(−) matings were found, none of which gave positive offspring, as must be expected when the absence of the factor is recessive. In cattle, because of the higher frequency of the B1 allele, no B1(−) × B1(−) matings were found. However, a study of specific matings in which one parent was heterozygous *B1/b1* as ascertained by pedigree analysis (*b1* is used to designate the absence of a detectable allelic product) and the other homozygous negative *b1/b1*, provided evidence of dominant inheritance. Furthermore, none of the data obtained indicated a sex difference making it clear that B1 is autosomally controlled in both species.

Table 2. *Inheritance of B_1 antigen in cattle*

Matings	No. of offspring* Total	$B_1(+)$	$B_1(-)$	χ^2	D.F.
+ × +	114	112 (111·6)	2 (2·4)	0·07	1
+ × −	8	7 (6·8)	1 (1·2)	0·04	1
− × −	0				

* Expected numbers are given in parentheses, and were calculated on the basis of a gene frequency of $B_1 = 0{\cdot}83$.

4. DISCUSSION

The alloantiserum produced in sheep by whole serum identified, after the tenth injection, an allotypic form (B1) carried on a serum component which was slow moving in electrophoresis. The specificity was also present in cattle, as revealed by the reaction of identity which resulted when cattle and sheep sera were checked in adjacent wells against sheep alloantiserum by DD.

In an attempt to delineate some of the physico-chemical characteristics of the molecule carrying the B1 specificity in both species, B1(+) sera from sheep and cattle were submitted to gel filtration (Sephadex G200), immunoelectrophoresis, immunoelectrophocusing, a test for lipoprotein (Aerosil) and affinity chromatography (Sepharose ConA). Results indicated that, in each species, the same determinant was carried on two molecules of different molecular weight but of identical electrophoretic mobility. In addition, the immunoelectrophoretic pattern of the B1 antigen from both sheep and cattle was similar to that of a human β2-glycoprotein, a kind of protein for which, as yet, no allotypic form has been identified in either sheep or cattle. Further, the isoelectric point of the molecule carrying the marker proved to be at pH 6·2 for both species. However, it has been already shown that the allotypic markers Mcal (cattle) and A1 (sheep) also carry the same immunodominant sugar-Mannose on two molecules of very different molecular weight and of similar electrophoretic mobility (Iannelli & De Benedictis, 1978; De Benedictis, 1979). In this connection, promising preliminary results, achieved by electrophoresis on SDS polyacrylamide gel of the B1 antigen from sheep and from cattle, would seem to indicate that the B1 sheep protein, eluting

in the second peak on Sephadex G200, could be a polymeric form of the B1 cattle protein, eluting in the third peak. Lastly, results from absorption tests with Aerosil and from affinity chromatography indicated that this antigen was not a lipoprotein and did not contain glycosil end groups interacting with ConcanavalinA.

Genetic data showed that the B1 marker was controlled by an autosomal dominant locus (*B1*) in both sheep and cattle, whilst the recessive allele (*b1*) brought about the absence of the marker. Further data on the immunochemical features of this protein in both species are being collected and it is hoped that they will clarify at least two questions: the molecular localization of the B1 specificity on the carrier molecules in both species and the possible biological role played by this allotype; further, an attempt is being made to identify the products of hypothetical genes allelic to *B1* by the immunization of homozygous sheep (*B1/B1*) with sera of B1(−) sheep.

Finally, the gel filtration results and the test with Aerosil showed that this antigen could not be identified either with the polymorphic low density lipoprotein already detected by Iannelli *et al.* (1978) in cattle or with the β-lipoprotein identified by Curtain (1971) in sheep; however the electrophoretic position and, in the case of the cattle at least, the molecular weight, indicated that the B1 marker could not be identified either with the Immunoglobulin allotypes detected by Blakeslee *et al.* (1971) in cattle and by Curtain (1971) in sheep or with the α-macroglobulin allotypes detected by Rapacz *et al.* (1968) in cattle and by Curtain (1971) in sheep.

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