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Identification and genetics of betaglobulin (BgC1) and alphaglobulin (AmiF1) antigenic markers in cattle

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Abstract. Alloantibodies detecting antigenic determinants called BgC1 and AmiF1 were obtained in cattle. The analysis of determinants' inheritance was done on 1754 offsprings after 72 sires and 1740 dams. The molecule-carriers of both traits were characterized using serological and physico-chemical methods. It was found that the identified determinants are markers of beta-globulin and alpha-microglobulin proteins, and of autosomal, dominant genes *BgC1* and *AmiF1* from independent loci, which control them.

Key words: antigenic determinants, cattle, gene markers, proteins.

Introduction

In a review (FABER, STONE 1976) of antigenic markers of genes of serum proteins in cattle, 22 characters, including 11 characters relating to immunoglobulins, were put together and compared indirectly. The majority of markers of this type, which were discovered later (IANNELLI 1978, IANNELLI, CAPPARELLI 1979, WĘGRZYN, WĘGRZYN 1985, WĘGRZYN et al. 1986, 1996) were related to alpha- and beta-globulin molecules. The following work describes two antigenic markers of beta-globulins and alpha-microglobulins in cattle, controlled by autosomal, dominant genes from independent loci.

Material and methods

In alloimmunizations of three recipient Black-and-White (BW) cows, full serum of BW donor was used as an antigen (immunizations) and proteins coated on recipient erythrocytes (reimmunizations). The donor and recipients

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were selected after marking in them nine previously described antigens of alpha-, beta- and immuno-globulins (WĘGRZYN 1973, WILLMANN-WĘGRZYN, WĘGRZYN 1975, WĘGRZYN et al. 1977, WĘGRZYN, WĘGRZYN 1978). Intramuscular injections (8 ml serum or 6 ml coated erythrocytes) were done using complete Freund's adjuvant (0.2-0.3 ml), at 7 to 10-day intervals, 5-6 injections in each series of immunizations. The recipients' reactions were monitored continuously by a precipitation test.

A double immunodiffusion test – DD (OUCHTERLONY 1953), gel absorptions, immunoelectrophoresis – IE (HIRSCHFELD 1960) were used in the serological analyses. The preparations were stained using Uriel and Masseyeff methods (after WIEME 1965).

The photographic documentation was made from fresh IE preparations and from a stained preparation of the control electrophoresis.

Column chromatography of serum with the studied antigens was performed on sephadex G-200 (KILLANDER, FLODIN 1962).

The presence or absence of a character was designated with "+" or "–". Domination of a character over its absence as well as gene–antigen determinant relationship has been assumed.

The inheritance of the detected antigens was analysed on family material, which included 1754 offsprings of 72 sires and 1740 dams of the Black-and-White (BW), Red-and-White (RW), Polish Red (PR) and Simmental breeds. The number of offsprings of a single bull was from 10 to 101. The offsprings were selected on the basis of blood groups. Segregations of characters in the offsprings from phenotypic matings of the type $+ \times +$, $+ \times -$ and $- \times -$ and from selected test matings of the type ♂ heterozygote × ♀ recessive homozygote were analysed. The χ^2 test was used to test the validity of the results obtained.

The abbreviations C1, *C1*, *C0*, F1, *F1*, and *F0*, relating to the nomenclature of the described markers and genes that control them, and used in the chapters Results, Discussion and in Tables 3 and 4, have only been introduced for the sake of this publication.

Results

Alloimmune sera containing specific precipitins were obtained from two recipients (C-63 and C-37) after the fourth injection and from the third recipient (C-64) after the fifth injection in reimmunizations.

An analysis of C-63 and C-64 sera showed that the former was of the double-antibody, while the latter – of the single-antibody type. Both sera contained antibodies identifying the same, hitherto unknown antigenic determinant. These antibodies were called anti-BgC1, while the character BgC1 (Bg = betaglobulins), detected by them, was abbreviated as C1. Serum C-63 additionally contained antibodies detecting the previously described marker BA8 (WILLMANN-WĘGRZYN, WĘGRZYN 1975). Absorption was not used in the case of this serum, because in DD the BgC1 antigen produced a markedly different precipitation arc from the BA8 antigen arc.

With natural sera, the third serum (C-37) resulted in precipitation reactions of varying intensity. The absorptions carried out showed that both weakly and strongly reacting natural sera mutually removed precipitins from serum C-37. The antibodies obtained were called anti-AmiF1, while the marker AmiF1 (Ami = alpha-microglobulins) detected by them, was abbreviated as F1.

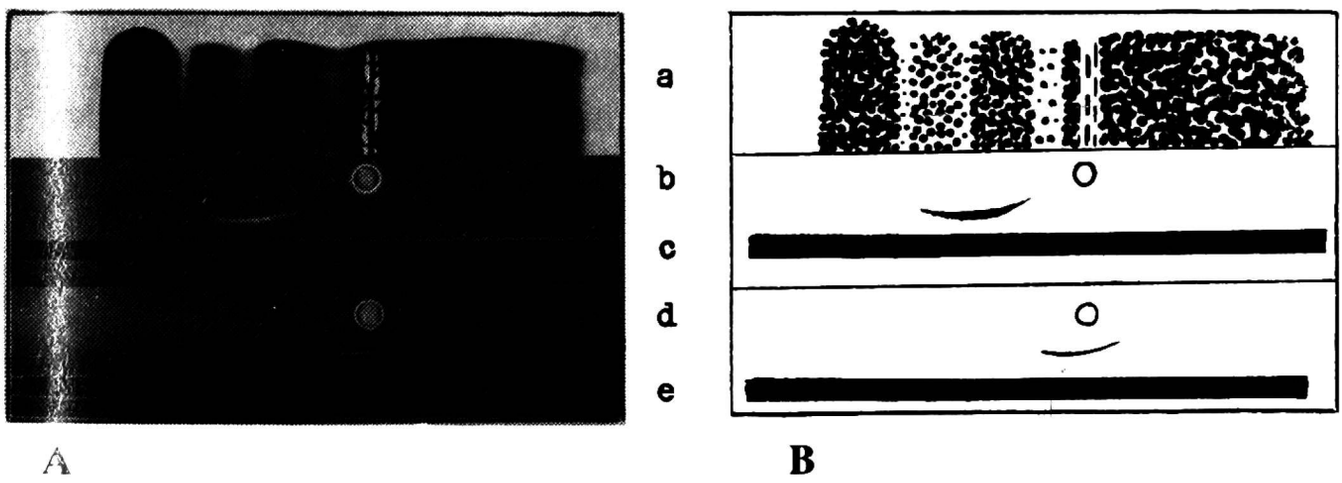

Fig. 1. An electrophoregram of natural cattle serum AmiF1$^+$BgC1$^+$ (a) and immunoelectrophoregrams of this serum (b, d) against anti-AmiF1 (c) and anti-BgC1 (e)
A – a photograph, B – a diagram

Both anti-BgC1 and anti-AmiF1 antibodies reacted with respective antigens in 1% gel made from Noble agar and 8% NaCl solution. Precipitates of both antigens were stained with amido black.

In IE, molecules carrying determinant C1 located in the zone of beta-globulins, in the starting point (Fig. 1d, e), while molecules with F1 were found in the fastest-migrating proteins of alpha$_2$-globulin fractions (Fig. 1b,c).

In sephadex G-200 chromatography, the antigen C1 was washed out at peak II, while F1 – at the transition point between peaks II and III, and in peak III.

The characters identified were compared with regard to their identity with the previously described seven markers of alpha-globulins and three markers

of beta-globulins (WĘGRZYN 1973, WILLMANN-WĘGRZYN, WĘGRZYN 1975, WĘGRZYN, WĘGRZYN 1985, WĘGRZYN et al. 1977, 1986, 1996). It was found that in the family material both C1 and F1 occurred in various combinations with previously discovered markers. Also molecule-carriers of the compared characters differed with regard to their physico-chemical properties.

Genes controlling determinants C1 and F1 were designated *BgC1* (*C1* in short) and *AmiF1* (*F1* in short), respectively, and their respective loci were designated *BgC* and *AmiF*. Alleles of these genes (recessive by convention), whose products had not yet been detected, were called *BgC0* and *AmiF0* (*C0* and *F0* in short). Gene frequencies of *C1* and *F1* were the highest in the BW breed (0.43 and 0.69, respectively), increasingly lower in RW and PR breeds, and the lowest in Simmentals (0.12 and 0.47, respectively) (Tables 1, 2).

In phenotypic matings of the parents positive for C1 (Table 1) and F1 (Table 2), over 75% of offsprings were found to have the character analysed. In the case of matings of the type + × –, there were at least 50% animals among the offsprings, positive for the respective antigen. In the case of the RW and PR breeds, distributions of C1 differed significantly from predicted values. It appeared that the offspring from the mating of parents without C1 and parents

Table 1. Distribution of BgC1 antigenic determinant in the offspring of Black-and-White (BW), Red-and-White (RW), Polish Red (PR) and Simmental cattle

Breed	Mating type	Number of matings	Observed number of offspring		Gene frequency[a]	χ^2 d.f. = 1
			+	–		
BW	+ × +	92	73	19		0.62
	+ × –	305	177	133	0.28	0.14
	– × –	192	0	193		
RW	+ × +	380	306	75		14.09**
	+ × –	233	130	104	0.43	6.70**
	– × –	34	1	33		
PR	+ × +	45	31	15		5.05*
	+ × –	167	85	85	0.21	2.37
	– × –	110	1	110		
Simmental	+ × +	5	5	0		1.41
	+ × –	51	34	17	0.12	3.72
	– × –	126	0	127		

* $P \leq 0.01$

** $P \leq 0.001$

[a] Genetic equilibrum and one gene-one antigenic determinant dependence were assumed.

Table 2. Distribution of AmiF1 antigenic determinant in the offspring of Black-and-White (BW), Red-and-White (RW), Polish Red (PR) and Simmental cattle

Breed	Mating type	Number of matings	Observed number of offspring		Gene frequency[a]	χ^2 d.f. = 1
			+	−		
BW	+×+	315	290	29		0.02
	+×−	234	177	59	0.56	3.43
	−×−	019	1	39		
RW	+×+	556	523	35		0.48
	+×−	89	71	18	0.69	0.58
	−×−	2	0	2		
PR	+×+	192	171	25		0.54
	+×−	110	69	42	0.50	1.01
	−×−	20	0	20		
Simmental	+×+	107	88	19		3.36
	+×−	66	42	25	0.47	0.21
	−×−	9	0	9		

[a] Genetic equilibrium and one gene-one antigenic determinant dependence were assumed

without F1 had individuals with phenotypes of their parents (463 and 70, respectively) and two unexpected $C1^+$ and one $F1^+$ individuals.

From the matings of parents with genotypes ♂ *C1/C0* × ♀ *C0/C0* and ♂ *F1/F0* × ♀ *F0/F0*, the numbers of offsprings positive and negative for each character, did not markedly differ from the 1:1 ratio (Table 3).

Table 3. Distribution of BgC1 and AmiF1 antigenic determinants in the offspring of the selected test matings*

Antigenic determinant	Mating type (deduced genotypes)	Number of matings	Observed number of offspring				χ^2 d.f. = 1
			+		−		
			♂	♀	♂	♀	
BgC1	♂*C1/C0* × ♀*C0/C0*	71×588	127	158	123	180	1.36
AmiF1	♂*F1/F0* × ♀*F0/F0*	27×155	37	52	30	36	3.41

* Test matings were chosen among cattle of four breeds. Genotypes of the parents were determined on the basis of the antigens segregating in the offspring. Gene-antigen determinant dependence was assumed.

Table 4. Segregation of BgC1 and AmiF1 antigenic determinants in the offspring from matings of double heterozygote fathers and recessive homozygote mothers*

Breed	Mating type (deduced genotypes)	Number of matings	Phenotypes and number of individuals			
			$C1^+F1^+$	$C1^+F1^-$	$C1^-F1^+$	$C1^-F1^-$
BW	♂ *C1/C0 F1/F0* × ♀ *C0/C0*	58	21	3	25	9
RW		34	11	2	19	2
PR	F0/*F0*	47	9	6	20	12

* - see Table 3

In the case of matings of double (as regards C1 and F1) heterozygote sires with recessive homozygote dams, four possible phenotypes, $C1^+F1^+$, $C1^+F1^-$, $C1^-F1^+$ and $C1^-F1^-$, occurred amoung the offspring (Table 4).

Discussion

The alloimmunization method used, viz. injections during immunizations with a full antierythrocytic serum and reimmunization with proteins of the same serum, which were coated on the recipients' erythrocytes, proved to be very effective. Precipitins identifying the previously described marker of beta-globulin BA8 and antibodies anti-BgC1 and anti-AmiF1, detecting two hitherto unknown antigenic determinants called BgC1 and AmiF1, were obtained.

Serological and physico-chemical analyses showed that C1 determinants are carried by molecules of beta-globulins with molecular weight (MW) of about 160 kD, while F1 determinants are carried by alpha-microglobulins with MW of about 100 kD. AmiF1 is the first antigenic marker of protein molecules, identified in cattle, with such a low molecular weight.

An independent analysis of phenotypes of each antigen from the offspring of the type + × +, + × – and – × – suggests that determinants C1 and F1 are transferred as units and can be determined by dominant genes. This is indicated by the numbers of positive offspring, which were in line with expectations: over 75% from the + × + matings and at least 50% from the + × – matings. The high values of χ^2 in the case of C1 in RW and PR breeds indicate that there is a lack of genetic balance in the populations under study. The identification of two $C1^+$ and one $F1^+$ offsprings from the mating of parents without

these characters does not seem to contradict the hypothesis of domination of characters over their absence and the gene-determinant relationship, because in such extensive material, documentation errors, connected with sampling and preparation of separate samples for hemolytic and precipitation tests, cannot be ruled out. There is also a possibility that individuals can occur which in fact do not originate from the stated parents and which are not excluded by blood groups.

The genetic control of C1 and F1 by the dominant genes *C1* and *F1*, respectively, and the gene-antigenic determinant relationship are also confirmed by studying the segregation of each character in offspring from test matings of the type heterozygote sire × recessive homozygote dam as well as a sire heterozygous for both characters × recessive homozygote dam. These results also indicate that the genes *C1* and *F1* are located at independent loci *BgC* and *AmiF* of autosomal chromosomes.

A direct serological and genetic comparison of C1 and F1 with markers of beta-globulins and alpha-globulins identified in cattle (WĘGRZYN 1973, WILLMANN-WĘGRZYN, WĘGRZYN 1975, WĘGRZYN, WĘGRZYN 1985, WĘGRZYN et al. 1977, 1986, 1996) indicates that the newly-discovered determinants are not determined by any of the genes controlling the previously described characters. For lack of a direct, serological comparison of this type of characters, nothing can be said about the identity of both markers, described with characters discovered in other laboratories. While an indirect comparison of physico–chemical properties of determinant molecule-carriers indicates that none of these characters is related to molecules similar to carriers BgC1 and AmiF1.

Markers of beta-globulins BgC1 and alpha-microglobulins AmiF1, like characters of this type determined previously, can be used both as markers of proteins in gene expression analysis and in physiological and molecular studies, and as markers of genes in the work on gene linkages and mapping, identification of individuals, characterization of breeds, etc.

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