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Linkage relationships in the bovine MHC region. High recombination frequency between class II subregions

Leif Andersson[1], Anne Lundén[1], Sunna Sigurdardottir[1], Christopher J. Davies[2], and Lars Rask[3]

[1] Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Biomedical Centre, Box 596, S-751 24 Uppsala, Sweden
[2] James A. Baker Institute for Animal Health, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA
[3] Department of Cell Research, Swedish University of Agricultural Sciences, Biomedical Centre, Box 596, S-751 24 Uppsala, Sweden

Abstract. Class II genes of the bovine major histocompatibility complex (MHC) have been investigated by Southern blot analysis using human DNA probes. Previous studies revealed the presence of bovine DO_β, DQ_α, DQ_β, DR_α, and DR_β genes, and restriction fragment length polymorphisms for each of these genes were documented. In the present study, the presence of three additional class II genes, designated DZ_α, DY_α, and DY_β, are reported. DZ_α was assumed to correspond to the human DZ_α gene while the other two were designated *DY* because their relationship to human class II genes could not be firmly established. The linkage relationships among bovine class II genes and two additional loci, *TCP1B* and *C4*, were investigated by family segregation analysis and analysis of linkage disequilibrium. The results clearly indicated that all these loci belong to the same linkage group. This linkage group is divided into two subregions separated by a fairly high recombination frequency. One region includes the *C4*, DQ_α, DQ_β, DR_α, and DR_β loci and the other one is composed of the DO_β, DY_α, DY_β, and *TCP1B* loci. No recombinant was observed within any of these subregions and there was a strong or fairly strong linkage disequilibrium between loci within groups. In contrast, as many as five recombinants among three different families were detected in the interval between these subregions giving a recombination frequency estimate of 0.17 ± 0.07. The fairly high recombination frequency observed between class II genes in cattle is strikingly different from the corresponding recombination estimates in man and mouse. The finding implies either a much larger molecular distance between some of the bovine class II genes or alternatively the presence of a recombinational "hot spot" in the bovine class II region.

Offprint requests to: L. Andersson

Introduction

The *BoLA* system is the major histocompatibility complex (MHC) of cattle. In homology with the situation in other mammalian species it is divided into a class I and a class II region. One class I locus (*BoLA-A*) has been established by serological studies (Amorena and Stone 1978, Spooner et al. 1978), while evidence for a bovine class II locus (*BoLA-D*) was obtained by cellular studies (Usinger et al. 1977). The knowledge regarding the genetic organization of *BoLA*, in particular the class II region, has recently increased considerably because of the use of human probes in genomic hybridizations (Andersson et al. 1986a, b, Andersson and Rask 1988). Based on the results which were obtained with a large set of different human class II probes, the following numbers of bovine class II genes were estimated: one to two DQ_α and DQ_β genes, one DR_α gene, at least three DR_β genes, and one DO_β gene; the number of *DQ* genes was found to vary between haplotypes (Andersson and Rask 1988). In this study the presence of a DZ_α gene and two other class II genes, tentatively designated DY_α and DY_β, are described.

So far, only limited data on the linkage relationships in the *BoLA* region have been reported. Early studies showed that *BoLA-A* and *BoLA-D* are linked (Spooner et al. 1978, Usinger et al. 1981) as expected from the organization of MHC genes in other species. Close linkage and fairly strong linkage disequilibrium between class I and class II genes have also been found by us (P. G. Lindberg and L. Andersson unpublished data); no recombinant between *BoLA-A* and *BoLA-DQ,DR* was found in material comprising 45 informative offspring. Close linkage and strong linkage disequilibrium have also been found among DQ_α, DQ_β, DR_α, and DR_β class II loci (Andersson et al. 1986a, b) as well as between blood group locus *M* and *BoLA-A* (Leveziel and Hines 1984). Furthermore, a locus designated *TCP1B* was recently reported to be linked to bovine MHC genes (Andersson 1988); *TCP1B* represents a restriction fragment length polymorphism (RFLP) of a

bovine homolog of the mouse *Tcp-1* gene which belongs to the *t* complex. In the present study the linkage relationship among *BoLA* class II genes was investigated by family segregation analysis. *TCP1B* and an RFLP of a gene for complement component four (C4), not previously described, are also included in the analysis.

Materials and methods

Animals. The results are based on the analyses of three sets of animals: (1) Family material from five sire half-sib families of the Swedish Red and White breed (SRB) comprised of, besides the bulls, 38 offspring and 37 different dams. Standard serological analyses as well as extensive RFLP typing have not revealed any indication of erroneous parentage in the families. (2) A population sample of the SRB breed comprised of 197 young breeding bulls. (3) A selected sample of 25 animals representing the American Holstein-Friesian breed (AHF). The AHF sample included the dam and eight full-siblings of one embryo-transfer family.

Hybridization probes. The probes utilized in the present study are compiled in Table 1. They are all human probes except the TCP1 probe, which was derived from the mouse. The purified probe fragments were labeled to high specific activity with $\alpha(-^{32}P)$ dCTP by nick-translation (Rigby et al. 1977). The genetic interpretations of RFLPs of DO_β, DQ_α, DQ_β, DR_α, DR_β, and *TCP1* genes and a proposed nomenclature for these RFLPs are described elsewhere (Andersson et al. 1986a, b, Andersson 1988, Andersson and Rask 1988; S. Sigurdardottir, A. Lundén and L. Andersson, unpublished data).

DNA isolation and analysis. Genomic DNA was isolated from blood or semen samples, and Southern blot analysis was carried out as previously described (Andersson et al. 1986a, Andersson 1988). The membranes were hybridized overnight in 40% formamide, 0.5 *M* NaCl at 42 °C, and subsequently washed in 0.7× standard sodium citrate at 60 °C.

Linkage analysis. The linkage relationships among the tested loci were investigated by a family segregation analysis. The statistical examination of the data followed the lod score method (Morton 1955). A lod score (*Z*) value of 3 or greater was considered significant evidence of linkage. The linkage relationships were also investigated by analyzing the presence of linkage disequilibria among the tested loci. The analysis was carried out using (i) the sample of parental animals in the family material of the SRB breed and (ii) the sample of breeding bulls of the SRB breed. In the former sample, it was possible to estimate linkage disequilibrium by direct counting of parental haplotypes, since the association between alleles could be deduced using family information; this approach was used only for those combinations of loci for which close linkage was indicated. In the sample of breeding bulls, linkage disequilibrium was tested for according to the method of Hill (1974). Linkage disequilibrium (D_{ij}) was estimated as $D_{ij}=X_{ij}-p_iq_j$, where, respectively, X_{ij}, p_i, and q_j are the observed frequencies of the haplotype A_iB_j, the allele A_i at the *A* locus, and the allele B_j at the *B* locus (cf. Hedrick et al. 1978, for estimates of linkage disequilibrium).

Results

Genetic polymorphism of DY_α. RFLPs of MHC class II α genes in cattle (as revealed using human probes and the restriction enzymes Bam HI, Eco RI, and Pvu II) have previously been reported for DQ_α and DR_α (Andersson et al. 1986a, b). Furthermore, in a recent study DQ_α and DR_α RFLPs were investigated using the enzyme Taq I (S. Sigurdardottir et al., unpublished data). In the latter study an RFLP was revealed with the DQ_α probe which did not correlate with the previously recognized DQ_α polymorphism, and it was therefore tentatively designated DY_α. The DY_α RFLP comprised two allelic fragments of 1.9 and 1.4 kb in size, respectively. The DY_α fragments hybridized quite strongly with the DQ_α probe but not at all with the DR_α and DZ_α probes at the stringency conditions employed (Fig. 1). They hybridized weakly with the DP_α probe (data not shown). Hybridizations carried out with exon-specific DQ_α probes indicated that the DY_α fragments hybridize with the second domain exon but not with the first domain exon.

Family data were consistent with codominant inheritance of two alleles which were designated DY^1_α and DY^2_α, corresponding to the 1.4 and 1.9 kb fragments, respectively. DY_α was polymorphic both in the SRB breed and in the AHF breed. The allele frequency of DY^2_α was estimated at 0.36 in the sample of SRB breed-

Table 1. Documentation of hybridization probes employed

Probe corresponding to	Type of clone	Designation of clone	Restriction sites employed	Size of probe fragment (bp)	Reference
C4	cDNA	pAT-A	Hind III/Sal I	5500	Belt et al. 1984
DO_β	Genomic	p107-2	Acc I/Stu I	317	Servenius et al. 1987
DP_α	cDNA	pDAα13B	Ava I/Eco RI	600	Trowsdale et al. 1985
DP_β	cDNA	pII-β-7	Hpa I/Rsa I	601	Gustafsson et al. 1984a
DQ_α	cDNA	pII-α-5	Rsa I/Stu I	584	Schenning et al. 1984
DQ_α 1st domain	cDNA	pII-α-5	Mst II/Rsa I	193	Schenning et al. 1984
DQ_α 2nd domain	Genomic	p102-2	Stu I	592	Jonsson et al. 1987
DQ_β	cDNA	pII-β-1	Ava I	627	Larhammar et al. 1982a
DR_α	cDNA	pII-α-1	Pst I/Sac I	598	Larhammar et al. 1982b
DR_β	cDNA	pII-β-3	Hind III/Sac I	790	Gustafsson et al. 1984b
DR_β 2nd domain	Genomic	p3101-2	Pvu II/Xba I	573	Andersson et al. 1987
DZ_α	Genomic	I68b	Pst I	1800	Trowsdale and Kelly 1985
TCP1	cDNA	pB1.4	Bam HI	790	Dudley et al. 1984

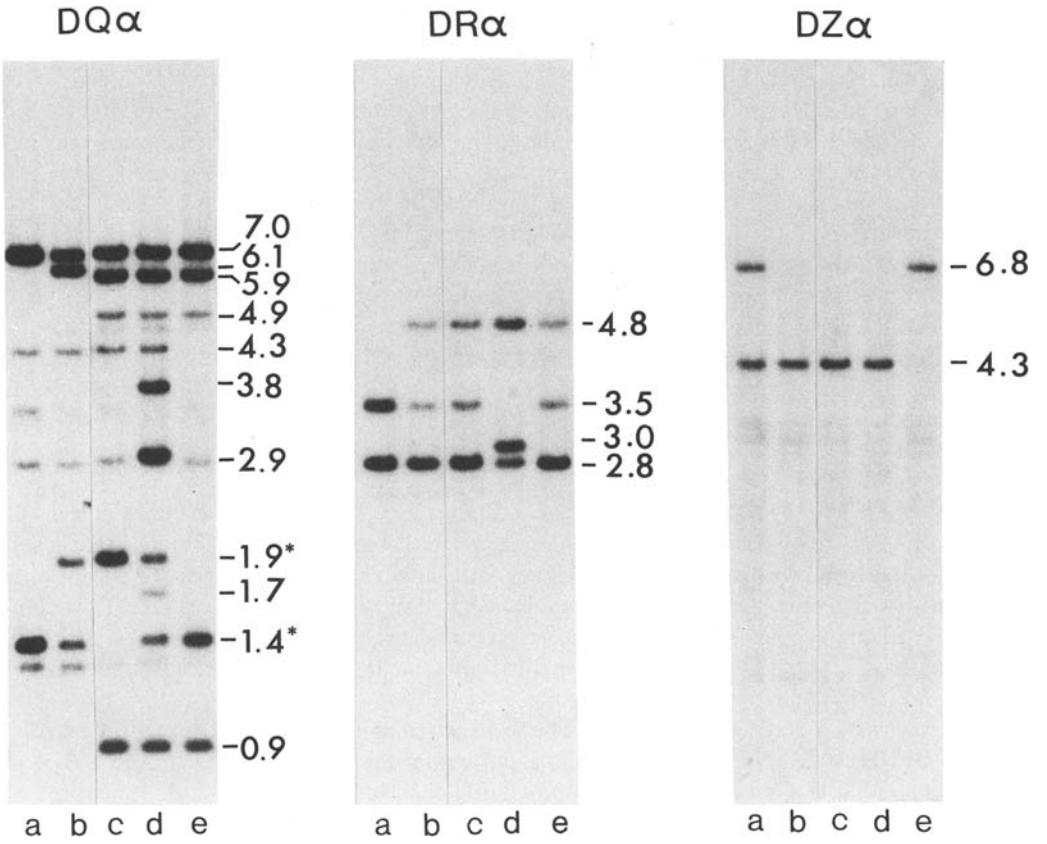

Fig. 1. Southern blot analysis of class II α chain genes in cattle. Genomic DNA samples were digested with Taq I and consecutively hybridized with human DQ_α, DR_α, and DZ_α probes. The animals were typed as follows: a: DQ_α 1/1, DR_α 1A/1A, DY_α 1/1, DZ_α 1/2; b: DQ_α 1/2, DR_α 1A/2, DY_α 1/2, DZ_α 1/1; c: DQ_α 1/9, DR_α 1A/2, DY_α 2/2, DZ_α 1/1; d: DQ_α 7/9, DR_α 1B/2, DY_α 1/2, DZ_α 1/1; e: DQ_α 1/9, DR_α 1A/2, DY_α 1/1, DZ_α 2/2. The estimated sizes of fragments are given in kilobases and the DY_α fragments are indicated by an *asterisk*

ing bulls, and there was an excellent agreement with expected Hardy-Weinberg proportions in this material.

Genetic polymorphism of DZ_α. Genomic DNA samples were separately digested with Pvu II and Taq I and hybridized to the human DZ_α probe. The probe hybridized quite strongly with cattle DNA and the results were consistent with a single bovine DZ_α gene. Two constant 9.5 and 1.6 kb fragments were obtained with Pvu II, whereas two variable 6.8 and 4.3 kb fragments were obtained with Taq I (Fig. 1). The DZ_α fragments cross-hybridized weakly with the DQ_α (Fig. 1) and DP_α (data not shown) probes but not with the DR_α probe (Fig. 1). The DZ_α RFLP was only found in the AHF breed. We have no family data supporting its inheritance, but the RFLP pattern was consistent with a simple two-allele polymorphism. The two alleles corresponding to the 4.3 and 6.8 kb fragments were designated DZ^1_α and DZ^2_α, respectively.

Genetic polymorphism of DY_β. Bovine DR_β RFLPs have previously been detected using Eco RI and Pvu II (Andersson et al. 1986b) and, subsequently, using Taq I (S. Sigurdardottir et al., unpublished data). In the latter study two variable fragments were found which did not correlate with the previously described DR_β polymorphism; they were designated DY_β. The two DY_β fragments are illustrated in Figure 2; they were 6.7 and 2.9 kb in size. The DY_β fragments hybridized with the DR_β full-length probe as well as with the DR_β second domain exon probe. At the stringency conditions employed, they also cross-hybridized weakly with the DQ_β and DP_β probes but not with the DO_β probe.

Family data were consistent with Mendelian inheritance of the two DY_β variants. They were assumed to be allelic since they segregated as alleles in one large sire family and since they have not been found to be inherited on the same chromosome. The DY_β variants were both found in the SRB breed as well as in the AHF breed; the frequency of both variants was quite low in the SRB breed.

Many individuals lacked both the 2.9 and the 6.7 kb DY_β fragments and therefore we looked for a third DY_β variant which should be present unless the two former variants represent gene duplications/deletions. The identification of such a fragment was difficult because the two DY_β variants were quite rare and because of the large

number of Taq I fragments hybridizing with the DR_β probe. However, a 2.0 kb fragment was missing in those individuals which exhibited both the 2.9 and 6.7 kb fragments or were assumed to be homozygous for one of the two fragments (cf. Fig. 2). On the basis of this finding, we postulate that there are three DY_β alleles, designated DY^1_β, DY^2_β, and DY^3_β, which are defined by the 2.0, 2.9, and 6.7 kb Taq I fragments, respectively.

The presence of the DY^2_β and DY^3_β alleles was found to correlate with a reduced intensity or absence of a 6.1 kb Pvu II fragment obtained with the DR_β probe (Fig. 3). That some individuals lack this fragment was previously reported but the finding was not interpreted genetically (Andersson et al. 1986b). The present results are consistent with the interpretation that the DY^1_β allele is represented by a 6.1 kb Pvu II fragment while the DY^2_β and DY^3_β alleles are represented by a 5.5 kb Pvu II fragment; the 5.5 kb fragment is assumed to comigrate with a constant DR_β fragment. Thus, according to our interpretation the Pvu II RFLP types of different genotypes should be as follows: (i) DY^1_β/DY^1_β homozygotes should exhibit a 5.5 and 6.1 kb fragment having about the same intensity, (ii) DY^1_β/DY^2_β and DY^1_β/DY^3_β heterozygotes should exhibit a strong 5.5 and a weak 6.1 kb fragment, (iii) other genotypes (DY^2_β/DY^3_β, DY^2_β/DY^2_β, and DY^3_β/DY^3_β) should have a strong 5.5 kb fragment but lack the 6.1 kb fragment (cf. Fig. 3).

Genetic polymorphism of C4. A full-length cDNA probe corresponding to a human gene for complement component four (C4; cf. Table 1) was used to screen for C4 RFLP in cattle. Genomic DNA samples representing about 20 animals each of the SRB and AHF breeds were separately digested with Pvu II and Taq I and hybridized with the C4 probe. Genetic polymorphism was only revealed with Taq I in this material. Digestions with Taq I gave four constant (1.1, 1.2, 3.2, and 4.2 kb) and two variable fragments (5.7 and 6.8 kb) as shown in Figure 4. The variable 6.8 and 5.7 kb fragments were obviously allelic forms and the corresponding alleles were designated $C4^1$ and $C4^2$, respectively. Family data were consistent with codominant Mendelian inheritance of these alleles. *C4* was polymorphic in both cattle breeds investigated, and the allele frequency of $C4^2$ was estimated at 0.49 in the SRB breed.

Linkage relationships. The results of the linkage analysis are summarized in Table 2. Significant evidence for genetic linkage was obtained in all comparisons except one, i. e., $DQ_\beta : DO_\beta$, DY_β. However, the results of this comparison also indicated the presence of linkage, but with the small sample size the data did not reach statistical significance.

The presence of close linkage and strong linkage disequilibrium among the DQ_α, DQ_β, DR_α, and DR_β loci was previously reported (Andersson et al. 1986a, b); no recombinant has yet been observed among this group of loci. The data given in Table 2 are based on the same family material as used in the previous study but complemented with ten additional offspring in one of the sire families.

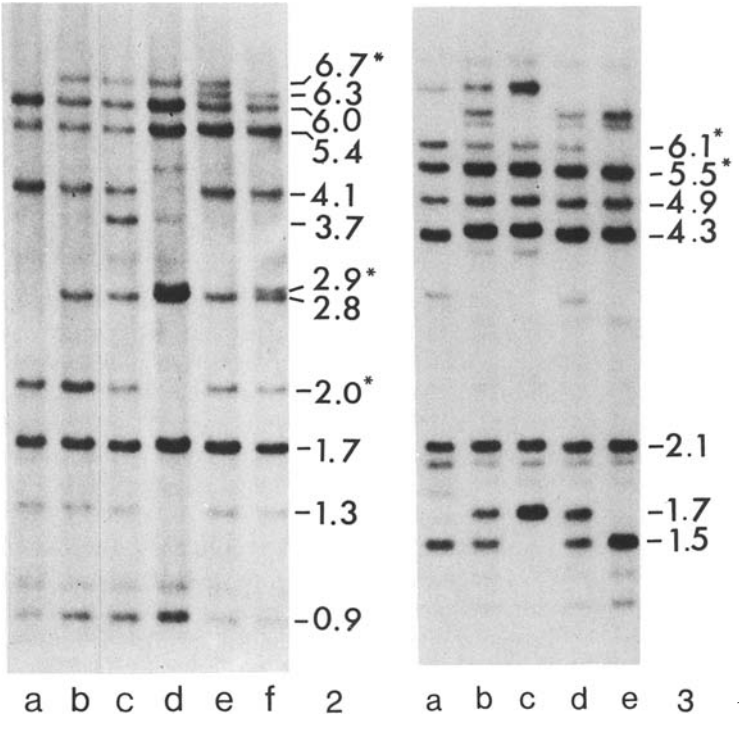

Fig. 2. Restriction fragment patterns of cattle genomic DNA digested with Taq I and hybridized with a human DR_β second domain probe. The following DY_β types are shown: a: 1/1, b: 1/3, c: 1/3, d: 2/3, e: 1/3, f: 1/2. The estimated sizes of fragments are given in kilobases and the DY_β fragments are indicated by an *asterisk*

Fig. 3. Restriction fragment patterns of cattle genomic DNA digested with Pvu II and hybridized with a human DR_β probe. The following DY_β types are shown: a: 1/1, b: 1/3, c: 1/3, d: 1/3, e: 2/3. The estimated sizes of fragments are given in kilobases and the positions of the DY_β fragments are marked by an *asterisk*. It should be noted that the 1.5 kb and 1.7 kb fragments represent a DR polymorphism (Andersson et al. 1986b) and that the variable fragments not indicated by size are DQ_β specific

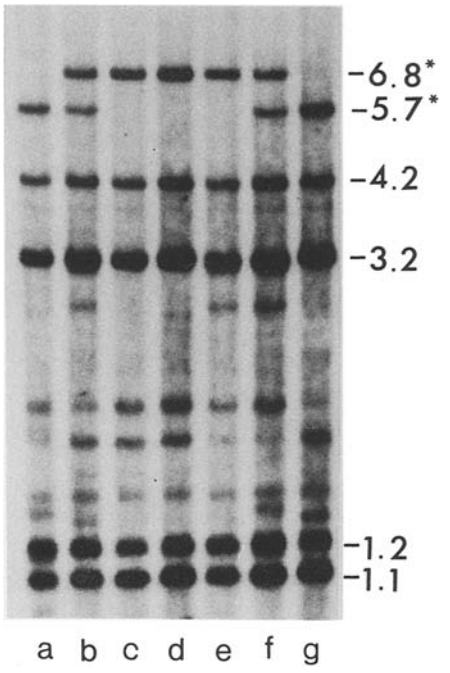

Fig. 4. Restriction fragment patterns of cattle genomic DNA digested with Taq I and hybridized with a human C4 probe. The following C4 types are shown: a: 2/2, b: 1/2, c: 1/1, d: 1/1, e: 1/1, f: 1/2, g: 2/2. The estimated sizes of fragments are given in kilobases and variable fragments are indicated by an *asterisk*

In the further analyses, DQ_β was chosen as a marker for the *DQ-DR* region since the DQ_β allele transmitted from the segregating parent could be determined in all offspring.

The family segregation data clearly showed that *C4* is genetically linked to the *DQ*, *DR* loci (Table 2). No recombinant between the *C4* and *DQ*, *DR* loci was found in this limited material. The linkage appears to be very close since a strong linkage disequilibrium between *C4* and *DQ* haplotypes was found in the sample of parental animals in the SRB family material. There were only a few exceptions to the rule that a given *DQ* haplotype was exclusively associated with one of the two *C4* alleles (data not shown).

Significant evidence for genetic linkage among the DO_β, DY_α, DY_β, and *TCP1B* loci was obtained (Table 2). The results are based on three informative sib groups. One SRB sire was heterozygous at DO_β, DY_β, and *TCP1B* while another SRB sire and the dam of the AHF full-sib family both were heterozygous at DY_α and DY_β. No recombinant was found, but the material is so limited that the recombination frequency between any pair of these loci could be substantial. For instance, the finding of no recombinant among 15 offspring gives a 95% confidence interval which includes recombination frequencies from 0–0.18 (estimated according to Sokal and Rohlf 1981). The possibility that the linkage may be close or at least fairly close is supported by the finding of highly significant linkage disequilibrium among these loci in the SRB breed (Table 3).

The family segregation analysis (Table 2) together with the analysis of linkage disequilibrium (Table 3) showed that the DO_β, DY_α, DY_β, and *TCP1B* loci are linked to the *DQ*, *DR* loci. The linkage analysis is based on three informative families and these data, by themself, approached statistical significance (Table 2). There were 7, 8, and 14 informative offspring in the three sib groups and the data indicated that the number of recombinants in these families were 1, 1, and 3, respectively. The result

Table 2. Summary of linkage analysis data involving the BoLA linkage group

Comparison	No. of families	No. of offspring	No. of putative recombinants	$\hat{Z}$*	$\hat{\theta}$†
$DQ_\beta : DQ_\alpha$	4	33	0	8.7	0
$DQ_\beta : DR_\alpha$	2	19	0	5.1	0
$DQ_\beta : DR_\beta$	4	33	0	8.7	0
$DQ_\beta : C4$	2	16	0	4.2	0
$DO_\beta : DY_\beta$	1	14	0	3.9	0
$DY_\alpha : DY_\beta$	2	15	0	3.6	0
$TCP1B : DO_\beta$, DY_β	1	13	0	3.6	0
$DQ_\beta : DO_\beta/DY_\beta$	3	29	5	2.0	0.17

* Maximum lod score. A $\hat{Z}$ value of 3 or greater is generally considered significant evidence of linkage

† The recombination fraction at which $\hat{Z}$ was obtained, i. e., the recombination fraction which best fit the available data

Table 3. Analysis of linkage disequilibrium among loci in the BoLA linkage group

Comparison $A_i : B_j$	Sample†	*n*	D_{ij}	χ^2_1
$DO^1_\beta : DY^1_\alpha$	F	42	0.066	30.1***
$DO^1_\beta : TCP1B^0$	F	42	0.042	28.8***
$DY^1_\alpha : TCP1B^0$	F	42	0.057	25.5***
	B	197	0.058	24.0***
$DQ^{1A} : DY^1_\alpha$	B	197	0.044	12.1***
$DQ^2 : DY^1_\alpha$	B	197	-0.022	2.8
$DQ^9 : DY^1_\alpha$	B	197	0.042	14.5***
$DQ^{1A} : TCP1B^0$	B	197	-0.002	0.0
$DQ^2 : TCP1B^0$	B	197	-0.025	6.9**
$DQ^9 : TCP1B^0$	B	197	0.002	0.1

** $P<0.01$; *** $P<0.001$

† Two population samples of the SRB breed were investigated. F represents the sample of parental animals in the family material and B represents the sample of breeding bulls (see Materials and methods). In the latter material, only the DQ_α, DQ_β, DY_α, and *TCP1B* loci have been tested so far

gives an estimate of the recombination frequency in this interval of 0.17 ± 0.07.

An examination of the SRB family data did not reveal any obvious linkage disequilibrium between *DQ* and any of the DO_β, DY_α, DY_β, or *TCP1B* loci (data not shown). Many of the *DQ* haplotypes were observed to be inherited together with any one of the alleles at these latter loci as expected from the high recombination frequency between these groups of loci. However, the possible presence of linkage disequilibrium between *DQ* haplotypes and DY_α and *TCP1B* alleles was also investigated in the much larger sample of SRB breeding bulls (sample B in Table 3). The analysis was carried out for each *DQ* haplotype separately by pooling the data to create a two-allele system, e. g., DQ^2 versus not DQ^2 (cf. Hedrick et al. 1978). Significant associations were revealed for several of the comparisons, in particular between *DQ* and DY_α; the results for the three most common *DQ* haplotypes are given in Table 3. The result may seem unexpected considering the fairly high recombination frequency indicated by the linkage analysis. However, the results are most likely explained by the population structure in the breed since these breeding bulls are the offspring of a limited number of sires. In this material there were only 41 different sires and some of them had ten or more offspring. This fact explains why even loosely linked loci tend to show linkage disequilibrium. In conclusion, the data on linkage disequilibrium in the sample of breeding bulls clearly support the presence of loose genetic linkage between *DQ*, *DR* and DO_β, DY_α, DY_β, and *TCP1B* loci.

At present we have no family segregation data that are informative with regard to the linkage relationships of DZ_α. However, there does not seem to be a close association between *DZ* and *DQ* polymorphism as illustrated by the genotypes in Figure 1.

Discussion

In the present study three bovine MHC class II related sequences, designated DY_α, DY_β, and DZ_α, were identified by Southern blot analysis. Furthermore, four loci, *C4*, DO_β, DY_α, and DY_β, were assigned to the linkage group including the bovine MHC (*BoLA*). This means that the *BoLA* linkage group is now composed of blood group locus *M*, *BoLA-A* (class I), *BoLA-*DQ_α, DQ_β, DR_α, DR_β, DO_β, DY_α, DY_β (class II), *C4* (complement component four), and *TCP1B*. DZ_α, detected in the present study, most likely belongs to this linkage group, but this has not yet been possible to test. The results of the present study show that this linkage group is divided into two regions that are separated by a fairly high recombination frequency. One region includes the *M*, *A*, *DQ*, *DR*, and *C4* loci while the other one is composed of the DO_β, DY_α, DY_β, and *TCP1B* loci. Five recombinants among three different families were detected in the interval between these two groups of loci and the recombination frequency was estimated at 0.17 ± 0.07. At present, there is no conclusive data concerning the order of genes within groups. The results of the present and previous studies (Leveziel and Hines 1984, Andersson et al. 1986a, b; P. G. Lindberg et al., unpublished data) show that there is a strong or fairly strong linkage disequilibrium between loci within each region while there is no or only weak linkage disequilibrium between loci in different regions.

The organization of the corresponding regions in man (*HLA*) and in the mouse (*H-2*) has been well established by segregation analyses and extensive molecular studies. The order of genes in the *HLA* region has been established as follows: centromere-DP_α, DP_β-DZ_α-DO_β-DX_α, DX_β-DQ_α, DQ_β-DR_β-DR_α-*C2*, *C4*, *Bf*-*B*-*C*-*A* (Bodmer et al. 1986, Hardy et al. 1986); *A*, *B*, and *C* are *HLA* class I loci, *C2*, *C4*, and *Bf* encode complement components, and all others are *HLA* class II genes. This genetic map is in close agreement with the organization of the corresponding *H-2* region in the mouse but with the exceptions that one class I locus, *H-2K*, maps centromeric to the class II genes and mouse homologs of DP_α and DZ_α have not been found (Kobori et al. 1986, Steinmetz et al. 1986). Comparison of these data with the present knowledge of the *BoLA* linkage group reveals several striking similarities such as the linkage of a similar set of class II genes, the close linkage of class I and class II genes, and the location of complement genes in the region.

The data on the *BoLA* region obtained so far are thus consistent with an organization quite similar to the one established for *HLA* and *H-2*. However, the finding of a high recombination frequency between the *DQ*, *DR* and DO_β, DY_α, DY_β genes in cattle was unexpected. The molecular distance between $A_{\beta 2}$ (the mouse homolog of DO_β) and A_β (the mouse homolog of DQ_β) is only about 20 kb (Larhammar et al. 1985), and the corresponding distance in man has been estimated to be less than 200 kb by pulsed-field electrophoresis (Hardy et al. 1986). The recombination distance in this interval has apparently not been estimated directly, but data on the recombination distance between A_β/DQ_β and flanking markers of $A_{\beta 2}/DQ_\beta$ (i. e., *H-2K* and *HLA-DP*) provide upper limits of about 1.5 cM in mice (Steinmetz et al. 1986) and about 3 cM in man (Termijtelen et al. 1983). Thus, the present estimate of about 17 cM in cattle is strikingly different. The finding is either explained by a much larger molecular distance between these loci in cattle or by the presence of a recombinational "hot spot." It is interesting that several recombinational "hot spots" have been found in the class II region of the mouse; one occurs within the intron between the $\beta 1$ and $\beta 2$ exons of the E_β gene and two "hot spots" are located in the interval between A_β and *H-2K* (Kobori et al. 1986, Steinmetz et al. 1986). In cattle, pulsed-field electrophoresis should be carried out

to provide information on the molecular distance between the *DQ*, *DR* and DO_β, DY_α, DY_β genes.

It is interesting to note that the appreciable recombination frequency between *DQ-DR* and *DO-DY* in cattle and the recombination frequency between *DQ-DR* and *DP* in man are in sharp contrast to the very close linkage between *DQ* and *DR* in both species. No recombinant has yet been found between these two loci and there is a strong linkage disequilibrium between them in both species. Interestingly, in man the differences in recombination frequency do not correlate with differences in the molecular distance between the different class II loci (Hardy et al. 1986). It is tempting to speculate that the strong association between *DQ* and *DR* alleles is adaptive. DQ and DR molecules have an identical or very similar function in the immune system and they are both highly polymorphic. It is well established that this genetic polymorphism is associated with significant differences between allelic class II molecules regarding their influence on the immune response (Benacerraf and Germain 1978, Zinkernagel and Doherty 1979). It is possible that certain *DQ* and *DR* alleles are closely associated because they interact favorably in their function.

The DY_α and DY_β sequences detected in the present study were denoted DY because their relationship to human class II genes was not clear. It should be noted that these sequences were both denoted DY although there are no data indicating that they are associated like DQ_α and DQ_β genes. DY_α and DY_β cross-hybridized primarily with the human DQ_α and DR_β probes, respectively, but with a weaker intensity than the presumed bovine DQ_α and DR_β genes. However, they were identified as unique class II sequences first when Taq I RFLPs were detected which mapped to the DO_β, *TCP1B* region rather than to the *DQ*, *DR* region. There are several possible explanations for the current observations. First, DY_α and DY_β may be bovine DQ_α and DR_β sequences which have been duplicated and transposed to the present location and which have diverged more rapidly from their human counterparts. Second, their map position suggests that they may correspond to the human *DP* genes. No clear *DP* homologs have yet been identified in cattle (Andersson and Rask 1988). The hybridization results did not support this possibility although weak cross-hybridizations were obtained with the DP probes. There is of course a possibility that bovine DQ_α and DR_β genes have donated sequences to the *DP* genes by gene conversion, unequal crossing-over, or a similar mechanism, causing the present reaction pattern with the human probes; there are a number of reports indicating that these types of mutations occur quite frequently among class II genes (Denaro et al. 1984, McIntyre and Seidman 1984, Gorski and Mach 1986, Wu et al. 1986). The most interesting possibility, although not the most likely one, is that DY_α and/or DY_β represent "new" members in the class II family unique for cattle or not yet detected in other species. It should be straightforward to clone and characterize the DY sequences and thereby clarify the relationship to class II genes in other species.

Previous studies on the bovine class II region revealed the presence of one to two DQ_α and DQ_β genes, one DR_α gene, at least three DR_β genes, and one DO_β gene (Andersson and Rask 1988). To this list are now added DZ_α, DY_α, and DY_β. Thus, there are at least four α and six β class II genes in the bovine genome. This number is even higher in those haplotypes which carry duplicated DQ_α and DQ_β genes (Andersson and Rask 1988). The results imply that the complexity of the class II region in cattle is comparable to the one in man, at least at the genomic level.

An important topic for future research is to relate the polymorphism and diversity of bovine class II genes detected at the genomic level with that expressed at the protein level. Studies on traits controlled by class II genes in other species, such as immune response and mixed lymphocyte reactions (MLR) should be of great interest. Class II polymorphism has in fact been investigated using class II alloantisera and MLR assays in the sample of 25 AHF animals included in the present study (Davies 1988; C. J. Davies et al., unpublished data). These studies showed that the serological and MLR typing correlated well with *DQ*, *DR* RFLPs. Furthermore, there was clear evidence that primary MLR reactions are controlled by genes in the *DQ*, *DR* subregion. No significant effect of the DO_β, DY_α, DY_β subregion was revealed. It may be worthwhile to study the effect of this latter subregion in secondary MLRs since that procedure is necessary in order to reveal DP polymorphism in man (Bach 1985). Thus, it is not yet known if any of the genes in the DO_β, DY_α, DY_β subregion are expressed and their functional significance in the bovine immune system is an open question.

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