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BRIEF COMMUNICATION

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Characterization of cattle cDNA sequences from two *DQA loci*

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The class II region of the major histocompatibility complex (*MHC*) of cattle encodes antigen-presenting molecules of two isotypes, DR and DQ. These highly polymorphic cell-surface glycoproteins bind peptide fragments from mainly exogenous antigens and present them to CD4 T cells to initiate an immune response. Each DR or DQ molecule can bind a range of antigenic peptides, defined by the shape and charge properties of the antigen binding cleft (Brown et al. 1993). Thus, the expression of a wide range of different class II molecules could increase the range of antigens presented to the immune system. Each class II haplotype expresses a single DR molecule, encoded by the *DRA* and *DRB3* genes, but one or more DQ products because of the duplication which occurs in about half of the common class II haplotypes (Andersson and Rask 1988). The *DQ* locus is duplicated in primates, but the *DQA2* and *DQB2* genes are transcriptionally silent (Kappes and Strominger 1988). In contrast, the *DQB* genes on duplicated cattle haplotypes are expressed (Bissumbhar et al. 1994; Xu et al. 1994; Marello et al. 1995). In order to correlate class II gene expression and polymorphism with immune function, we are cloning and transfecting the class II genes expressed by a pair of immunologically characterized Holstein-Friesian cattle. The animals (numbers 10795 and 10814) have well-characterized responses to immunization with a model peptide antigen, FMDV15, derived from foot-and-mouth-disease virus (Glass et al. 1991, 1992; Glass and Millar 1994), and had been extensively typed as part of the fifth BoLA workshop (Davies et al. 1994). The *MHC* types carried by the animals were:

10795 BoLA-A11, *DRB3*0102*, DQA1A, DQB1 (class II haplotype *DH24A*);
BoLA-A36, *DRB3*1201*, DQA12, DQB12 (class II haplotype *DH8A*).

10814 BoLA-A11, *DRB3*0102*, DQA1A, DQB1 (class II haplotype *DH24A*);
BoLA-A32, *DRB3.2*15*, DQA1E, DQB1 (class II haplotype *DH15B*).

Presentation of the FMDV15 antigen by mouse L cells transfected with the *DRA-DRB3* gene pair from the shared *DH24A* haplotype has been described previously (Fraser et al. 1996). Here we report the *DQA* sequences expressed by these animals, determined from polymerase chain reaction (PCR)-amplified cDNA clones.

The lack of extensive DNA sequence data for the cattle *DQA* genes led us to use the available sequences from cattle and sheep to design primers which could amplify full-length *DQA* genes from cattle cDNA preparations. To improve the chances of amplifying all possible *DQA* sequences, one forward and two reverse primers were designed. All three primers contained degenerate bases to take into account positions which were polymorphic in the *DQA* sequences used. The forward primer DQAFWD (5′-CCA CCT TGA GAA SAG GAT GRT CCT G-3′) annealed at the 5′ end of the *DQA* gene and included the start codon (underlined). The reverse primers DQAREV1 (5′-ACT TKG SCA GAA AMT AGY TCT AGG-3′) and DQAREV2 (5′-TGA GAT GAT AYA GCA AYC TTA AGT CC-3′) annealed in the 3′ untranslated region, approximately 70 and 140 base pairs (bp), respectively, beyond the termination codon.

Full-length *DQA* sequences were amplified from first-strand cDNA from both animals using a high-fidelity PCR system (Expand High Fidelity, Boehringer Mannheim, Lewes, UK) to reduce the frequency of PCR artefacts. Amplifications using the DQAFWD-DQAREV1 and DQAFWD-DQAREV2 primer pairs produced clean products of the expected sizes (880 and 950 bp, respectively) from animal 10795, but only the larger product was obtained from animal 10814 (Fig. 1). Despite several experiments using different RNA and cDNA preparations,

The nucleotide sequence data reported in this paper have been submitted to the EMBL, GenBank, and DDBJ nucleotide sequence databases and have been assigned the accession numbers Y07819, Y07820, and Y07898

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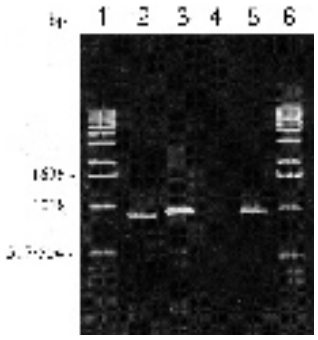

Fig. 1 Agarose gel electrophoresis of *DQA* PCR products. cDNA equivalent to about 0.5 μg of total RNA was amplified by PCR using the primers shown below. Approximately 5% of each PCR reaction was used. Lane 1,6 1 kb ladder size markers (Life Technologies, Paisley, UK). Relevant fragment sizes are indicated in base pairs (*bp*) at the side of the gel.
Lane 2 DQAFWD - DQAREV1 amplification from animal 10795
Lane 3 DQAFWD - DQAREV2 amplification from animal 10795
Lane 4 DQAFWD - DQAREV1 amplification from animal 10814
Lane 5 DQAFWD - DQAREV2 amplification from animal 10814

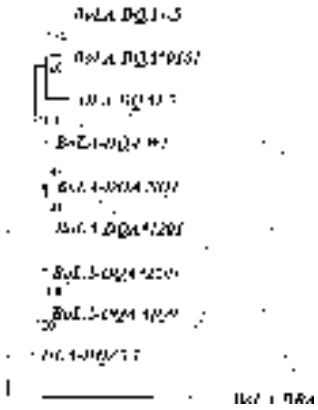

Fig. 2 Phylogeny of *DQA* protein sequences. The analysis was based on an alignment of the amino acid sequences of the published full-length cattle *DQA* sequences and representative sheep *DQA1* and *DQA2* sequences. Sequence names are those used in the text and references therein: the prefixes *BoLA* and *OLA* indicate cattle and sheep sequences respectively. The cattle *DRA* sequence is included as an outgroup. The tree shown was derived by the neighbor-joining method, but maximum parsimony analysis gave a best tree with the same topology. The number at each branch-point indicates the percentage of bootstrap trials in which the sequences to the *right* of the branch were grouped together (total number of trials was 500)

no product was obtained from animal 10814 with the DQAFWD-DQAREV1 primer pair, suggesting that it did not express any *DQA* sequences complementary to the DQAREV1 primer. The products obtained were cloned into the TA vector pCR3 (Invitrogen, Leek, The Netherlands).

Five recombinant clones from each TA cloning were initially analyzed, and all contained *DQA*-like sequences with an open reading frame of the expected size. Two distinct sequences were amplified from animal 10795 with the DQAFWD-DQAREV1 primer pair, represented by clones R1-1 and R2-2, while three sequences were amplified by the DQAFWD-DQAREV2 primers, R1-1, R2-2 and a third sequence represented by clone R2-15. In contrast, all of the clones derived from animal 10814 were identical, with the same sequence as clone R2-15. Allele names for these three sequences are suggested in accord with the system described by Mikko and Andersson (1995), based on the associated *DQA* restriction fragment length polymorphism types: R2-15 is named *DQA***0101*, R2-2 is named *DQA***1201*, and R1-1 is named *DQA***2201*.

The three new sequences were compared with all of the available full-length *DQA* sequences from cattle, and sequences representing the *DQA1* and *DQA2* loci of sheep (Fabb et al. 1993). Phylogenetic analysis of the peptide sequences, using the neighbor-joining method (Fig. 2; Felsenstein, 1988), demonstrated that the three sequences determined here represented the products of *DQA1*-like and *DQA2*-like loci, and that the *DQA1*-like sequences could be divided into two groups. Interestingly, the cattle cDNA clone α5 (Xu et al. 1993), and the *DQA***0101* sequence resembled the sheep *DQA1.2* sequence (Fabb et al. 1993) more closely than the other *DQA1*-like sequences from cattle – NQ1 (Nishino et al. 1995), W1 (van der Poel et al. 1990), and *DQA***1201* (Fig. 2).

The *DQA***2201* allele closely resembles the sheep *DQA2.1* sequence and the cattle cDNA sequence MQ9 (Morooka et al. 1995), and differs from the MQ9 sequence at only six amino acid positions. These differences are spread throughout the molecule: two residues in the signal peptide, one in the α1 domain, two in the α2 domain, and one in the cytoplasmic domain (Fig. 3).

*DQA***0101* appears to be a *DQA1* allele, clustering with the sheep *DQA1.2* and the cattle *DQA* clone α5 sequences (Fig. 2), and it shares sequence motifs with the α5 cDNA not found in the other *DQA1*-like sequences *DQA***1201*, NQ1, and W1 (Fig. 3). Nevertheless, *DQA***0101* and α5 are distinct from each other with sixteen amino acid differences, only half of which are in the polymorphic α1 domain.

The group of sequences which includes *DQA***1201* appear to form a subset of cattle *DQA1* alleles distinct from the *DQA***0101* group, but which appear to be well conserved. The three sequences *DQA***1201*, NQ1, and W1 differ from each other at only five amino acid positions, four of which are in the α1 domain (Fig. 3).

The differences between the *DQA***0101* and *DQA***1201* sequences may be haplotype-related, since *DQA***1201* is associated with a duplicated *DQ* haplotype and *DQA***0101* is found on two unduplicated haplotypes. As such, the sequence groups represented by *DQA***0101* and *DQA***1201* may be considered to be either different lineages of a single *DQA* locus or alleles of distinct *DQA* loci. The α1 domain of the *DQA***0101* peptide sequence is identical to the BNI1 sequence of Gelhaus and co-workers (1995), and the *DQA***1201* sequence is identical to BNI9. Since these exon 2 sequences were amplified using primers containing flanking intron sequences (Gelhaus et al. 1995), it is likely that the *DQA***0101* and *DQA***1201* sequences represent different lineages of *DQA1* genes in cattle. There are three major human *DQA1* lineages all of which show

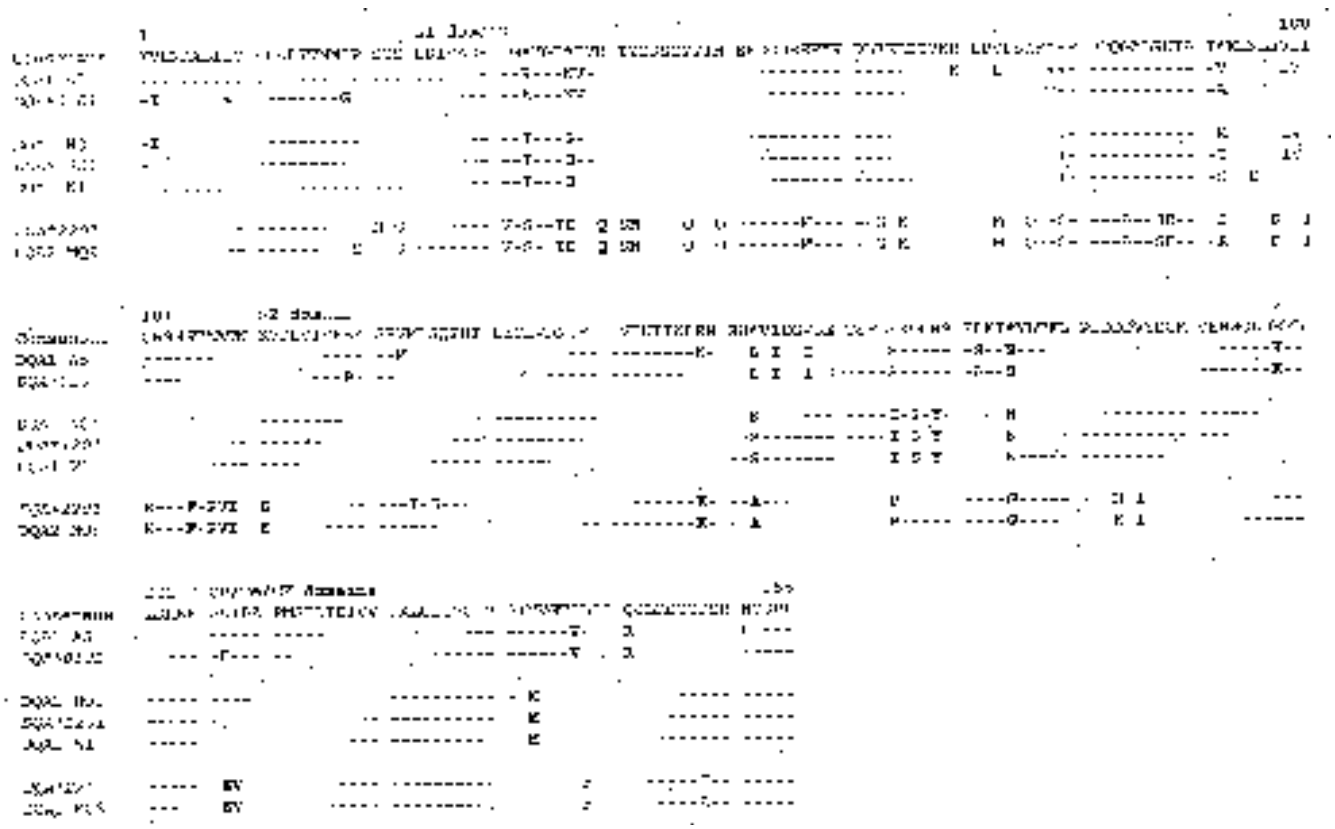

Fig. 3 Multiple alignment of full-length cattle *DQA* amino acid sequences. The alignment was generated by the GCG program PILEUP, and displayed using the program PRETTY. Residues identical to the consensus sequence are indicated by *dashes* (–), and areas where sequence data were not available are shown as *dots* (.). Positions where no consensus sequence was found are shown by *asterisks* (*). Residues characteristic of each sequence group are shown in *bold*, and are based on alignments which included sequences from *DQA* exon 2 genomic clones (Gelhaus et al. 1995; K.T. Ballingall, accession numbers Z79052–Z79526, unpublished information)

pairing preferences in the assembly of DQA-DQB heterodimers (Kwok et al. 1993).

The isolation of clones representing a single *DQA* sequence from the *MHC* heterozygous animal 10814 left open the possibility that a second allele was expressed in this animal, but had not been detected in the five clones sequenced. Seventeen further clones from animal 10814, analyzed by sequencing of the polymorphic exon 2 region, were also identical to the *DQA*0101* sequence. To ensure that selective PCR amplification was not excluding a second expressed allele in this animal, PCR primers were designed to amplify exon 2 from cDNA, annealing at sites that were absolutely conserved in all of the available sheep and cattle *DQA* sequences. The primers were: DQAEX2FWD (5′-GTG AAG ACA TTG TGG CTG ACC AC-3′) at the exon 1-exon 2 boundary; and DQAEX3REV (5′-GGA GAC TTG GAA AAC ACA GTC A-3′) approximately 15 bp inside exon 3. In addition, *DQA1*-specific exon 2 primers (Gelhaus et al. 1995) were used to determine whether a second, untranscribed, *DQA1* allele was present in animal 10814. Direct sequencing of exon 2 from the 280 bp genomic PCR products, the 297 bp cDNA products, and the full-length cDNA products from both animals showed the expected polymorphism in animal 10795, but none in animal 10814 (not shown).

The isolation of the *DQA*0101* sequence from both animals identifies this sequence with the shared *DH24A* haplotype, and consequently implies that the *DQA*1201* and *DQA*2201* sequences are associated with the *DH8A* haplotype. Since the *DH8A* haplotype has duplicated *DQ* genes (Andersson and Rask 1988; Davies et al. 1994), the isolation of three *DQA* sequences from animal 10795 is in accord with the haplotype information, and formally demonstrates the expression of multiple *DQA* genes from a single cattle haplotype for the first time. In addition, since only one *DQA* sequence could be amplified from animal 10814, it seems likely that the *DH15B* haplotype also carries the *DQA*0101* encoded sequence. The suggestion that the *DH15B* and *DH24A* haplotypes share a DQ product is supported by the behavior of a group of allo-reactive T-cell clones which were specific for products of the *DH15B* haplotype. Four of seven clones only recognized cells from animals carrying the *DH15B* haplotype, but the remaining three clones recognized cells from animals expressing either the *DH15B* or *DH24A* haplotypes, suggesting that these clones were specific for a shared restriction element (Glass et al. 1992).

The functional significance of *DQ* gene duplication for heterodimer formation and T-cell responses will be investigated by transfection of the cloned *DQA* genes, in combination with the *DQB* genes from the same animals.

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