Allelic polymorphism in the CD45 locus in African cattle

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ABSTRACT

African cattle are a highly divergent population possibly due to introgression by Asian *Bos indicus* (humped) cattle and more recently European *B. taurus* (humpless) cattle. Due to co-evolution, African cattle are generally more tolerant to African pathogens than those of European origin. The purpose of this study was to identify genes associated with pathogen tolerance in indigenous cattle. The leukocyte common antigen, CD45, a highly abundant molecule on the surface of cells of the immune system was studied. CD45 is involved in signal transduction resulting in lymphocyte activation. Molecules involved in immune response are highly likely to come under evolutionary pressure in the face of antigen challenge.

Peripheral blood leukocytes from 179 cattle from a range of African *B. indicus* (Boran), African *B. taurus* (N'Dama), European *B. taurus* (Jersey, Friesian and Guernsey) and Asian *B. indicus* (Sahiwal) breeds were screened for staining with two anti-bovine CD4RO monoclonal antibodies (IL-A116 and IL-A150). All the *B. taurus* animals screened stained uniformly with the mAbs, however differential staining was observed in the *B. indicus* population suggesting polymorphism in the CD45 locus. Nucleotide sequence analysis identified highly divergent Asian, African and European allelic families. This describes significant allelic polymorphism at the CD45 locus of a species and provides additional evidence for distinct African sub-species of cattle. Moreover, high frequencies of non-synonymous (amino acid altering) substitutions and an excessive nucleotide substitution rate imply that natural selection may play a major role in generating diversity at the bovine CD45 locus.

Key words: African cattle, Cd45, allele, polymorphism

RESUME

Les bovins africains présentent une grande divergence dans leur population ceci probablement due à *l'introgression* par l'espèce asiatique *Bos indicus* et plus récemment par l'espèce européenne *B. taurus*. Par mécanisme de la co-évolution, les bovins africains sont généralement plus tolérants contre les pathogènes d'origine africaine qu'européenne. L'objectif de cette étude était d'identifier les gènes qui sont associés à la tolérance des pathogènes parmi les bovins indigènes. L'antigène leucocytaire, le CD_{45}, molécule très abondante à la surface des cellules du système immunitaire a constitué la base de cette étude. Cet antigène (CD_{45}) participe dans le processus de transduction des signaux résultant ainsi à l'activation des lymphocytes. Les molécules qui participent dans le système immunitaire sont plus susceptibles à la pression évolutionnaire lorsque elles affrontent les antigènes.

Des cellules leucocytaires du sang périphérique de 179 bovins repartis entre les espèces africaines *B. indicus* (Borau); *B. taurus* (N'Dama); européennes *B taurus* (Jersey, Friesiau and Guernsly) et Asiatiques *B indicus* (Sahiwal) ont été testées avec deux anticorps monoclonaux anti-bovine (mAbs) CD4 RO (IL-A116 et IL-A150). Tous les animaux de l'espèce *B. taurus* testés ont présenté une coloration uniforme avec le mAbs, cependant une coloration différentielle a été observée dans la population de *B. indicus* suggérant l'existence d'un polymorphisme sur le site de CD_{45}. Les analyses de la séquence nucléotidique ont permis de mettre en évidence l'existence d'une très grande divergence de familles allèliques parmi les espèces asiatiques, européennes et africaines. Ceci décrit de manière significative un polymorphisme allélique au site de l'antigène CD_{45} et met en évidence la présence des sous-espèces distinctes parmi les espèces africaines. De plus, la grande fréquence de substitution des acides aminés et un taux de substitution excessif des nucléotides suggèrent que la sélection naturelle devrait jouer un rôle majeur dans l'apparition de la diversité dans le site de l'antigène CD45 des bovins

Mots clés: CD_{45}, allele, polymorphisme, bovin

INTRODUCTION

Domestic cattle may have originated from a wild auroch (*Bos primegenius*) that inhabited Asia, Europe and possibly North Africa. The earliest archaeological evidence of domestication is associated with the early civilizations in the near and middle East and is believed to have taken place around 8000 years before present (bp) (Epstein 1971, Epstein and Mason 1984). The first cattle to be domesticated were humpless (*B. taurus*). The *B. indicus* cattle evolved later by developing an arid adopted physiology (hump) (Epstein 1971). According to the theory, cattle were introduced into Africa from 1000 years bp.

A second theory postulates that cattle were domesticated in two independent events in the Middle East and Europe (Loftus et al. 1994a, Loftus et al. 1994b). The theory is supported by mitochondrial and microsatellite DNA analyses that show the existence of two cattle lineage, European-African and Asian that diverged between 117 and 275,000 years bp (Bradley et al. 1996). African and European cattle separated 22 - 26.000 years bp, which predates the proposed time for the initial domestication process and suggests an independent domestication of each continental subspecies of the wild auroch. A third theory proposes an African origin for African cattle and is supported by drawings of cattle in caves that date as far back as the fourth millennium bp (Grigson 1991).

Significant genetic variation between the closely related African and European cattle may have arisen by mutation and may have been perpetuated through pathogen driven selection, since certain African breeds are tolerant to African pathogens to which European cattle are highly susceptible (Dolan 1998, Anene et al. 1991). In screening for cattle genes that may be responsible for pathogen tolerance, we set out to identify polymorphism within genes of immunological significance. One such candidate gene encodes the highly abundant tyrosine phosphatase, CD45, that is involved in signal transduction via the T and B lymphocyte antigen receptors (Thomas 1989) resulting in lymphocyte activation (Chan et al 1994). Here, we describe the analysis of the extracellular coding region of CD45 from a diverse range of cattle breeds.

MATERIALS AND METHODS

Cattle. Blood was collected from Kenyan Boran, (African *B. indicus*); ILRI Gambian N'Dama (Jordt et al. 1986) (African *B. taurus*); Sahiwal (Asian *B. indicus*); Friesians, Guernsey and Jersey (European *B. taurus*) representing African, Indian and European cattle. Peripheral blood monocytes were prepared as described by (Godderris and Morrison 1988).

Monoclonal antibodies (mAbs) and FACScan staining. Monoclonal antibodies IL-A116 (Bembridge et al. 1993) and IL-A150 (N. MacHugh, unpublished results) directed against the bovine CD45RO molecule and mAb CC76 directed against a non-polymorphic determinant on bovine CD45R (Howard et al. 1991) were used. Peripheral blood leukocytes, obtained following hypotonic lysis of red blood cells, were stained with the mAbs IL-A116, IL-A150 and CC76 followed by a flouorescences conjugated sheep anti-mouse immunoglobulin (Sigma, Irvine, UK) and analysed by flow cytometry (FACScan, Becton, Dickinson).

Complementary DNA preparation. First-strand Complementary DNA (cDNA) was synthesized from 2 µg of total RNA extracted from $2x10^6$ cells using the RNAZol™B kit (Biogenesis Ltd, New Fields, England). The cDNA was synthesized using Promega's reverse transcriptase kit (Promega, Madison, WI).

Amplification of the extracellular region of bovine CD45(BoCD45).
Oligonucleotide primers, 45F (ATG TAT TTG TGG CT[C/T] AAA CT) and 45R (TTC TCA TCA TCC CTT TCA ACG) were designed to amplify a 1.4 kilo base (kb) fragment encoding the extracellular domain, the transmembrane and 60 bp of the intracellular domain of bovine CD45RO gene. The PCR mix included, cDNA (2µl of a 20 µl cDNA reaction), primers at 25 pM, 1.2 units of Taq polymerase (Promega) and PCR buffer (2 mM $MgCl_2$, 200 µM, dNTp) in a 50µl reaction. Amplification conditions comprised an initial denaturation at 94°C for 2 minutes followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 1 min and extension at 72°C for 1.5 min, with a final extension at 72°C for 4 min.

Cloning and nucleotide sequence analysis. PCR fragments of BoCD45RO were cloned into the pGEM T-easy vector (Promega) and the nucleotide sequence derived from seven independent clones was determined using fmol™ sequencing kit (Promega). Sequence data was analysed using the DNASIS package (Hitachi software, San Bruno CA).

CD45 exon 9 PCR-RFLP. Oligonucleotide primers 45X9F (AGA ACA AAT AC(A/G) GAG (A/G)TG TG) and 45X9R (CCC TGG TGG TAC CTC (C/T) AA G) were designed to amplify exon 9 of the bovine CD45 gene from genomic DNA. The PCR reaction mix include, 100 ng of genomic DNA, primers at 25 pM, 1.2 units of Taq polmerase (Promega) and PCR buffer

Table 1: Staining of PBL from a range of cattle breeds with the CD45RO mAbs IL-A116 and IL-A150.

Breeds	Number sampled	IL-A116^{++} IL-A150^{++}	116^{+} 150^{-}	116^{++} 150^{-}	116^{-} 150^{-}
Boran, African *B. indicus*	95	59	10	12	14
Sahiwal, Asian *B. indicus*	20	17	0	3	0
N'Dama African *B. taurus*	20	20	0	0	0
Friesian, European *B. taurus*	20	20	0	0	0
Jersey, European *B. taurus*	8	8	0	0	0
Guernsey, European *B. taurus*	8	8	0	0	0

$^{++}$ Strong staining, $^{+}$weak staining, $^{-}$no staining

(2mM $MgCl_2$, 200 µM dNTP) in a 50 µl reaction. Amplification conditions comprised of an initial denaturation of 94°C for 2 min followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 1min and extension at 72°C for 1 min, with a final extension at 72°C for 4 min. Amplification products were restriction digested with B*st*N1 to accurately discriminate between bovine CD45 allelic families. The fragments were analysed on 8% polyacrylamide gels using Hoefer SE 250 electrophoresis systems (Hoefer scientific systems, San Francisco, CA).

RESULTS

Differential CD45RO staining in African and Asian *B. indicus*. PBL obtained from African *B. indicus* (Kenyan Boran), Asian *B. indicus* (Sahiwal) African *B. taurus* (ILRI Gambian N'Dama) and European *B. taurus* (Friesian, Guerney and Jersey) cattle were stained with CD45RO specific mAbs IL-A116 and IL-A150. The results, summarised in Table 1, reveal striking variations in antibody specificity between taurine and indicine cattle. All the taurine (African and European) animals stained uniformly with both mAbs. In contrast, the staining patterns obtained from the indicine (Boran and Sahiwal) cattle were highly variable. All the animals tested stained intensely with the CD45R positive control mAb CC76.

Genetic basis for the differential staining. To determine if the differential mAbs staining was associated with allelic polymorphism at the BoCD45 locus, 1.4kb fragments of the extracellular domain, the transmembrane and 60 bp of the intracellular domain were amplified from four animals. These were two *B. taurus* Friesians (IL-A116 strong positive and IL-A150 strong positive), a single *B. indicus* Boran (IL-A116 weak positive and IL-A150 negative) and a single *B. indicus* Sahiwal (IL-A116 strong positive and IL-A150 negative). Five CD45RO allelic families were identified and grouped in three divergent allelic families as shown in Figure 1. Family 1was represented by two closely related sequences identified from the Friesian cattle. Allelic families 2 and 3 were both identified from the Boran animal. These families were highly divergent from each other and from allelic family 1. The sequence obtained from the Sahiwal *B. indicus* animal had high homology to family 2 (Table 2).

Sequence analysis of BoCD45RO. Consensus nucleotide sequences (Figure 1) and amino acid sequences were generated for each of the three families of bovine CD45. It was clear that firstly, the three sequences vary in size. Family 1 had 1385 base pairs (461 amino acids), family 2, 1361 base pairs (453 amino acids) and family 3, 1391 base pairs (463 amino acids). This was as a result of several nucleotide insertions and deletions, including a 24 base pair deletion (8 amino acid deletion) in family 2. By comparison with the human CD45 sequence (Hall et al. 1988) the deletion lies within exon 9, hence is unlikely to result from the exon splicing associated with CD45 (Thomas 1989). The results have subsequently been confirmed by PCR amplification of exon 9 from genomic DNA, which gives fragments of varying sizes. These are a 209 base pairs fragment representing family 1, 185 base pairs for family 2 and 212 base pairs for family 3. Digestion by the restriction enzyme B*st*N 1 accurately distinguishes between the families and confirms the existence of the allelic families at the BoCD45 locus. Three additional base pair (1 amino acid) insertions are evident in family 3 at positions 238-240 and 505-507.

Table 2: Percentage nucleotide and (amino acid) identity between the OCD45 allelic families

	Family 1	Family 2	Family 3
Family 1	-		
Family 2	97(94)	-	
Family 3	95(90)	94(88)	

DISCUSSION

The diverse nature of domesticated cattle suggests a complex origin relating to multiple domestication events of ancestral populations in Asia, Europe and Africa. Mitochondrial DNA analyses have identified three lineages; Asian *B. indicus*, European *B. taurus* and African *B. taurus* (Loftus et al. 1994). The Asian lineage diverged from the European and African lineages approximately 117-275,000 years bp, while the European and African lineages diverged between 22,000 and 26,000 years bp (Bradley et al. 1996). Here, we have described three CD45 families: one associated with Asian *B. indicus* cattle, the second associated with European *B. taurus* and a third found exclusively in African cattle. The distribution is similar to that identified by mitochondrial DNA analysis. However, CD45 analysis shows a recent divergence of the indicine and taurine lineages as compared to the African lineage. Moreover, there is a wide distribution of the African allele in diverse populations of both taurine and indicine African cattle, implying a common ancestry.

Microsatellite, mitocondrial DNA and Y-chromosome analysis have shown excessive and progressive *B. indicus* introgression in African cattle (with the exception of West African N'Dama) (MaHugh et al. 1997), so much so that the male transmitted Y-chromosomes are of *B. indicus* origin (Bradley et al. 1995) while the female transmitted mitochondrial DNA is of *B. taurus* origin. This indicates a male dominated indicine introgression. However, the African allelic CD45 family remains dominant in Africa despite considerable *B. indicus* genetic input suggesting the existence of a selective advantage for the allelic.

The high rate of nucleotide substitutions and the ratio of non-synonymous to synonymous substitutions in bovine CD45 suggest that natural selection may be operating at this locus. Intense selection for the CD45 African allele in African cattle requires an equally intense driving force. The most likely candidate is a pathogen with a broad African distribution affecting survival and reproduction. The major constraint to cattle production across central and southern African is trypanosomiasis, caused by the protozoan parasite T*rypanosoma* (Hursey and Slingenberg 1995). Trypanosomiasis is characterized by progressive anaemia and immunosuppression, which greatly affects survival and reproductive efficiency (Rowland et al. 1993). It has been shown that immunosuppression may be included by ligation of the extracellular portion of CD45 molecule (Lazarovits et al. 1996, Basadonna et al. 1998), hence the intriguing possibility of an interaction between the trypanosome and the abundant leukocyte surface protein, CD45.

In summary, we have identified polymorphism at the bovine CD45 locus. Three allelic families have been described. The families correspond to continental cattle populations in Asia, Europe and Africa. In contrast to mitochondrial DNA analysis, the most divergent CD45 allelic family is African in origin, implying an African origin for African cattle. The exceptionally high rate of nucleotide substitutions and the high ratio of non-synonymous substitutions to synonymous imply that natural selection may be operating at the CD45 locus. Such selection may be pathogen driven. With the wide continental distribution of the African allele, the pathogen must have as wide a distribution. We suggest that the African Trypanosome may be the driving force behind such selection.

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