

The bovine T cell receptor alpha/delta locus contains over 400 V genes and encodes V genes without CDR2

Peter Reinink · Ildiko Van Rhijn

Received: 9 May 2009 / Accepted: 17 June 2009 / Published online: 2 July 2009
© The Author(s) 2009. This article is published with open access at Springerlink.com

Abstract $\alpha\beta$ T cells and $\gamma\delta$ T cells perform nonoverlapping immune functions. In mammalian species with a high percentage of very diverse $\gamma\delta$ T cells, like ruminants and pigs, it is often assumed that $\alpha\beta$ T cells are less diverse than $\gamma\delta$ T cells. Based on the bovine genome, we have created a map of the bovine TRA/TRD locus and show that, in cattle, in addition to the anticipated >100 TRDV genes, there are also >300 TRAV or TRAV/DV genes. Among the V genes in the TRA/TRD locus, there are several genes that lack a CDR2 and are functionally rearranged and transcribed and, in some cases, have an extended CDR1. The number of bovine V genes is a multiple of the number in mice and humans and may encode T cell receptors that use a novel way of interacting with antigen.

Keywords T cell receptor diversity · Complementarity-determining regions · Artiodactyls · Cattle

Introduction

One of the factors that determines whether a successful T cell response will be generated upon first encounter with an antigen is the availability of T cell receptors (TR) that interact with the target antigen. The potential T cell repertoire is determined by the number of V, D, and J

genes that is available to take part in the process of rearrangement and by the extent to which coding region ends are shortened and nontemplate encoded nucleotides are added. Successfully rearranged α and β chains form $\alpha\beta$ TR, and γ and δ chains are used by $\gamma\delta$ TR.

Ruminants, chicken, and pigs have a high percentage of circulating $\gamma\delta$ T cells, and these $\gamma\delta$ T cells are known to be structurally and functionally more diverse than $\gamma\delta$ T cells in mice and humans (Hein and Dudler 1993, 1997). Many authors suggest that the diversity and relative importance of $\alpha\beta$ and $\gamma\delta$ T cells is reversed in these so-called $\gamma\delta$ high species and thus expect limited diversity of the TR α and β chains (Su et al. 1999). In fact, the number of V genes in the chicken TRA/TRD and TRB loci is limited and each locus contains only two subgroups of V genes (Gobel et al. 1994; Kubota et al. 1999; Tjoelker et al. 1990). Less is known about the diversity of ruminant $\alpha\beta$ T cells. A survey of TR β chain transcripts does not suggest a limited TRBV gene repertoire in cattle, but instead multiple subgroups and multiple genes within subgroups were identified (Houston et al. 2005; Tanaka et al. 1990).

The six complementarity-determining regions (CDR) of the TR are the most variable parts of the TR and interact directly with the antigen-presenting element/antigen complex. The CDR1 and CDR2 are directly encoded by the V genes (germline encoded), while the CDR3 is encoded by the V–D–J junction which is formed during the process of rearrangement. In humans and mice, a comparable level of junctional diversity is present, and the number of V genes directly encoding the CDR1 and CDR2 is in the same range. The human TRA/TRD locus contains 49 TRAV genes, five TRAV/DV genes, and three TRDV genes, so a total of 57 V genes of which 49 are functional and lies on chromosome 14, spanning 0.9 Mb from the first TRAV till TRAC (Lefranc and Lefranc 2001; IMGT/GENE-DB,

Electronic supplementary material The online version of this article (doi:10.1007/s00251-009-0384-9) contains supplementary material, which is available to authorized users.

P. Reinink · I. Van Rhijn (✉)
Department of Infectious Diseases and Immunology,
Faculty of Veterinary Medicine, Utrecht University,
Yalelaan 1,
3584CL Utrecht, The Netherlands
e-mail: i.vanrhijn@uu.nl

Giudicelli et al. 2005; IMGT Repertoire, <http://www.imgt.org/textes/IMGTrepertoire/LocusGenes/tabgenes/human/geneNumber.html>). The mouse TRA/TRD locus on chromosome 14 spans 1.6 Mb from the first TRAV till TRAC and contains 104 V genes, of which 78–89 are functional (Bosc and Lefranc 2003; Giudicelli et al. 2005).

The bovine T cell receptor β (TRB) and T cell receptor γ (TRG) loci have been described and are located on chromosome 4. The two TRG loci are located at 4q3.1 and 4q1.5–2.2 (Conrad et al. 2007), the TRB locus at 4q24 (Antonacci et al. 2001; Conrad et al. 2002), and the bovine TRA/TRD locus lies on chromosome 10 (Fries et al. 2001; Van Rhijn et al. 2007). The structure of the most downstream part of the TRA/TRD locus, containing TRDC and TRDV4, has been described in detail (Herzig et al. 2006), but the size of the locus and the organization and number of its V genes is unknown. Automated gene prediction methods resulted in 71 functional TRAV/DV genes and 51 TRDV1 genes in the Btau4.0 assembly of the bovine genome version (Elsik et al. 2009).

Like for sheep, multiple bovine TRDV genes, belonging to four TRDV subgroups, have been described (Herzig et al. 2006; Ishiguro et al. 1993; Van Rhijn et al. 2007). The artiodactyl TRDV1 subgroup is highly expanded compared to humans and mice (Antonacci et al. 2005). Hein and Dudler (1997) identified Vd1.1 till Vd1.26. Van Rhijn et al. (2007) identified Vd1.27 till Vd1.37. In addition to these 37 TRDV1 genes (identified as rearranged cDNAs), two TRDV2, two TRDV3, and one TRDV4 genes have been described (Herzig et al. 2006; Van Rhijn et al. 2007). Bovine TRAV genes have been described by Ishiguro et al. (1990), but no information on the number of genes and subgroups is available so far.

Using the Btau4.0 assembly of the bovine genome, we set out to describe the V genes of the bovine TRA/TRD locus and found that it contains a fourfold to fivefold higher number of V genes than humans and mice and contains V genes with extended CDR1 and very short or absent CDR2.

Materials and methods

Databases and searches

In order to find bovine TRAV genes an initial series of BLAST-Like Alignment Tool (BLAT) searches was performed in the bovine genome (Ensemble, Btau4.0 assembly version 52), using the transcripts of all human TRAV genes. (Partially) overlapping hits were joined, the hits in the TRB and TRG loci excluded, and from the resulting set of V genes, a preliminary phylogenetic tree was generated. One representative bovine V gene of 17 branches of this tree

was used to perform a series of Basic Local Alignment Search Tool (BLAST) searches in the bovine genome. Also, the published TRDV1, TRDV2, TRDV3, and TRDV4 sequences were used to perform BLAST searches. After the removal of genes with overlapping genomic location, the V exons of the thus identified bovine V genes till the second cysteine (2nd-CYS 104, definition available at IMGT®, <http://www.imgt.org>) were downloaded and translated *in silico* to check for frameshift mutations or internal stop codons in the V exon. The nucleotide sequences of the bovine V exons were arranged into subgroups with 75–100% sequence identity. To check for V gene expression, expressed sequence tags and other cDNAs of TR α and TR δ chains were identified by performing BLAST searches with the constant region (TRAC and TRDC) of the TR α and TR δ chains or with individual V genes.

Software

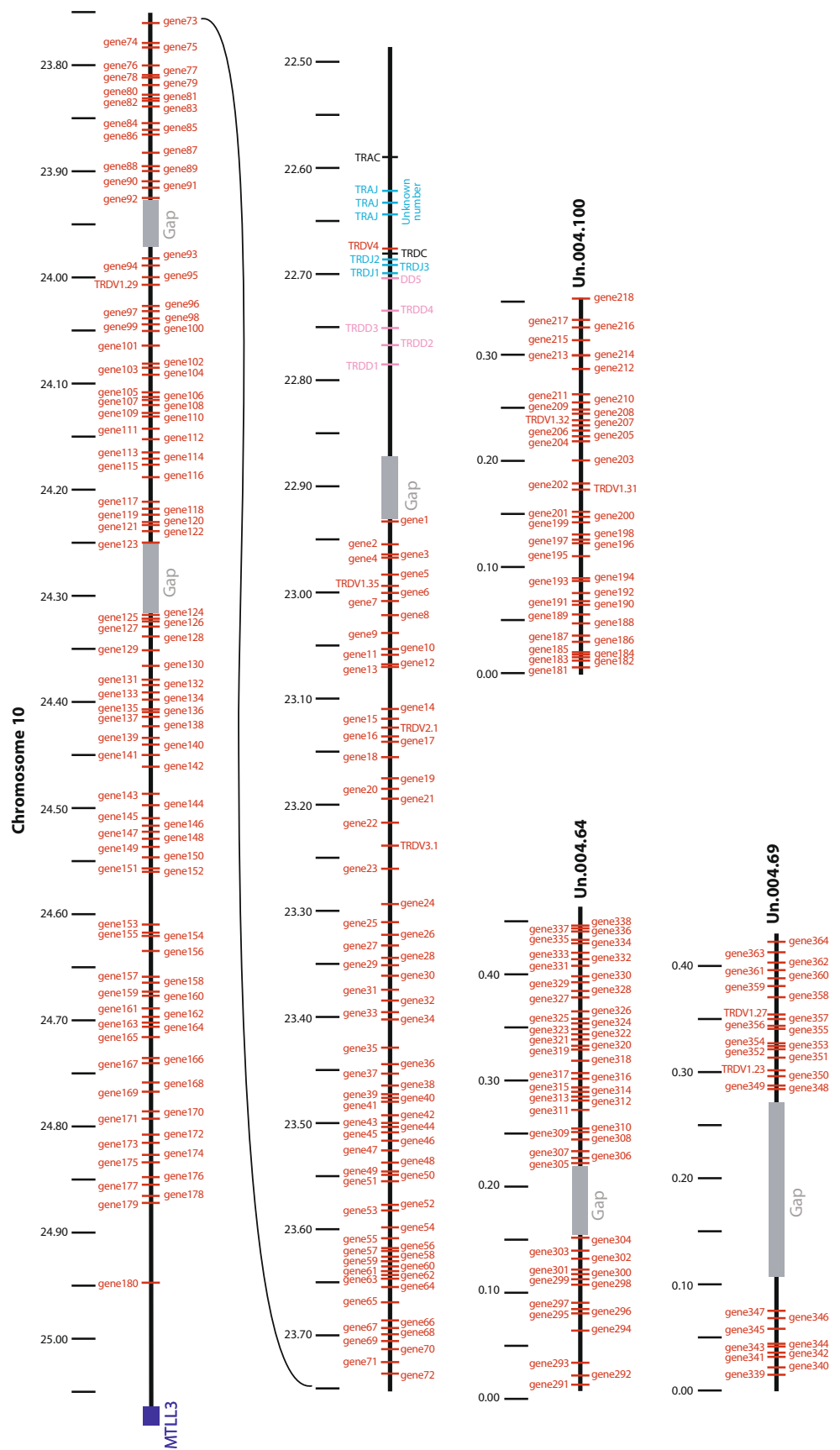
Alignments were performed with ClustalW available at <http://www.ebi.ac.uk/Tools/clustalw2>. The circular phylogenetic tree in Fig. 2 was based on a ClustalW-generated alignment using iTOL (Letunic and Bork 2007) available at <http://itol.embl.de>. Translations were performed using the Expasy translate tool (<http://www.expasy.ch/tools/dna.html>). Subgroup classification was determined using IMGT/V-QUEST (Brochet et al. 2008) available at <http://www.imgt.org>. Amino acid alignments were made in accordance with the standardized IMGT alignment scheme for human V genes (IMGT/DomainDisplay tool available at <http://www.imgt.org/3Dstructure-DB/cgi/DomainDisplay.cgi>) using the IMGT/DomainGapAlign tool (<http://imgt3d.igh.cnrs.fr/3Dstructure-DB/cgi/DomainGapAlign-include.cgi>). The IMGT/Collier-de-Perles tool (<http://www.imgt.org/3Dstructure-DB/cgi/Collier-de-Perles.cgi>) was used to create graphical representations of selected V regions (Kaas et al. 2007; Ruiz and Lefranc 2002).

Results

Numbers of genes and size of locus

Initial BLAT searches in the bovine genome using human TRAV sequences resulted in 217 bovine V genes. Subsequent BLAST searches with representative bovine V gene sequences among these 217 genes and with all known bovine TRDV genes resulted in a total of 402 bovine V genes. Some, but not all, previously described genes were 100% identical to a gene on this list. All novel genes were numbered 1–388 (Supplementary Table 1 of the Electronic supplementary material). The total number of previously

Fig. 1 The bovine TRA/TRD locus. Map of the TRA/TRD locus on chromosome 10 and the three biggest contigs that have not yet been assigned to a chromosome. A complete list of the V genes and their exact locations are provided in Supplementary Table 1 of the Electronic supplementary material. Gaps with a size >45,000 bp are shown in gray. Red V genes, pink D genes, light blue J genes, dark blue METTL3 gene



described genes plus these novel TRAV and TRDV genes is 430. The fact that some previously described genes were not found in the genome in a 100% identical form is most likely due to polymorphisms. Most bovine V genes were found on chromosome 10, but also on contigs that had not yet been assigned to a chromosome (Fig. 1), and two on chromosome 21 (not shown). Even though these latter two genes did not

contain internal stop codons or frameshift mutations that qualify them as pseudogenes, their location outside the TRA/TRD locus qualifies them as orphans. There are no homologs of TRDC genes on chromosome 21, so it is not possible that these two genes can be used in a functional TR α or δ chain. A comparable situation has been described for human TRBV genes on chromosome 9p (Robinson et al. 1993).

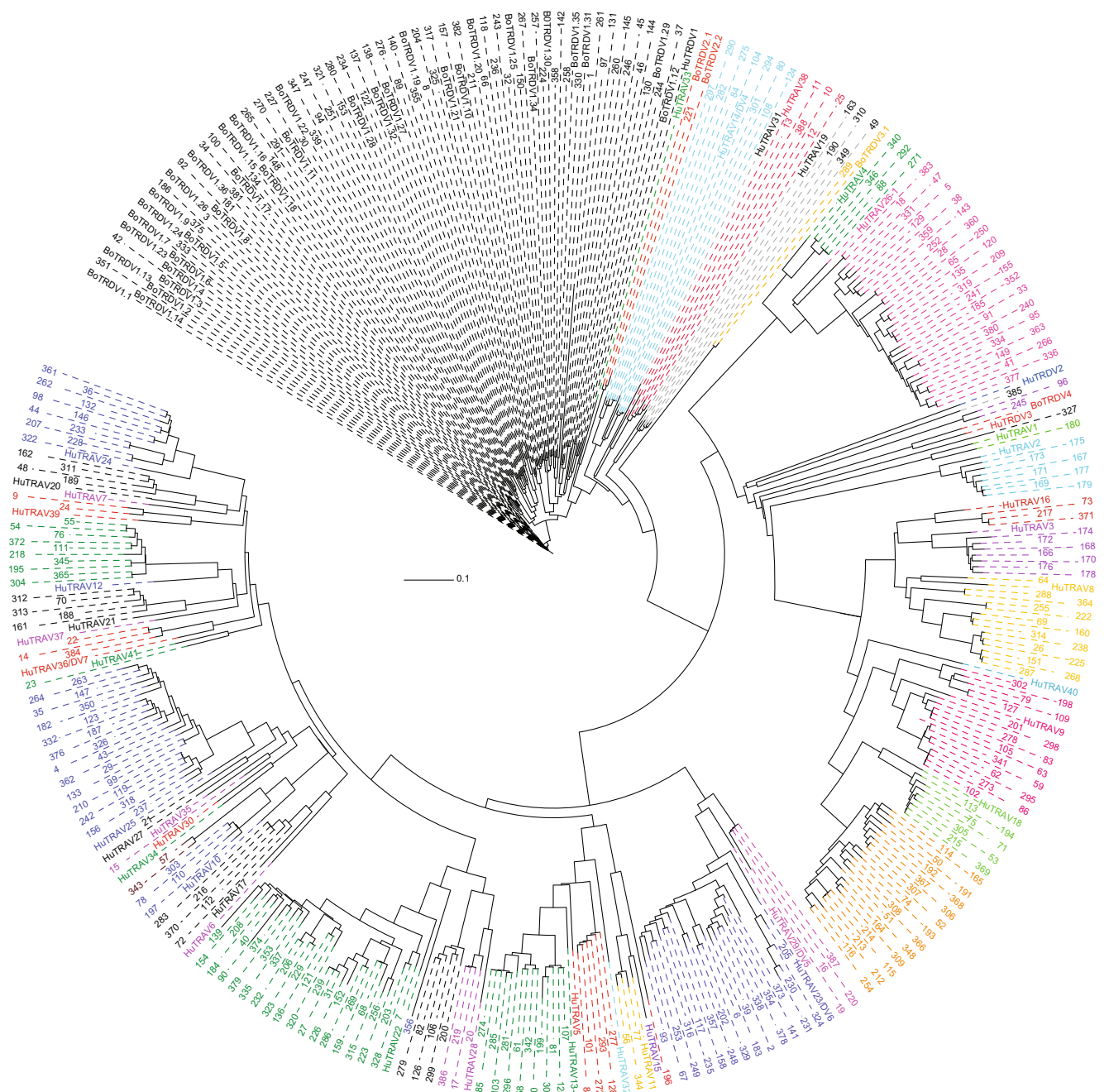


Fig. 2 Phylogenetic tree of all bovine V genes of the TRA/TRD locus. Tree of all bovine TRAV and TRDV genes and one representative human TRAV or TRDV gene of each human subgroup. The tree is based on the nucleotide sequences of the V-EXON till the

second cysteine (2nd-CYS 104). All novel genes are numbered 1–391 and all previously described genes are shown under their previously published name. The V genes in each color-coded segment of the circle belong to one subgroup

Table 1 Bovine and human V gene subgroup assignments

	Number of human genes	Number of bovine genes
Human TRAV subgroup		
HuTRAV1	2	1
HuTRAV2	1	6 + 1Ψ
HuTRAV3	1	6 + 1Ψ
HuTRAV4	1	3 + 2Ψ
HuTRAV5	1	6
HuTRAV6	1	–
HuTRAV7	1	–
HuTRAV8	6 + 1Ψ	7 + 7Ψ
HuTRAV9	2	13 + 5Ψ
HuTRAV10	1	4
HuTRAV11	1Ψ	3
HuTRAV12	3	–
HuTRAV13	2	10 + 5Ψ
HuTRAV14/DV4	1	8 + 3Ψ
HuTRAV15	1Ψ	–
HuTRAV16	1	3
HuTRAV17	1	2 + 3Ψ
HuTRAV18	1	7 + 1Ψ
HuTRAV19	1	4 + 1Ψ
HuTRAV20	1	4
HuTRAV21	1	5
HuTRAV22	1	28 + 4Ψ + 1 ^a
HuTRAV23	1	8 + 17Ψ
HuTRAV24	1	5 + 6Ψ
HuTRAV25	1	17 + 5Ψ + 1 ^a
HuTRAV26	2	29 + 5Ψ
HuTRAV27	1	1
HuTRAV28	1Ψ	4
HuTRAV29/DV5	1	4
HuTRAV30	1	–
HuTRAV31	1Ψ	–
HuTRAV32	1Ψ	–
HuTRAV33	1Ψ	–
HuTRAV34	1	–
HuTRAV35	1	1
HuTRAV36/DV7	1	1 + 2Ψ
HuTRAV37	1Ψ	–
HuTRAV38/DV6 ^b	2	6
HuTRAV39	1	2
HuTRAV40	1	–
HuTRAV41	1	–
Human TRDV subgroup		
HuTRDV1 ^c	1	93 + 9Ψ + 2 ^a
HuTRDV2	1	–
HuTRDV3 ^d	1	1
Bovine TRDV subgroup		
BoTRDV1 ^c	1	93 + 9Ψ + 2 ^a

Table 1 (continued)

	Number of human genes	Number of bovine genes
BoTRDV2	–	3
BoTRDV3	–	2 + 1 ^a
BoTRDV4 ^d	1	1
New bovine subgroup		
Gene 50 subgroup	–	23 + 1Ψ
Gene 54 subgroup	–	9 + 1 ^a
Gene 57 subgroup	–	2Ψ
Gene 82 subgroup	–	6
Gene 96 subgroup	–	2Ψ
Gene 196 subgroup	–	1Ψ
Gene 259 subgroup	–	1 ^a
Gene 284 subgroup	–	1 ^a
Gene 327 subgroup	–	1
Gene 356 subgroup	–	1Ψ
Gene 385 subgroup	–	1Ψ
Total number of V genes in TRAV/DV locus	57	430

The interspecies subgroups were named after the human subgroup, and the novel bovine subgroups (subgroups without human members) were named after the member with the lowest number. The total number of bovine and human genes in each subgroup is listed, as well as the total number of V genes in the locus

Ψ pseudogene

^a Incomplete sequence

^b This subgroup consists of one TRAV and one TRAV/DV

^c Bovine TRDV1 and human TRDV1 subgroups can be considered to form one interspecies subgroup because human TRDV1 is >75% identical to multiple bovine TRDV1 genes. However, this does not hold for all bovine TRDV1 genes

^d Human TRDV3 is homologous to bovine TRDV4 and form an interspecies subgroup based on >75% identity at the nucleotide level

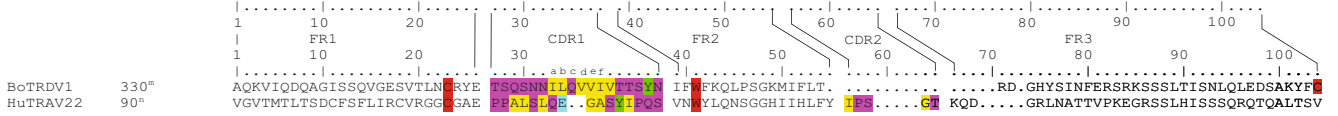
Because the exact linear organization of chromosome 10 is not yet known, the provisional numbering of the genes does not reflect their order on chromosome 10. At the upstream end of the TRA/TRD locus on chromosome 10

Table 2 Summary and statistics of the bovine and human TRA/TRD loci

Statistics	Human locus	Bovine locus
Known vs. new	57 known	42 known + 388 new
TRDV vs. TRAV including AV/DV	3 TRDV + 54 AV/DV	111 TRDV + 319 AV/DV
Pseudogenes	8	85
Functional genes	49	337
Incomplete genes	0	8

a

Subgroup	gene number	FR1										CDR1				FR2			CDR2			C''		FR3			E		F	
		1	10	20	30	40	50	60	70	80	90	100	30	40	50	60	70	80	90	100	70	80	90	100	80	90	100	110	120	
Representatives																														
HuTRAV1	180	AGKGVKQPTELMAIEGASAQVN	TYQ	TSG	VNG	LFN	YQQHDGGAPVFLSY	NVL	...DGL	ETR	GHFSSFLRRSDAHSYLLKELHMKDFASY																
HuTRAV2	169	SKEQVFSPTVVSLEGAVAEIS	NHS	ISN	YND	FLNFPHFPFAPRLLIK	GS	KE	GRYNTMYER	..FSSLLILQVQTADAGVYY																
HuTRAV3	168	AQSVTQPEAEVPAEAGDPPVK	CTYS	VSG	SPY	LFNYVQHRNLGLQFLIK	ISG	..DLV	KGN	YGFEAEFNKSTSFHLKPKSPVSGDSALYF																	
HuTRAV4	271	SLAKTSQPIFIDSVEGQEVNIS	NHT	ITA	SEY	LFNYRQFPNQGPFLIQ	CYK	FN	...	EVASLLIPDPRKFSFLSLPQASLRDTAVYY																	
HuTRAV5	272	GEKVEQYPSFQSVQEGDNCV	INTY	DSA	SIY	FFNYKQEPGKGLQLLIH	ILSN	..VDK	KEG	QGLVILLNKKNHLSLNIATNPGDSATYF																	
HuTRAV8	69	AQSVTPDDHIAVSEGARLELK	SNYS	SSV	SPY	LFNYIQYPNQGLQLL	KVSG	..DNLV	SGI	KGFEAEFRNSETSFHLRKPAAHWKDSAKYF																	
HuTRAV9	273	GNSVTQMDQVRSSEGTSTVIN	CTYS	ISG	YFN	LFNYVQYLGGEPPELLK	AMKA	...NDK	GTN	KGFEATYNTETTSFHLKASVQESDSAVYY																	
HuTRAV10	303	KNQVEQSPPLVLVEGENCTFQ	ENFT	SSP	FNN	LRNYTQDTGRGLVSLIT	MTYS	..DNK	KSN	GRYTATVDAKHSLSHITAQQLSDPAFYI																	
HuTRAV11	344	QYTLDSQPSFSLIQRTHADLN	CTYQ	KKT	FNY	FFNYKQEPGKGLVLSL	IQSS	..QKE	EAD	KNFKELGKQEVSYVLSIASHPGDSATYF																	
HuTRAV13	85	GKQVEQSP..TILSQEENSTFI	TCTY	DGN	SNY	LFNYIQYPPGKGLQLLI	ILSN	..KAK	EED	QRLTATLNRKTKRHSLSLHIATPAGDSAVYY																	
HuTRAV14	104	AQKVTQDQPPMSVQEKETVTL	NTYD	TSG	IYS	FFNYKQHSQVGMFLIP	QDSY	..NKFN	ATE	GHYSLNFQKASKFIFTLTIASQGLDSAVYY																	
HuTRAV16	371	AQSVTPDDHIAVSEGARLELK	SNYS	VSG	SPY	LFNYIQYPNQGLQLL	KVSG	..DNLV	SGI	KGFEAEFRNSETSFHLRKPAAHWKDSAKYF																	
HuTRAV17	72	NQQGKQLGTLISIQEENVTMN	CSYK	SLI	LTA	LQNYRQDSRRGFVHLIL	MRSN	..ERC	KHS	QRHLFTLDNISKSSLSIMASQTEDTATYL																	
HuTRAV18	53	GDSVTEGVTLSLPEKASLTLC	TYQ	SSY	SGE	LHNYVQYQNKLELELLK	SSY	..NOK	VTS	RGFEATHISSDSSFSHLKSSVQTSDSAVYY																	
HuTRAV19	310	AQKVQNSQSEISLVLEKEDVT	LNCAYE	ANS	IYX	LFNYKQPPSGEITFLIH	QDSY	..NELN	TTK	DQYFLNFQKATSSSIETISGGLDSAVYY																	
HuTRAV20	311 ^a	EDQVEQSPQILRIQEGDLSLN	CSY	SS	RG	LHNYVQYPGKGLQLLI	LXSV	..GDE	KQK	ERLRATLLK..KGSSLSLHIATPAGDSATYL																	
HuTRAV21	161	KQDVEQSPPALNVRGDSVVLN	CTYT	DSA	LXE	LQNYRQDPGKGLTLLLS	IQAN	..QKE	QAS	GRITVSLDKSRHSALYIAAQSQSDSYF																	
HuTRAV22	121	GVDVEQSPPALSLQEGASVTL	QNFS	TSS	OS	VNYLQNSGGHILQFLY	IPE	GT	...	KQD....GRLNATTVLKRERSSLHISISSQRTQALTSY																	
HuTRAV23	253	QQQVKSQSLTVQEGEISILN	CSYE	DSL	EDY	FFNYQYPPGKGPAPFSIA	IRSV	..ENE	MKD	GRLTVFLNKRKQLSLHISISSQRTQALTSY																	
HuTRAV24	262	LLTVEQSPPALSLQEGDSTNF	CSFP	SSS	FNA	LHNYRWEPAKTGPVLLK	ISVN	..GDE	KQK	GRMVRTVGTAKRHSLSLHIATPAGDSATYL																	
HuTRAV25	318	GOQISQIPQFLPLQEGENFTY	KNSS	SIL	SS	LQNYKQRPGGSPVFLMI	LAKG	..GKV	KTE	QRLTGRGLRTRKHGSLHLAAQLSDVGYF																	
HuTRAV26	41	DAKTTQ..PSSMDCAEGEDAN	FNHS	IIGG	KDY	LHNYRQNPQSPQVVIH	GLR	GT	...	VNR....SMASLTASDRKSSSTLVLPQVTLRDAVYY																	
HuTRAV27	21	TQQLQEQPFHLIQEGGNVTM	NSS	GLE	TE	FQNYRQEQPQGVLLMFT	LTK	KEV	...	KKRIRAQFEAKKSSLSLHIATPAGDSATYL																	
HuTRAV28	386	KNVQSPQPIRLIQEGDLSLN	SNYS	ISM	TS	VNYLQNSGGHILQFLY	IAS	GM	...	QKQ....GRLNATTVLKRERSSLHISISSQRTQALTSY																	
HuTRAV29	220	GQKVKQNPMSLSVTEGGISIL	NDYE	DIM	LNY	FFHRRNYPAKSPTEFLIS	IQS	..LEK	NED	GRFTVFHNRNAKHSLSLHIATPAGDSATYL																	
HuTRAV35	15	AQQLNSQPSMISIQEGEDVSN	NSS	SM	NE	LHNYKQDAGEGVLFLY	LKLG	..GEL	ARN	GRLMATVPEKRSLSLHIATPAGDSATYL																	
HuTRAV36	384	DDHVMQSPPSLVHVEGASNT	LSYK	VLN	SH	LHNYKQEKQVPTFLFA	LISY	..GIE	KKS	GRLVGTLDKRELLSLHITVTELGDSTYL																	
HuTRAV38	12	AQTVTQPPQESVQETGTVTLL	CTYS	TSES	GYL	LFNYKQPPSGEMVIFL	QYAN	..EQON	ATN	DRYSVNPQKRSLSLHIATPAGDSATYL																	
HuTRAV39	9 ^b	TCLVQSQPPLSLITREQQTG	NCHS	VLT	SDI	FLNYRQDQ..KSLESLEF	LMSN	..RAV	RKK	GGLTASPDKACRSPLHITASHPGLSATYF																	
HuTRAV41	23	KNLVEQSPRYLSAQEGDLVIT	NLYL	EGM	FT	FLNYRQDQ..QGKLSLEF	LISL	..EM	KRK	GRVSATINSRERSSLSLHIATPAGDSATYL																	
50	51	GDSVNTQEGPVTVEGALLTLN	CTYQ	ASYS	IYX	LFNYVQHLNKAPQLLLK	CSM	..DQK	PKS	EGFQATLSDSSSFHLKQKRAVQTSDSAVYY																	
54	372	QNTVEQSPASLPVPEGAASLS	CTYS	DVN	SQV	LFNYRQPPGKGEPEFLQ	VYA	..NNN	KEE	GKFTVQSNKTRKHSLSLHIATPAGDSATYL																	
82	279	GKVEQSPVSLVLEGANSTLNF	S	DYV	QV	FQNYRQPPGSLTIRLFF	IAS	GT	...	KKN....ERMSSTVNSKERYSTLHITASQLEDAATYL																	
327	327	THTWLFLPNPRAAAGKALMG	CRGL	S	G	TVRYRSMNLRQSSNI	CGA	..VAL	LSI	QSDSHLLEFFIPYIYTKQLVGGLVQVPI																	
BoTRDV1	247	AQKVTQDQSDIISQVQSVIFN	QYQG	ICHE	IYXS	LFNYKQLPRQMTPLIH	QN	VAL	EHNAGY..DRYSVNPQKRSLSLHIATPAGDSATYL																	
BoTRDV2	221	ADKVTAEQTTVAREAREAVT	IGCTYE	ASRH	IYL	LFNYRQPPGKREPLIF	QD	S	NNANARR..DRYSVNPQKRSLSLHIATPAGDSATYL																	
BoTRDV3	289	NNVESADQVTVPFKKEGESV	TVEKFS	VSY	IYI	MYFYRQPPSGEMIMIN	IS	S	QNKHSRE..GRYSVEYKPNQMLKTLISALTSLSAVYY																	
Atypical genes																														
HuTRAV2	175 ^c	EQAGTSVSYCGLFPGSVVAEIS	NHS	MSN	YVG	FFLYLHFPGFAPRLLIK	VS	KE	SEQ.....GCYNTMYER..FSSLLILQVHMADAGVYY																	
HuTRAV8	222 ^d	SQVLTQLNVHITVSEGFRLELN	SNYS	ESV	QDR	LFNYVQYPNQGLQFLIK	ITSK	..DSL	ADI	NCFEAEFRNSETSFHWKRSVHWKDSAWDTL																	
HuTRAV11	77 ^e	QYKLDQSPSFLSTQERTHSDLN	CTYQ	KKT	FYN	FFNYKQEPGKGLVLSL	IQSS	..QKE	EAD	KPFKELGKQEVSYVLSIASHPGDSATYF																	
HuTRAV22	139 ^f	GVDVEQSPPALSLQEGANSTL	SNFP	TSP	QD	VNYLQNSGGHILQFLY	IPE	GT	...	QD....RRLKGGTLLIECHSLHMSLQTTDTSISVSY																	
HuTRAV22	27 ^b	GVDVEQSPPALTPQEGASSTL	NFS	TSA	DS	VNYLQKWPGR..HLIY	IPE	GT	...	RQG....GRLNATTVLKRERSSLHISFRLTDSGYF																	
HuTRAV22	31 ^b	GVDVEQSPPALTPQEGASSTL	NFS	TSA	DS	VNYLQKWPGR..HLIY	IPE	GT	...	RQG....GRLNATTVLKRERSSLHISFRLTDSGYF																	
HuTRAV22	239 ^b	GVDVEQSPPALTPQEGASSTL	NFS	TSA	DS	VNYLQKWPGR..HLIY	IPE	GT	...	RQG....GRLNATTVLKRERSSLHISFRLTDSGYF																	
HuTRAV23	248 ^g	QQQVKSQSLTVQEGEISILN	SCE	KSF	INC	FLNYQYPPGKGPAPFLV	VNE	...	MEE....GRFTSILNKSAQQLSHIAAQPFGDSATYL																	
HuTRAV25	119 ^h	GOQISQIPQFLPLQEGENFTY	KNSS	SIL	SS	LQNYKQRPGGSPVFLMI	LAKG	..GEV	KTE	QRLTGRGLRTRKHGSLHLAAQLSDVGYF																	
HuTRAV25	210 ^h	GOQISQIPQFLPLQEGENFTY	KNSS	SIL	SS	LQNYKQRPGGSPVFLMI	LAKG	..GEV	KTE	QRLTGRGLRTRKHGSLHLAAQLSDVGYF																	
HuTRAV25	242 ^h	GOQISQIPQFLPLQEGENFTY	KNSS	SIL	SS	LQNYKQRPGGSPVFLMI	LAKG	..GEV	KTE	QRLTGRGLRTRKHGSLHLAAQLSDVGYF																	
HuTRAV26	252 ⁱ	AAKTTQ..PSSMDCAEGEDVNL	PNNY	IIGG	NDY	LHNSYQNPQSPQVVIH	VHG	..SPT	VNS	SMASLNTASNRKCSSTLVLPQVTLRDTTIVYY																	
HuTRAV26	360 ^j	AAKTTQ..PSSMDCAEGEDVNL	PNNY	IIGG	NDY	LHNSYQNPQSPQVVIH	VHG	..SPT	VNS	SMASLNTASNRKCSSTLVLPQVTLRDTTIVYY																	
HuTRAV39	24 ^k	AELKVEQSPPSLIIQEGPTG	INFDHS	SPT	SDT	FLNYRQDQ..QGKLSLEF	LIMS	..NRI	VRK	KGGLTASFDIKAHGSPHHTASHPGLSATYF																	
50	191 ^k	GDSVNTQEGPVTVEGALLTLN	CTYQ	TASL	APY	LFNYIQHLNKAPQFLMK	GLTA	..DKK	VEH	EGFQATLSDSSSFHLKQKRAVQTSDSAVYY																	
BoTRDV1	142 ^l	AQKVIQDQDPDIFTQIABAVT	MNQQYE	TASV	IYIN	LFNYKQPPSGEMIFL	RD.GHYSVNPQRSLKSSLSLHIATPAGDSATYL																	
BoTRDV1	258 ^l	AQKVIQDQDPDIFTQIABAVT	MNQQYE	TASV	IYIN	LFNYKQPPSGEMIFL	RD.GHYSVNPQRSLKSSLSLHIATPAGDSATYL																	
BoTRDV1	358 ^l	AQKVIQDQDPDIFTQIABAVT	MNQQYE	TASV	IYIN	LFNYKQPPSGEMIFL	VG.SRCSINPQRSLKSSLSLHIATPAGDSATYL																	



b

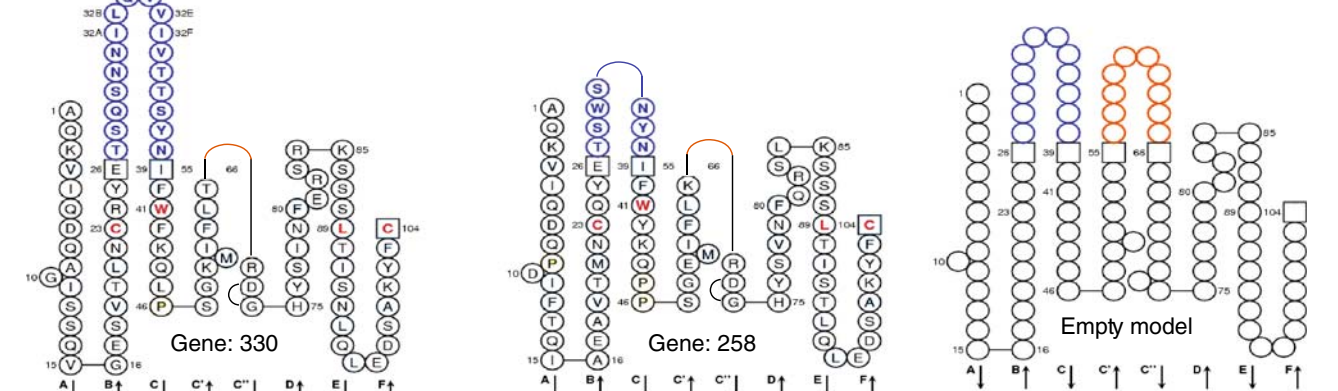


Fig. 3 Alignment of amino acid sequences of bovine V genes. **a** Alignment of predicted bovine TRAV and TRDV protein sequences. One representative of each subgroup is included in the alignment (*upper part*, labeled “Representatives”). In addition, some V genes with special features are shown (*lower part*, labeled “Atypical genes”). The CDR1 and CDR2 amino acids are colored as follows: *gray* positively charged R groups, *light blue* negatively charged R groups, *light green* aromatic R groups, *pink* polar uncharged R groups, *yellow* nonpolar aliphatic R groups, *red* conserved cysteines (1st-CYS 23 and 2nd-CYS 104) and conserved tryptophan 41. *a* Deletion in FR1 and CDR1 compared to the human homolog. *b* Deletion in FR2 compared to the human homolog. *c* Conserved Trp 41 is a Leu. *d* Conserved Cys 104 is a Val. *e* Conserved Cys 104 is a Trp. *f* Conserved Cys 23 is a Ser. *g* Deletion in FR2 and CDR2 compared to the human homolog. *h* Conserved Cys 104 is a Tyr. *i* Conserved Trp 41 is a Ser. *j* Conserved Cys 23 is a Phe. *k* Deletion in FR3 compared to the other genes of the subgroup. *l* Deletion in the FR2, CDR2 and FR3 compared to the human homolog. *m* Insertion of six amino acids in CDR1 and deletion in the FR2, CDR2, and FR3 compared to its human homolog. *n* Insertion of four amino acids in CDR1 and conserved Cys104 is a Val. **b** IMGT Collier-de-Perles of two atypical genes were created to compare their 2D structure with the 2D structure of a standard gene. Conserved amino acids (1st-CYS 23, Trp 41, hydrophobic amino acid 89, 2nd-CYS 104) always have the same position, based on the IMGT unique numbering for V-DOMAIN (Lefranc et al. 2003) and are marked *red*. CDR1 is shown in *dark blue* and the CDR2 in *orange*

lies the methyltransferase-like 3 (METTL3; Fig. 1), zinc finger protein (SALL2; not shown), and olfactory receptor (OR) loci in conserved synteny with human and mouse. The total size of the bovine TRA/TRD locus between the first V gene till the TRAC is 2.4 Mb.

Subgroups and homology to human genes

The nucleotide sequences of the novel and the previously described bovine V genes were used to generate a phylogenetic tree and were arranged in subgroups of >75% nucleotide identity (Fig. 2; Tables 1 and 2). For comparison, the human TRAV and TRDV were included. Of the 41 human TRAV subgroups, 11 are not represented in the bovine genome. Thirty human TRAV subgroups have bovine members and can thus be classified as interspecies subgroups. We found 11 bovine subgroups that are not represented in the human genome. As shown previously by others, the bovine and human TRDV1 subgroups are homologous to each other, as well as the bovine TRDV4 and human TRDV3 subgroups (Herzig et al. 2006; Su et al. 1999).

In bovine, in contrast to the highly expanded TRDV1 subgroup, the number of genes for the other subgroups is very limited and only two TRDV2, two TRDV3 (including one incomplete), and one TRDV4 genes have been described in the past. We found one additional TRDV2 gene (gene 221) and no additional TRDV3 and TRDV4 genes. The total number of bovine TRDV genes is 111.

Most likely, the other 319 V genes are TRAV or TRAV/DV. Using a polymerase chain reaction-based approach, the existence of bovine V genes that are used in α and δ chains (TRAV/DV genes) has already been shown previously (Herzig et al. 2006).

Description of protein sequences and individual genes: some V genes lack a CDR2

Upon in silico translation of all bovine V genes, 86 were determined to be pseudogenes based on frameshift mutations or internal stop codons in the V-EXON of the V genes, and 336 V genes that did not contain such mutations were assessed as full-length functional genes. Of eight genes, the full-length coding sequence was not available. It is possible that the number of pseudogenes is slightly underestimated because some additional V genes may have mutations in the leader exon (L-PART1), encoding part 1 of the leader. The predicted amino acid sequences of the novel bovine V genes that are not pseudogenes were aligned. One representative bovine V gene of each subgroup is shown in Fig. 3a, upper part. Some individual V genes are aberrant in the sense that they have a mutated conserved first or second cysteine, a mutated conserved tryptophan, or considerable insertions or deletions compared with their subgroup members. These genes are shown separately (Fig. 3a, lower part).

The impact of the insertions or deletions of two particular V genes was studied by creating an “IMGT Collier-de-Perles” representation (Fig. 3b), illustrating that gene 330 and gene 258 have a nine-amino-acid deletion leading to the loss of the complete CDR2 and part of FR3. This nine-amino-acid deletion is present in a total of four V genes that are all part of the bovine TRDV1 subgroup. Gene 330 has an extremely long CDR1 (18 amino acids), which is considerably longer than the limit of 12 amino acids set by IMGT for the usual CDR1. Gene 330 combines these features, so it has an extremely long CDR1 and no CDR2.

Because the nine-amino-acid CDR2 and partial FR3 deletion was found in four V genes, we were interested to see if these genes are functionally rearranged and used by T cells. In the databases, there were two mRNA sequences present (accession numbers BC142414 and EF175173) that consisted of one of the V genes with a nine-amino-acid deletion in the CDR2/FR3, both functionally rearranged to different TRDD and TRDJ and spliced to TRDC, suggesting that these genes are functional.

The bovine homologs of the V genes used by the invariant TR α chain of mucosal-associated invariant T cells (MAIT) cells (human TRAV1–2, mouse TRAV1) and NKT cells (human TRAV10, mouse TRAV11, or TRAV11D) have been previously identified and were

confirmed in the current study to be the closest possible bovine homologs of the human TRAV1–2 and TRAV10, respectively (and similarly of the mouse TRAV1 and TRAV11 or TRAV11D, respectively). Bovine gene 180 is the only bovine gene of the interspecies subgroup to which human TRAV1–2 and mouse TRAV1 belong, the V gene used by MAIT TR (Ishiguro et al. 1990; Tilloy et al. 1999). Interestingly, the V gene used by MAIT cells is the first one in the locus (in mice and cattle) or the second one (in human) and is interspersed between olfactory receptor genes (Glusman et al. 2001; Parra et al. 2008). Among the four bovine genes that form a subgroup with human TRAV10, three had already been identified as candidate V genes for hypothetical bovine NKT TR α chains (Looringh van Beeck et al. 2009)

Discussion

It has been previously shown that the number of TRDV genes and the potential and actual variability of $\gamma\delta$ TR in the artiodactyls sheep, cattle, and pigs is much higher than in other species (Antonacci et al. 2005; Hein and Dudler 1993; Van Rhijn et al. 2007; Yang et al. 1995), and it has been suggested that this may relate to the fact that they are “ $\gamma\delta$ high” species. In this study, we show that the TRAV genes in cattle are also much more plentiful than in mice and humans, and the numbers of genes identified by our method of manual annotation greatly exceed a previous prediction based on automated gene annotation using the same assembly of the genome (Elsik et al. 2009). In addition to the high number of V genes described in this study, an excess of heterozygosity in the bovine TRA/TRD locus has been demonstrated (Fries et al. 2001). Despite the fact that the actual variability of $\alpha\beta$ TR in artiodactyls remains to be determined, the existence of such a high number of bovine V genes elicits the question whether this implies an extended functionality and what evolutionary forces may have shaped this diversity.

From the available crystals of murine and human classical major histocompatibility complex (MHC) proteins with bound peptides and the $\alpha\beta$ TR recognizing these complexes (Kaas et al. 2004; Rudolph et al. 2006), it is known that CDR1 and CDR2 mainly interact with the surface of the MHC protein, whereas CDR3 interacts with the peptide. Even though there is some variation in docking angle, interaction of all six CDR with the MHC–peptide complex is possible because the $\alpha\beta$ TR docks approximately in a straight line on top of the MHC–peptide complex and the CDR have approximately the same length. This docking mechanism of TR on classical MHC–peptide complexes is highly similar in humans and mice and supported by a large set of data (Rudolph et al. 2006).

However, for nonclassical antigen-presenting elements and/or $\gamma\delta$ TR, extrapolations to other species are difficult because there is only a limited amount of data available and some nonclassical antigen-presenting elements and T cell populations are not distributed among all species. A few cases of direct recognition of a target molecule by an individual $\gamma\delta$ TR have been described and include the murine nonclassical MHC proteins T10 and T22, which are absent in humans; the human MHC-I-like CD1c, which is absent in mice; and allo-MHC (Bluestone et al. 1988; Ito et al. 1990; Schild et al. 1994; Spada et al. 2000). The murine $\gamma\delta$ TR G8, recognizing the T10 and T22 proteins (Adams et al. 2005), has been crystallized and shows that the very long CDR3 of the δ chain is responsible for most of the contact between the molecules. Because of the unequal length of the CDR loops, the TR interacts at an angle with its target molecule. No crystallographic data on bovine TR or antigen-presenting elements are available. However, based on a comparison with the known mode of interaction of human and murine TR with a classical MHC–peptide complex, the four CDR2-less bovine V genes are unlikely to recognize classical MHC–peptide complexes.

Acknowledgements Ildiko Van Rhijn is supported by the Wellcome Trust [GR078283].

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- Adams EJ, Chien YH, Garcia KC (2005) Structure of a gammadelta T cell receptor in complex with the nonclassical MHC T22. *Science* 308:227–231
- Antonacci R, Massari S, De Iaco R, Ciccicarese S (2001) Assignment of the TRB@ locus encoding the T-cell receptor beta chain to sheep, cattle, goat and river buffalo chromosomes by in situ hybridization. *Cytogenet Cell Genet* 94:82–83
- Antonacci R, Lanave C, Del Faro L, Vaccarelli G, Ciccicarese S, Massari S (2005) Artiodactyl emergence is accompanied by the birth of an extensive pool of diverse germline TRDV1 genes. *Immunogenetics* 57:254–266
- Bluestone JA, Cron RQ, Cotterman M, Houlden BA, Matis LA (1988) Structure and specificity of T cell receptor gamma/delta on major histocompatibility complex antigen-specific CD3+, CD4-, CD8- T lymphocytes. *J Exp Med* 168:1899–1916
- Bosc N, Lefranc MP (2003) The mouse (*Mus musculus*) T cell receptor alpha (TRA) and delta (TRD) variable genes. *Dev Comp Immunol* 27:465–497
- Brochet X, Lefranc MP, Giudicelli V (2008) IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis. *Nucleic Acids Res* 36:W503–W508

- Conrad ML, Pettman R, Whitehead J, McKinnel L, Davis SK, Koop BF (2002) Genomic sequencing of the bovine T cell receptor beta locus. *Vet Immunol Immunopathol* 87:439–441
- Conrad ML, Mawer MA, Lefranc MP, McKinnel L, Whitehead J, Davis SK, Pettman R, Koop BF (2007) The genomic sequence of the bovine T cell receptor gamma TRG loci and localization of the TRGC5 cassette. *Vet Immunol Immunopathol* 115:346–356
- Elsik CG, Tellam RL, Worley KC, Gibbs RA, Muzny DM, Weinstock GM, Adelson DL, Eichler EE, Elnitski L, Guigo R, Hamernik DL, Kappes SM, Lewin HA, Lynn DJ, Nicholas FW, Raymond A, Rijnkels M, Skow LC, Zdobnov EM, Schook L, Womack J, Alioto T, Antonarakis SE, Astashyn A, Chapple CE, Chen HC, Chrast J, Camara F, Ermolaeva O, Henrichsen CN et al (2009) The genome sequence of taurine cattle: a window to ruminant biology and evolution. *Science* 324:522–528
- Fries R, Ewald D, Thaller G, Buitkamp J (2001) Assessment of the nucleotide sequence variability in the bovine T-cell receptor alpha delta joining gene region. *Anim Biotechnol* 12:29–49
- Giudicelli V, Chaume D, Lefranc MP (2005) IMGT/GENE-DB: a comprehensive database for human and mouse immunoglobulin and T cell receptor genes. *Nucleic Acids Res* 33:D256–D261
- Glusman G, Rowen L, Lee I, Boysen C, Roach JC, Smit AF, Wang K, Koop BF, Hood L (2001) Comparative genomics of the human and mouse T cell receptor loci. *Immunity* 15:337–349
- Gobel TW, Chen CL, Lahti J, Kubota T, Kuo CL, Aebersold R, Hood L, Cooper MD (1994) Identification of T-cell receptor alpha-chain genes in the chicken. *Proc Natl Acad Sci U S A* 91:1094–1098
- Hein WR, Dudler L (1993) Divergent evolution of T cell repertoires: extensive diversity and developmentally regulated expression of the sheep gamma delta T cell receptor. *EMBO J* 12:715–724
- Hein WR, Dudler L (1997) TCR gamma delta+ cells are prominent in normal bovine skin and express a diverse repertoire of antigen receptors. *Immunology* 91:58–64
- Herzig CT, Blumerman SL, Baldwin CL (2006) Identification of three new bovine T-cell receptor delta variable gene subgroups expressed by peripheral blood T cells. *Immunogenetics* 58:746–757
- Houston EF, Connelley T, Parsons K, MacHugh ND, Morrison WI (2005) Analysis of T-cell receptor BV gene sequences in cattle reveals extensive duplication within the BV9 and BV20 subgroups. *Immunogenetics* 57:674–681
- Ishiguro N, Tanaka A, Shinagawa M (1990) Sequence analysis of bovine T-cell receptor alpha chain. *Immunogenetics* 31:57–60
- Ishiguro N, Aida Y, Shinagawa T, Shinagawa M (1993) Molecular structures of cattle T-cell receptor gamma and delta chains predominantly expressed on peripheral blood lymphocytes. *Immunogenetics* 38:437–443
- Ito K, Van Kaer L, Bonneville M, Hsu S, Murphy DB, Tonegawa S (1990) Recognition of the product of a novel MHC TL region gene (27b) by a mouse gamma delta T cell receptor. *Cell* 62:549–561
- Kaas Q, Ruiz M, Lefranc MP (2004) IMGT/3Dstructure-DB and IMGT/StructuralQuery, a database and a tool for immunoglobulin, T cell receptor and MHC structural data. *Nucleic Acids Res* 32:D208–D210
- Kaas Q, Ehrenmann F, Lefranc MP (2007) IG, TR and IgSF, MHC and MhcSF: what do we learn from the IMGT Colliers de Perles? *Brief Funct Genomic Proteomic* 6:253–264
- Kubota T, Wang J, Gobel TW, Hockett RD, Cooper MD, Chen CH (1999) Characterization of an avian (*Gallus gallus domesticus*) TCR alpha delta gene locus. *J Immunol* 163:3858–3866
- Lefranc MP, Lefranc G (2001) The T cell receptor FactsBook. Academic, London, pp 1–398
- Lefranc MP, Pommie C, Ruiz M, Giudicelli V, Foulquier E, Truong L, Thouvenin-Contet V, Lefranc G (2003) IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. *Dev Comp Immunol* 27:55–77
- Letunic I, Bork P (2007) Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* 23:127–128
- Looringh van Beeck FA, Reinink P, Hermesen R, Zajonc DM, Laven MJ, Fun A, Troskie M, Schoemaker NJ, Morar D, Lenstra JA, Vervelde L, Rutten VP, van Eden W, Van Rhijn I (2009) Functional CD1d and/or NKT cell invariant chain transcript in horse, pig, African elephant and guinea pig, but not in ruminants. *Mol Immunol* 46:1424–1431
- Parra ZE, Baker ML, Hathaway J, Lopez AM, Trujillo J, Sharp A, Miller RD (2008) Comparative genomic analysis and evolution of the T cell receptor loci in the opossum *Monodelphis domestica*. *BMC Genomics* 9:111
- Robinson MA, Mitchell MP, Wei S, Day CE, Zhao TM, Concannon P (1993) Organization of human T-cell receptor beta-chain genes: clusters of V beta genes are present on chromosomes 7 and 9. *Proc Natl Acad Sci U S A* 90:2433–2437
- Rudolph MG, Stanfield RL, Wilson IA (2006) How TCRs bind MHCs, peptides, and coreceptors. *Annu Rev Immunol* 24:419–466
- Ruiz M, Lefranc MP (2002) IMGT gene identification and Colliers de Perles of human immunoglobulins with known 3D structures. *Immunogenetics* 53:857–883
- Schild H, Mavaddat N, Litzenberger C, Ehrlich EW, Davis MM, Bluestone JA, Matis L, Draper RK, Chien YH (1994) The nature of major histocompatibility complex recognition by gamma delta T cells. *Cell* 76:29–37
- Spada FM, Grant EP, Peters PJ, Sugita M, Melian A, Leslie DS, Lee HK, van Donselaar E, Hanson DA, Krensky AM, Majdic O, Porcelli SA, Morita CT, Brenner MB (2000) Self-recognition of CD1 by gamma/delta T cells: implications for innate immunity. *J Exp Med* 191:937–948
- Su C, Jakobsen I, Gu X, Nei M (1999) Diversity and evolution of T-cell receptor variable region genes in mammals and birds. *Immunogenetics* 50:301–308
- Tanaka A, Ishiguro N, Shinagawa M (1990) Sequence and diversity of bovine T-cell receptor beta-chain genes. *Immunogenetics* 32:263–271
- Tilloy F, Treiner E, Park SH, Garcia C, Lemonnier F, de la Salle H, Bendelac A, Bonneville M, Lantz O (1999) An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class Ib-restricted alpha/beta T cell subpopulation in mammals. *J Exp Med* 189:1907–1921
- Tjoelker LW, Carlson LM, Lee K, Lahti J, McCormack WT, Leiden JM, Chen CL, Cooper MD, Thompson CB (1990) Evolutionary conservation of antigen recognition: the chicken T-cell receptor beta chain. *Proc Natl Acad Sci U S A* 87:7856–7860
- Van Rhijn I, Spiering R, Smits M, Blokland MT, de Weger R, van Eden W, Rutten VP, Koets AP (2007) Highly diverse TCR delta chain repertoire in bovine tissues due to the use of up to four D segments per delta chain. *Mol Immunol* 44:3155–3161
- Yang YG, Ohta S, Yamada S, Shimizu M, Takagaki Y (1995) Diversity of T cell receptor delta-chain cDNA in the thymus of a one-month-old pig. *J Immunol* 155:1981–1993