Short communication

Transcriptional profiling of inflammatory cytokine genes in African buffaloes (*Syncerus caffer*) infected with *Theileria parva*

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ABSTRACT

Theileria parva (*T. parva*) causes East Coast fever (ECF), which is of huge economic importance to Eastern and Southern African countries. In a previous bovine model, inflammatory cytokines were closely associated with disease progression in animals experimentally infected with *T. parva*. The African Cape buffalo (*Syncerus caffer*), the natural reservoir for *T. parva*, is completely resistant to ECF despite a persistently high parasitaemia following infection with *T. parva*. Characterizing basic immunological interactions in the host is critical to understanding the mechanism underlying disease resistance in the African Cape buffalo. In this study, the expression level of several cytokines was analyzed in *T. parva*-infected buffaloes. There were no significant differences in the expression profiles of inflammatory cytokines between the infected and uninfected animals despite a remarkably high parasitaemia in the former. However, the expression level of IL-10 was significantly upregulated in the infected animals. These results indicate a correlation between diminished inflammatory cytokines response and disease resistance in the buffalo.

1. Introduction

Inflammatory cytokines including IL1β, IL6 and TNFα are produced by immune cells such as the macrophages, lymphocytes, and natural killer cells, and are involved in the inflammatory responses in the host (Rostworowski et al., 1997). Inflammatory cytokines cover a wide spectrum of biological activity that helps to eliminate pathogens in infections and against tumour cells (Arai et al., 1990). However, massive systemic release of inflammatory cytokines (cytokine storm) can cause severe vascular damage with increased vascular permeability in the host (Wang and Ma, 2008). Furthermore, the resultant vascular damage can lead to haemorrhage, shock, cachexia and disseminated intravascular coagulation. This hypercytokinemia is considered a major cause of death in severe diseases such as malaria (Ghosh and Shetty, 2008), severe acute respiratory syndrome (Huang et al., 2005), Ebola virus infection (Baize et al., 2002) and highly pathogenic avian influenza (To et al., 2001).

Theileria parva causes East Coast fever (ECF), a lymphoproliferative disorder of cattle that is characterized by pyrexia, dyspnoea and cachexia, and severely limits livestock production in much of Sub-Saharan Africa (Norval et al., 1992). In a previous report, we showed that inflammatory cytokines are closely associated with disease progression in cattle experimentally infected with *T. parva* (Yamada et al., 2009). Specifically, aberrant expression of

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IL1β, IL6 and TNFα correlated positively with the severity of clinical signs in the infected cattle. Although the African Cape buffalo is the reservoir for *T. parva*, it is naturally resistant to ECF, and exhibits few clinical signs despite high parasitaemia (Allsopp et al., 1999; Latif et al., 2002). However, the reasons behind disparate sensitivity and disease progression during *T. parva* infection between African buffaloes and domestic cattle are still unknown. More specifically, it is unclear whether elevation of inflammatory cytokine develops in *T. parva*-infected African buffaloes. Therefore, there is a need to investigate the immunological events, including dynamic changes of inflammatory cytokines, during infection, since cytokines are directly involved in disease severity (Bielefeldt et al., 1989; Jongen-Lavrencic et al., 1996). However, there is no information available about cytokines in the African buffalo. Here, we report the molecular cloning of inflammatory cytokines IL1β, IL6 and TNFα from African buffaloes and sequence comparisons with the existing sequences of other mammalian species in the GenBank. Based on this genetic information, we developed a quantitative real-time PCR method with the aim to examine the relationship between cytokine profile and disease resistance and determined whether elevation of inflammatory cytokine occurs in *T. parva*-infected African buffaloes.

2. Material and methods

2.1. Animals

Blood samples were collected from six buffaloes at the Nairobi National Park, Nairobi, Kenya, under a strict wildlife control regimen in accordance with Kenya Wildlife Service guidelines on using animals for research work. All animals were asymptomatic males (no fever, anemia or swollen lymph nodes) of unknown age, and infested with *Rhipicephalus appendiculatus* ticks, the vector of *T. parva*. Blood was collected into sterile tubes with anti-coagulant (lithium heparin) and maintained at 4 °C until arrival at the laboratory. Genomic DNA was extracted from whole blood using the Wizard Genomic DNA Purification kit (Promega). *T. parva* infection was tested by microscopic examination of Giemsa-stained blood smear, and confirmed by amplifying *T. parva p104* gene in a nested-PCR (Skilton et al., 2002). Parasite load was further confirmed by real-time PCR as described previously (Konnai et al., 2006).

2.2. Cloning and sequencing of African buffalo cytokine genes and sequence analysis

Buffy coats were collected after centrifugation at 14,000 rpm for 10 min. Total RNA was isolated from buffy coats using the TRIzol (Invitrogen) and stored at −80 °C until use. An aliquot of total RNA (1 μg) was reverse-transcribed using Moloney murine leukaemia virus reverse transcriptase (10 U/μL, Takara) and 200 pmol oligo-dT in a total volume of 20 μL. IL1β, IL6 and TNFα cDNAs were amplified by PCR using primers designed based on bovine cytokine sequences reported in the GenBank: IL1β (IL1β-for 5′-CTT CAT TGC CCA GGT TTC TG-3′ and IL1β-rev 5′-CAG TCC TCG GGG TTA TTC AG-3′), IL6 (IL6-for 5′-GAA CGA

AAG AGA GCT CCA TCT G-3′ and IL6-rev 5′-GCC CAG GAA CTA CCA CAA TC-3′), TNFα (TNFα-for 5′-CGT CTG GAC TTG AAC CCT TC-3′ and TNFα-rev 5′-ACT GAG GTG GGA GAA GAT GC-3′). The reaction mixtures for PCR contained 100 mM Tris–HCl, 50 mM KCL (pH 8.3), 0.1% Triton-X 100, 1.5 mM MgCl$_2$, each deoxynucleoside triphosphate (dNTP) at a concentration of 120 μM and 2.5 U Taq polymerase (Takara). Cycling conditions for PCR were 40 cycles of 30 s at 94 °C, 1 min at annealing temperatures depending on cytokines (IL-1β: 54 °C, IL-6: 54 °C at the rate of 0.2 °C/cycle, TNF-α: 60 °C) to be amplified and 1 min at 72 °C, followed by the final extension for 7 min. Resultant PCR products were separated on 2% agarose gels containing 0.5 μg/mL ethidium bromide and visualised under ultraviolet light. The amplified bands corresponding to cytokine cDNAs were excised from the gel and purified using the Gene-clean-III Kit (MP Biomedicals). The purified cDNA fragments were ligated into the pGEM T-Easy-vector (Promega), and transformed into a competent *Escherichia coli* strain DH-5α. Purified plasmids were sequenced using the CEQ 2000 Dye Terminator Cycle Sequencing method with the Quick Start kit (Beckman Coulter). Sequences were analysed using the CEQ 2000 DNA analysis system (Beckman Coulter) and aligned using ClustalW (Thompson et al., 1994). The secretory signal peptide and cleavage site for the mature protein were predicted using SignalP (http://www.cbs.dtu.dk/services/SignalP/). The phylogenetic tree was constructed by the neighbour-joining method with MEGA5 software (Tamura et al., 2011). The sequences of the cytokine genes of African buffaloes were submitted to the GenBank database under accession numbers AB571651 (IL1β), AB571652 (IL6) and AB571653 (TNFα).

2.3. Expression analysis of cytokine and iNOS mRNA by quantitative real-time PCR

To investigate the expression levels of cytokine mRNA, quantitative RT real-time PCR was performed using the LightCycler 480 System II (Roche Diagnostics). Oligonucleotide primers for inflammatory cytokine genes were designed from the sequence information obtained above and other primers were designed based on the sequences of bovine genes using Primer 3. Details of oligonucleotide primer sequences, including predicted product lengths by amplification and accession numbers are listed in Table 1. cDNA was synthesised from the RNA samples as described above. The cDNA template was mixed with 5 μL SYBR Premix DimerEraser (Takara) and 0.3 μL each primer (10 pmol/μL) in a total volume of 10 μL. Each amplification procedure was performed in triplicate. '2nd Derivative Max' was used to determine the value of crossing point. Glucuronidase, beta (Gusb) and heat shock protein 90 (Hsp90) were used as internal control genes. Relative amounts were calculated using the comparative threshold cycle method (Livak and Schmittgen, 2001). Each cytokine and inducible nitric oxide synthase (iNOS) mRNA expressions were evaluated as ratios obtained by dividing the concentration of cytokine mRNA by those of internal control genes. Two-tailed unpaired Student's *t*-test and

Table 1
Sequences of oligonucleotide primers.

Gene	Primer sequence (5′-3′)	Annealing temperature (°C)	Size of amplified fragment (bps)	Database accession number
IL-1β	F: ACCTTCATTGCCCAGGTTTCT R: TGTTTGGGGTCATCAGCCTCAA	55	120	AB571651
IL-2	F: TTTTACGTGCCCAAGGTTAA R: CGTTTACTGTTGCATCATCA	55	217	M12791[a]
IL-4	F: CAAAGAACACAACTAAGAAG R:AGGTCTTTCAGCGTACTTGT	55	181	AB571122
IL-6	F: TCCAGAATGAGTATGAGG R: CATCCGAATAGCTCTCAG	55	236	AB571652
IL-10	F: TGTTGACCCAGTCTCTGCTG R: GGCATCACCTCTTCCAGGTA	55	94	NM_174088[a]
IL-12p40	F: AACCTGCAACTGAGACCATT R: ATCCTTGTGGCATGTGACTT	55	186	U11815[a]
IFN-γ	F: ATAACCAGGTCATTCAAAGG R: ATTCTGACTTCTCTTCCGCT	55	218	AB571121
TNF-α	F: TAACAAGCCAGTAGCCCACG R: GCAAGGGCTCTTGATGGCAGA	55	277	AB571653
TGF-β1	F: CTGCTGAGGCTCAAGTTAAAAGTG[b] R: CAGCCGGTTGCTGAGGTAG	60	89	M36271[a]
TGF-β2	F: GCCGAGTTCAGAGTCTTTCGTTT[b] R: GCGCTGGGTTGGAGATGTTA	60	89	M19154[a]
iNOS	F: AGCGGAGTGACTTTCCAAGA R: TTTTGGGGTTCATGATGGAT	55	97	NM_001076799[a]
Gusb	F: CAGATGCCATTGAAGGGTTT R: TTTGGTCCAGAACCACATGA	55	99	NM_001083436[a]
Hsp90	F: GCCAAGTCTGGCACTAAAGC R: AGGCAGAGTAGAAGCCCACA	55	95	NM_001079637[a]

[a] Primers were designed based on bovine genes from the EMBL/GenBank databases.
[b] The primer sequences are cited from Ravelich et al. (2006).

two-tailed unpaired Welch's *t*-test were used for comparison tests. *p* values of <0.05 were considered significant.

3. Results and discussion

For the first time, we cloned inflammatory cytokines from African buffalo (Fig. 1). The 801 bp African buffalo IL1β cDNA contained an open reading frame (ORF) with 266 amino acids (AAs). The deduced AA sequence includes six cysteine residues, one possible N-linked glycosylation site and carboxyl terminal endpoints for active proteins. The cDNA of African buffalo IL6 was cloned and found to be 627 bp, with an ORF encoding 208 AAs. A 208 AA sequence of African buffalo IL6, bearing a N-linked glycosylation site with four cysteine residues, was deduced from a 627 bp ORF. The 705 bp African buffalo TNFα cDNA contained an ORF of 234 AAs. The deduced AA sequence includes four cysteine residues, which are also conserved in the other species.

Six African buffaloes were captured and *T. parva* infection was individually diagnosed by PCR and blood smear examination. The clinical signs of *T. parva*-infection, such as fever, anaemia and swelling of lymph nodes, were not observed in any the animals. Three animals were PCR positive and had microscopically detectable piroplasms on blood smears, indicating high parasitaemia (Table 2). Quantitative real-time PCR showed that each of the *T. parva* positive animals had high parasite DNA load ranging from $10^{-0.636}$ to $10^{0.915}$ pg/0.5 mL whole blood, which corresponds to a lethal dose (>10^{-1} pg) from the results obtained from the experimentally infected cattle with severe signs

(Yamada et al., 2009). To verify if differences in the levels of cytokine expressions could correlate with the high parasitaemia, we used real-time PCR to measure the mRNA expressions of IL1β, IL6 and TNFα, in whole blood cells isolated from *T. parva*-infected African buffaloes, since these cytokines are elevated in *T. parva*-experimentally infected cattle (Yamada et al., 2009). The mRNA expression of IL10 was significantly higher than those of uninfected animals, while iNOS induction was only detectable in *T. parva*-infected animals (Table 2). On the other hand, TNFα expression level was lower than that of uninfected animals. IL6 was undetectable in both animals. Furthermore, to evaluate other anti-protozoan molecules, we investigated relative differences in expression of IFNγ, IL2, IL4 IL12p40, transforming growth factor (TGF) β1 and TGF-β2 in African buffaloes infected with *T. parva*. The mean IFNγ, IL2, IL4, TGF-β1 and TGF-β2mRNA expression levels in infected animals were higher than those of uninfected animals, although there were large individual differences.

In a previous report, we reported that in a disease-susceptible cattle model, aberrant expression of the pro-inflammatory cytokines IL1β, IL6 and TNFα was closely correlated with the increase in *T. parva* DNA load and severe clinical signs, such as pyrexia, dyspnoea and cachexia (Yamada et al., 2009). Although African buffaloes become infected with *T. parva*, the infected animals exhibit fewer clinical signs and recover from parasite, which is fatal in cattle. Thus, the African buffalo is regarded as an important reservoir host of *T. parva* (Latif et al., 2002; Munang'andu et al., 2009). Unexpectedly, *T. parva*-infected African buffaloes had high levels of parasitaemia despite

Table 2

Theileria parva status of African buffaloes used in the study.

Animal ID	T. parva			Ratio (target gene/internal controls)			
	Blood smear	Nested PCR	Protozoan *p104* DNA (pg/0.5 mL)	IL-1β	IL-2	IL-4	IL-6
1	−	−	−	0.1898	0.0000	0.0000	N.D
2	−	−	−	0.0606	0.0001	0.0008	N.D
3	−	−	−	0.0823	0.0001	0.0018	N.D
(Average ± SD)				(0.1109 ± 0.0692)	(0.0001 ± 0.0000)	(0.0008 ± 0.0009)	
4	+	+	$10^{-0.636}$	0.4226	0.0004	0.0042	N.D
5	+	+	$10^{0.915}$	0.2892	0.0014	0.0140	N.D
6	+	+	$10^{0.891}$	0.1547	0.0000	0.0038	N.D
(Average ± SD)				(0.2888 ± 0.1340)	(0.0006 ± 0.0007)	(0.0073 ± 0.0057)	

Animal ID	Ratio (target gene/internal controls)						
	IL-10	IL-12p40	IFN-γ	TNF-α	TGF-β1	TGF-β2	iNOS
1	0.0163	0.0117	0.0632	0.1301	0.3421	0.0005	N.D
2	0.0167	0.0020	0.0198	0.1621	0.0581	0.0004	N.D
3	0.0070	0.0200	0.0218	0.1814	0.2852	0.0005	N.D
(Average ± SD)	(0.0133 ± 0.0055)	(0.0112 ± 0.0090)	(0.0349 ± 0.0245)	(0.1579 ± 0.0259)	(0.2285 ± 0.1503)	(0.0005 ± 0.0001)	
4	0.0398	0.0130	0.2415	0.1448	2.6253	0.0008	0.0105
5	0.0398	0.0104	1.1708	0.0300	0.6188	0.0030	0.0053
6	0.0246	N.D	0.0557	0.0247	2.0385	0.0030	0.0114
(Average ± SD)	(0.0347 ± 0.0087)[*]		(0.4893 ± 0.5974)	(0.0665 ± 0.0679)	(1.7609 ± 1.0317)	(0.0023 ± 0.0013)	(0.0091 ± 0.0033)

+: positive; −: negative; N.D: not detected.

[*] $p < 0.05$.

(A) IL-1β

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CTTCATTGCCCAGGTTTCTGAAACAGCCATGGCAACCGTACCTGAACCCATCAATGAAATGATGGCCTACTACAGTGACGAGAATGAGATGTTATTTGAGGCTGATGGCCCCAAACAGAT
                       M  A  T  V  P  E  P  I  N  E  M  M  A  Y  Y  S  D  E  N  E  M  L  F  E  A  D  G  P  K  Q  M
GAAGAGCTGCATCCAACACCTGGACCTCGGTTCCATGGGAGATGGAAACATCCAGCTGCAGATTTCTCACCAGCTCTACAACAAAAGCTTCAGGCAGGTGGTGTCGGTCATTGTGGCCAT
  K  S  C  I  Q  H  L  D  L  G  S  M  G  D  G  N  I  Q  L  Q  I  S  H  Q  L  Y  N  K  S  F  R  Q  V  V  S  V  I  V  A  M
GGAGAAGCTGAGGAACAGTGCCTACGCACATGTCTTCCATGATGATGACCTGAGGAGCATCCTTTCATTCATCTTTGAAGAAGAGCCTGTCATCTTTGAAACATCCTCCGACGAGTTTCT
  E  K  L  R  N  S  A  Y  A  H  V  F  H  D  D  D  L  R  S  I  L  S  F  I  F  E  E  E  P  V  I  F  E  T  S  S  D  E  F  L
GTGTGATGCACCCGTGCAGTCAGTAAAGTGCAAACTCCAGGACAGAGAGCAAAAATCCCTGGTGCTGGCTAGCCCATGTGTGCTGAAGGCTCTCCACCTCCTCTCACAGGAAATGAGCCG
  C  D  A  P  V  Q  S  V  K  C  K  L  Q  D  R  E  Q  K  S  L  V  L  A  S  P  C  V  L  K  A  L  H  L  L  S  Q  E  M  S  R
AGAAGTGGTGTTCTGCATGAGCTTCGTGCAAGGAGAGGAAAGAGACAACAAGATTCCTGTGGCCTTGGGTATCAAGGACAAGAATCTATACCTGTCTTGTGTGAAAAAAGGTGATACGCC
  E  V  V  F  C  M  S  F  V  Q  G  E  E  R  D  N  K  I  P  V  A  L  G  I  K  D  K  N  L  Y  L  S  C  V  K  K  G  D  T  P
CACCCTGCAGCTGGAGGAAGTAGACCCCAAAGTCTACCCCAAGAGGAATATGGAAAGGCGATTTGTCTTCTACAAGACAGAAATCAAGAATACAGTTGAATTTGAGTCCGTCCTGTACCC
  T  L  Q  L  E  E  V  D  P  K  V  Y  P  K  R  N  M  E  K  R  F  V  F  Y  K  T  E  I  K  N  T  V  E  F  E  S  V  L  Y  P
TAACTGGTACATCAGCACTTCTCAAATCGAAGAAAGACCCGTCTTCCTGGGACATTTTAGAGGTGGCCAGGATATAACTGACTTCAGAATGGAAACCCTCTCTCCTTAAAGAAAGCCATA
  N  W  Y  I  S  T  S  Q  I  E  E  R  P  V  F  L  G  H  F  R  G  G  Q  D  I  T  D  F  R  M  E  T  L  S  P  #
TCCCAGGGAGTCCACGTGGGCTGAATAACCCCGAGGACT
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(B) IL-6

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GAACGAAAGAGAGCTCCATCTGCCCTCCAGGAACAGCTATGAATTCCCGCTTCACAAGCGCCTTCACTCCATTCGCTGTCTCCCTGGGGCTGCTCCTGGTGATGACTTCTGCTTTCCCTA
                         M  N  S  R  F  T  S  A  F  T  P  F  A  V  S  L  G  L  L  L  V  M  T  S  A  F  P  T
CCCCGGGTCCCCTGGGAGAAGATTTCAAAAATGACACCACCCCAGGCAGACTACTTCTGACCACTCCAGAGAAAACCGAAGCTCTCATTAAGCGCATGGTCGACAAAATTTCTGCAATGA
  P  G  P  L  G  E  D  F  K  N  D  T  T  P  G  R  L  L  L  T  T  P  E  K  T  E  A  L  I  K  R  M  V  D  K  I  S  A  M  R
GAAAGGAGATATGTGAGAAGAATGACGAGTGTGAAAGCAGCAAGGACACACTGGCAGAAAATAAGCTGAATCTTCCAAAAATGGAGGAAAAGGACGGATGCTTCCAATCTGGGTTCAATC
  K  E  I  C  E  K  N  D  E  C  E  S  S  K  D  T  L  A  E  N  K  L  N  L  P  K  M  E  E  K  D  G  C  F  Q  S  G  F  N  Q
AGGCGATTTGCTTGATCAGAACCACTGCTGGTCTTCTGGAGTATCAGATATACCTGGACTACCTCCAGAATGAGTATGAGGGAAATCGGGAAAATGTAAGGGATTTGAGGAAAAATATCA
  A  I  C  L  I  R  T  T  A  G  L  L  E  Y  Q  I  Y  L  D  Y  L  Q  N  E  Y  E  G  N  R  E  N  V  R  D  L  R  K  N  I  R
GAACACTGATCCAGATCCTGAAGCAAAAGATCGAAGATCTAATAACCACTCCAGCCACAAACACTGACCTGCTGGAGAAGATGCAGTCCTCAAACGAGTGGGTAAAGAACGCAAAGATTA
  T  L  I  Q  I  L  K  Q  K  I  E  D  L  I  T  T  P  A  T  N  T  D  L  L  E  K  M  Q  S  S  N  E  W  V  K  N  A  K  I  I
TCCTCATCCTGAGAAACCTTGAGAATTTCCTGCAGTTCAGCCTGAGAGCTATTCGGATGAAGTAGCTGGGGCTCCCATGATTGTGGTAGTTCCTGGGC
  L  I  L  R  N  L  E  N  F  L  Q  F  S  L  R  A  I  R  M  K  #
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(C) TNF-α

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CGTCTGGACTTGAACCCTTCTGAAAAAGACACCATGAGCACCAAAAGCATGATCCGAGATGTGGAGCTGGCGGAGGAGGTGCTCTCCGAGAAAGCAGGGGGCCCCCAGGGCTCCAGAAGT
                       M  S  T  K  S  M  I  R  D  V  E  L  A  E  E  V  L  S  E  K  A  G  G  P  Q  G  S  R  S
TGCTTGTGCCTCAGCCTCTTCTCCTTCCTCCTGGTTGCAGGAGCCACCACGCTTCTGCCTGCTGCACTTCGGGGTAATCGGCCCCCAGAGGGAAGAGCAGTCCCCAGGTGGCCCCTCC
  C  L  C  L  S  L  F  S  F  L  L  V  A  G  A  T  T  L  F  C  L  L  H  F  G  V  I  G  P  Q  R  E  E  Q  S  P  G  G  P  S
ATCAACAGCCCTCTGGTTCAGACACTCAGGTCCTCTTCTCAAGCCTCCAGTAACAAGCCAGTAGCCCACGTTGTAGCCGACATCAACTCTCCAGGGCAGCTCCGGTGGTGGGACTCATAT
  I  N  S  P  L  V  Q  T  L  R  S  S  S  Q  A  S  S  N  K  P  V  A  H  V  V  A  D  I  N  S  P  G  Q  L  R  W  W  D  S  Y
GCCAATGCCCTCATGGCCAACGGCGTGAAGCTAGAAGACAACCAGCTGGTGGCCTGCTGATGGGCTTTACCTCATCTACTCACAGGTCCTCTTCAGGGGCCAAGGCTGCCCTTCCACC
  A  N  A  L  M  A  N  G  V  K  L  E  D  N  Q  L  V  V  P  A  D  G  L  Y  L  I  Y  S  Q  V  L  F  R  G  Q  G  C  P  S  T
CCCTTGTTCCTCACCCACACCATCAGCCGCATTGCAGTCTCCTACCAGACCAAGGTCAACATCCTGTCTGCCATCAAGAGCCCTTGCCACAGGGAGACCCCAGAGTGGGCTGAGGCCAAG
  P  L  F  L  T  H  T  I  S  R  I  A  V  S  Y  Q  T  K  V  N  I  L  S  A  I  K  S  P  C  H  R  E  T  P  E  W  A  E  A  K
CCCTGGTACGAACCCATCTACCAGGGAGGGGTCTTCCAGCTGGAGAAGGGAGATCGCCTCAGTGCTGAGATCAACCTGCCGGACTACCTGGACTATGCCGAGTCTGGGCAGGTCTACTTT
  P  W  Y  E  P  I  Y  Q  G  G  V  F  Q  L  E  K  G  D  R  L  S  A  E  I  N  L  P  D  Y  L  D  Y  A  E  S  G  Q  V  Y  F
GGGATCATCGCCCTGTGAGGGTGCAGGACATGCATCTTCTCCCACCTCAGT
  G  I  I  A  L  #
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Fig. 1. Nucleotide and deduced amino acid sequences of cDNA encoding African buffalo (A) IL-1β, (B) IL-6 and (C) TNF-α. The start codon (ATG) and the stop codon are in bold with single underline. The putative signal peptide site is underlined.

their asymptomatic status. The parasitaemia levels corresponded to those observed in experimentally infected cattle with severe clinical signs (Yamada et al., 2009). However, aberrant up-regulation of pro-inflammatory cytokines was not observed in the *T. parva*-infected buffaloes, although we could not directly compare the findings in asymptomatic African buffaloes of unknown age with chronic *T. parva* infection and 1-year-old cattle with acute experimental *T. parva* infection. In particular, IL6 was not detectable in the *T. parva*-infected African buffaloes, although aberrant up-regulation of IL6 was observed in the case of experimental infected cattle (80-fold increase). Recently, nucleotide sequence polymorphisms in genes of some cytokines have been shown to determine the specificity of an individual's immune response and to play roles in conferring resistance or susceptibility to several diseases in human (van Deventer, 2000). Likewise, one hypothesis is that differences in genetic background could elicit different immune responses, resulting in either resistance or susceptibility to *T. parva*-infection. However, the amino acid sequences of IL1β, IL6 and TNFα of the African buffalo were comparable to those of cattle and a high degree of homology was observed (more than 97%). It remains to be determined whether the inflammatory cytokine genetic differences could influence resistance or susceptibility to *T. parva*-infection in the same way human infections do.

As a preliminary test of the hypothesis that other antiprotozoan molecules are associated with the susceptibility to *T. parva*-infection, we also examined the expression levels of IFNγ, IL2, IL4 IL12p40 and iNOS in African buffaloes infected with *T. parva*. Interestingly, higher expression levels of IFNγ and IL2mRNA were observed as compared to uninfected animals regardless of high parasitaemia. In addition, iNOS was specifically detected in the infected animals. Th1-type cytokines, including IFNγ and IL2, and iNOS have a critical role in the host inflammatory response for elimination of parasites, especially in haemoprotozoan parasite infection (Green et al., 1994; Brown et al., 1999; Ahmed, 2002). These observations raise the possibility that the cell-mediated immune response may contribute to

disease resistance in *T. parva* infection, which is known to be the case in *T. parva* infected cattle. However, the infected animals had high parasitaemia. Such changes may be protozoan-induced, but insufficient responses were induced as the result of *T. parva* infection in the animals. Previously, McKeever et al. (1997) investigated the expression patterns of several cytokine in cloned *T. parva*-infected lymphoblast cell lines, and showed that only IL10 was universally expressed. IL10 has been shown to be unregulated in response to infection with *T. parva in vitro*, suggesting it plays a critical role when naive cattle are exposed to *T. parva* (McKeever et al., 1997). The role of IL-10 in the pathogenesis of the disease is unclear, but IL-10 may be induced as part of a homeostatic network that protects tissues from collateral damage by excessive inflammation (Mege et al., 2006). Chaussepied et al. (2010) recently reported differential induction of host TGFβ transcription between disease-resistant *Bos indicus* and the susceptible Holstein–Friesian (*Bos taurus*) cattle infected with *Theileria annulata*. In this study, mRNA expression levels of TGFβ1 and TGFβ2 genes were higher in infected than that of uninfected animals, although this data was inferred from a limited number of animals. TGFβ is known as a negative regulatory cytokine for the immune system by Foxp3+regulatory T cell (Nakamura et al., 2001). Inflammation is enhanced by the action of pro-inflammatory cytokines, including IL1β, IL6 and TNFα, and is resolved by anti-inflammatory cytokines, such as IL-4, IL-10 and TGFβ (Yoshimura et al., 2003). The role of TGFβ in the pathogenesis of the disease is unclear, but TGFβ may be involved in a mechanism that limits collateral damage from excessive inflammation. Characterizing cytokine expression in a larger number of animals infected with *T. parva* is necessary to identify distinct trends in the expression of these immune regulators.

In summary, we show that some pro-inflammatory cytokines of the African Cape buffalo are maintained at low levels regardless of high parasitaemia with *T. parva*, in contrast with susceptible breeds of cattle that often present with hypercytokinemia in response to *T. parva*. These findings suggest that the African Cape buffalo is disease tolerant rather than resistant to *T. parva* infection.

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