J. Vet. Sci. (2001), **2**(2), 139–141

JOURNAL OF
Veterinary
Science

Short communication

Cloning a new allele form of bovine TNF-α

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Although little is known on the function of γδ T lymphocytes, there is increasing evidence that γδ T lymphocytes are early responders and modulators of immune responses against pathogens and cytokines such as IL-2, IL-7, IL-15 and TNF-α. To study the role TNF-α on γδ T lymphocytes, we cloned bovine TNF-α. Sequence analysis revealed that a new allele form of bovine TNF-α was cloned which has 3 additional nucleotide sequences as well as 3 nucleotide substitutions compared with previously reported bovine TNF-α. Further studies are needed to document the functional significance of a new allele form of TNF-α in cattle.

Key words: New allele, bovine, TNF-α

T cells can be distinguished on the expression of αβ or γδ forms of T cell receptor. While αβ T lymphocytes are well characterized with respect to phenotype and function, little is known about γδ T lymphocytes. Since γδ T lymphocytes are predominantly localized in epithelia, it has been hypothesized to play in the first line of defense against infectious agents [12,13,14]. Although it is not clear the role of γδ T lymphocytes, there is increasing evidence that γδ T lymphocytes are early responders and modulators of immune responses to infectious agents [1,5,6,9,11, 15,19,21]. In most species examined thus far, γδ T lymphocytes comprise only a small proportion (<10%) of T lymphocytes in peripheral blood [8]. In contrast, γδ T lymphocytes comprise 30-60% of peripheral blood lymphocytes in cattle [18], 20-60% in sheep [17], and 40-60% in pigs [2]. The large population of γδ T lymphocytes in ruminants and pigs is attributed to the presence of a unique subpopulation of $WC1^+$ γδ T lymphocytes that express CD3 and CD5 but not CD2 or CD6 [3,4,16,20,25]. Comparative studies revealed the presence of $WC1^-$ γδ T lymphocytes that express CD2, CD3, CD5, and CD6 [4,16]. In cattle, $WC1^+$ γδ T lymphocytes are present in high concentration in blood lymphocytes, but comprise only 3 to 5% of lymphocytes in the spleen [3]. In contrast, $WC1^-$ γδ T lymphocytes comprise only 3% to 6% of blood lymphocytes, but may comprise 35% or more of lymphocytes in the spleen [16,24,25]. In addition to the differences in phenotype and tissue distribution, $WC1^+$ and $WC1^-$ γδ T lymphocytes differ in usage of Vγ and Jγ segments, and Cγ chains [10]. These observations suggest that $WC1^+$ and $WC1^-$ γδ T lymphocytes represent separate lineages of γδ T lymphocytes with distinct roles in host defense. Based on the phenotype and tissue distribution, $WC1^-$ γδ T lymphocytes in ruminants appear similar to γδ T lymphocytes characterized in other species and may play a similar role in host defense. However, no information is available which subset of γδ T lymphocytes will show similar responses against cytokines. To determine the role of TNF-α on $WC1^+$ and $WC1^-$ γδ T lymphocytes, we constructed an expression cDNA library in ZAP Express Vector and cloned bovine TNF-α. Bovine macrophages were cultured for 1-5 days in DMEM supplemented 2 mM L-glutamine, 13% bovine serum and mRNA was isolated using FastTrack™ mRNA isolation kit (Invitrogen). A cDNA library was constructed according to manufacturer's protocol using Gubler and Hoffman's method. Double strand cDNA was fractionated on a 1% agarose gel. cDNA fractions between 0.75 kb-2 kb and larger than 2 kb were harvested separately using gel extraction kit (Qiagen). One hundred ng of purified cDNA was ligated with 1 μg of ZAP Express Vector (Stratagene). The titer of the primary cDNA library was 3.5×10^6 pfu for 0.75-2 kb fragment and 4.5×10^5 pfu for larger than 2 kb fragment. The primary cDNA library was amplified and used for PCR to clone bovine TNF-α. Bovine TNF-α was amplified with F primer 5'-GAA GCT AGC ATG AGC ACC AAA AGC ATG ATC CGG-3' and R primer 5'-GAA CTC GAG TCA CAG GGC GAT GAT CCC AAA GTA-5'. PCR mixture (100 μl) contained 10 μl of 10 × PCR buffer, 3 μl of 50 mM $MgCl_2$, 1 μl of 10 mM dNTPs, 15 pmol of each primers, 5 μl of amplified cDNA library (2.2×10^9 pfu/ml and 2.8×10^9 pfu/ml for small and large fragment, respectively), and 2.5 units of Taq DNA Polymerase. PCR was run for 30 cycles with the condition of denaturation

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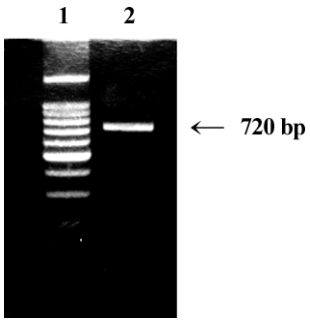

Fig. 1. Gel electrophoresis of PCR products 1) marker 2) amplified TNF-α. Elecrophoresis was performed in 1% agarose, 1 × TAE containing 0.5 μg/ml ethidium bromide.

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ATGAGCACCAAAAGCATGATCCGGGATGTGGAGCTGGCGGAGGAGGTGCTCTCCGAGAAA
M  S  T  K  S  M  I  R  D  V  E  L  A  E  E  V  L  S  E  K

GCAGGGGGCCCCCAGGGCTCCAGAAGTTGCTTGTGCCTCAGCCTCTTCTCCTTCCTCCTG
A  G  G  P  Q  G  S  R  S  C  L  C  L  S  L  F  S  F  L  L

GTTGCAGGAGCCACCACGCTCTTCTGCCTGCTGCACTTCGGGGTAATCGGCCCCCAGAGG
V  A  G  A  T  T  L  F  C  L  L  H  F  G  V  I  G  P  Q  R

GAAGAGCAGTCCCCAGGTGGCCCCTCCATCAACAGCCCTCTGGTTCAAACACTCAGGTCC
E  E  Q  S  P  G  G  P  S  I  N  S  P  L  V  Q  T  L  R  S

TCTTCTCAAGCCTCAAGTAACAAGCCGGTAGCCCACGTTGTAGCCGACATCAACTCTCCG
S  S  Q  A  S  S  N  K  P  V  A  H  V  V  A  D  I  N  S  P

GGGCAGCTCCGGTGGTGGGACTCGTATGCCAATGCCCTCGTGGCCAACGGTGTGAAGCTG
G  Q  L  R  W  W  D  S  Y  A  N  A  L  V  A  N  G  V  K  L

GAAGACAACCAGCTGGTGGTGCCTGCTGACGGGCTTTACCTCATCTACTCACAGGTCCTC
E  D  N  Q  L  V  V  P  A  D  G  L  Y  L  I  Y  S  Q  V  L

TTCAGGGGCCAAGGCTGCCCTTCCACCCCCTTGTTCCTCACCCACACCATCAGCCGCATT
F  R  G  Q  G  C  P  S  T  P  L  F  L  T  H  T  I  S  R  I

GCAGTCTCCTACCAGACCARGGTCAACATCCTGTCTGCCATCAAGAGCCCTTGCCACAGG
A  V  S  Y  Q  T  R  V  N  I  L  S  A  I  K  S  P  C  H  R

GAGACCCCAGAGTGGGCTGAGGCCAAGCCCTGGTACGAACCCATCTACCAGGGAGGAGTC
E  T  P  E  W  A  E  A  K  P  W  Y  E  P  I  Y  Q  G  G  V

TTCCAGCTGGAGAAGGGAGATCGCCTCAGTGCTGAGATCAACCTGCCGGACTACCTGGAC
F  Q  L  E  K  G  D  R  L  S  A  E  I  N  L  P  D  Y  L  D

TATGCCGAGTCTGGGCAGGTCTACTTTGGGATCATCGCCCTGTGA
Y  A  E  S  G  Q  V  Y  F  G  I  I  A  L  *
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Fig. 2. Composite nucleotide sequence and deduced amino acid sequence of bovine TNF-α (GenBank Accession Number AF348421). The substituted amino acid sequences and nucleotide sequences are bold and underlined.

94°C 30 second, annealing 62°C 30 second, extension 72°C 30 second. PCR product was cloned into PCR 2.1 (Invitrogen) and sequenced using ABI 373 (Applied Biosystem). Sequence analysis of bovine TNF-α revealed that a new allele form of TNF-α was cloned. The size of

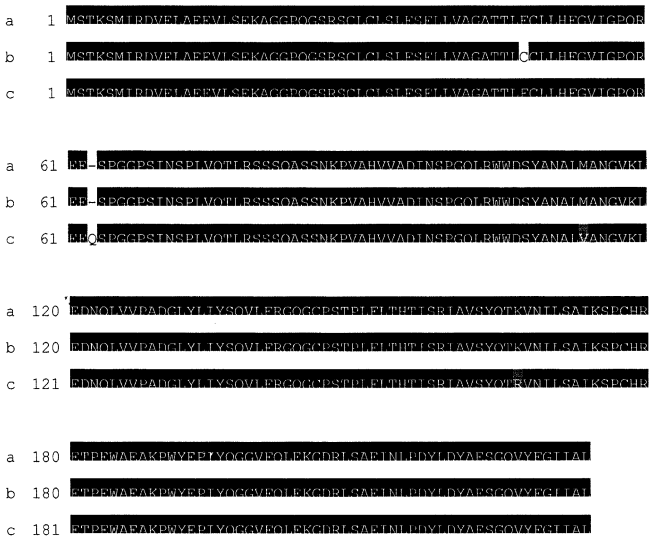

Fig. 3. Multiple sequence alignment of bovine TNF-α a) GenBank Accession number S24642, b) AAB84086 and c) AF348421.

the PCR products was 723 bp (Fig. 1). DNA sequence analysis revealed that new allele form of TNF-α has three more DNA sequences encoding +63Q as well as three nucleotide substitutions compare with previously reported bovine TNF-α sequences (GenBank Accession Number AF348421) (Fig. 2). TNF-α allele has substitutions at positions +340 (A/G), +500 (A/G), and +576 (T/C). These single nucleotide polymorphisms (SNP) caused two amino acid substitutions at positions +114 (M/V) and +167 (K/R). Multiple sequence alignment showed that a new TNF-α allele encodes two different amino acids and one more amino acid compared with previously reported bovine TNF-α (Fig. 3). Interestingly, one of the allele forms of TNF-α in sheep also encodes one more amino acid +63Q [26]. Recently, many studies have examined the relationship between cytokine gene polymorphism, cytokine gene expression in vitro, and the susceptibility to and clinical severity of diseases in human and mouse. Comparative sequence analysis revealed the presence of allele forms of TNF-α in promoter region and/or encoding region in human, mouse, cat, dog, horse and cattle. Although there is increasing evidence that the polymorphism of promoter region causes differential expression of TNF-α and is associated various diseases in human and mouse [7,22], little information is available on the biological significance of allele form of TNF-α. In conclusion, a new allele form of bovine TNF-α was cloned from an expression cDNA library, which contains three more nucleotides and three nucleotide substitutions. Since no information is available on the biological significance of this allele form of TNF-α in cattle, further researches are needed to study on the function of TNF-α allele form in the activation of lymphocytes.

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