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Original Article

In silico analysis of Ta9 gene polymorphism in an Iranian *Theileria annulata* schizont-infected cell line S15 vaccine strain and native isolates

Habibi, G.

Department of Parasite Vaccine Research and Production, Razi Vaccine and Serum Research Institute, Karaj, Iran

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Corresponding Author: g.habibi@rvsri.ac.ir

ABSTRACT

Bovine theileriosis is a tick-borne disease caused by obligate intracellular parasites related to the genus *Theileria*. Cellular immune responses protect cattle against pathogens through the activation of immune cells. Nowadays, live, attenuated vaccine of *Theileria annulata* (*T. annulata*) is being produced in Iran and is recommended for active cattle immunization. Detection of the immunogenic antigens and epitopes recognized by CD8+ T Lymphocytes is vital for the development of recombinant and subunit vaccines. Herein, sequences of the genes encoding Ta9, which is an important antigen recognized by bovine CD8+ T cells specific for *T. annulata,* in Iranian S15 vaccine strains, several Iranian isolates, as well as reference Ta9 DNA sequences registered in GeneBank were compared through polymerase chain reaction (PCR). The obtained data from DNA sequences were analyzed by using "Nucleotide", "Blast n", "BioEdit" and "IEDB" softwares. The results showed high level of variation in nucleotides and amino acids level. The observed polymorphism in Ta9 gene sequences of Iranian vaccine strains and some isolates from Iran demonstrated that this antigen contains polymorphic sequences and is active along with the specific major histocompatibility complex (MHC) of the host. Polymorphic sequences and specific epitopes of Ta9 gene for CD8+ T cell provides an explanation for incomplete protection observed after inoculation of heterologous parasites in vaccinated cattle. These results have important implications for the application of Ta9 antigen for developing novel subunit vaccines.

Keywords: Antigen, Iran, Polymorphism, Ta9, *Theileria annulata*, Vaccine

INTRODUCTION

Bovine theileriosis is a tick-borne disease caused by intracellular protozoan parasite *Theileria annulata* and *T. parva*. Both parasites infect and transform leukocytes, causing acute lymphoproliferative disorders that lead to high rates of mortality and economic losses (Spooner et al., 1989; Morrison, 1996). Although the two parasites infect different leukocyte subsets and are transmitted by a broad spectrum of tick species, the

biology of the host-parasite relationship and the resultant disease processes are essentially similar (Spooner et al., 1989). The genus *Theileria* is a member of obligate intracellular protozoan parasites, there is some evidence regarding the fact that CD8 T cell-mediated immune responses are vital for host cell resistance (McKeever et al., 1994; Morrot and Zavala, 2004). Cattle immunization with live attenuated *T. annulata*-infected cell lines generate strong parasite-specific CD8 T cell responses characteristic for the recipient's parasitized cells, coinciding with the clearance of immunizing infection (Preston et al., 1983; Ahmed et al., 1999; Machugh et al., 2008). The immunized cattle produce strong CD8 T-cell responses specific for schizont-infected cells (Emery et al., 1981; Morrison et al., 1987). Development of various patterns of specific CD8 T-cell responses in immunized cattle of different major histocompatibility complex (MHC) genotypes reveals that there are disparate patterns of parasite strain specificity (Goddeeris et al., 1990; Taracha et al., 1995). Several *Theileia parva* antigens, including Tp1 and Tp2, are recognized in animals of the respective MHC genotypes (Graham et al., 2008). Additionally, it was demonstrated that the target epitopes are polymorphic; therefore, such immunodominance can exert a major influence on the whole parasite strain specificity of the CD8 T-cell response. It was recently reported that cattle carrying the A10 class I MHC haplotype, immunized with the C9 clone of *T. annulata*, generate strong A10 restricted CD8 T cell responses that exhibit parasite strain specificity (Machugh et al., 2008). These findings suggest that variability in CD8 T cell target antigens may facilitate escape from protective immune responses, and thus is critical for vaccination. The bovine theileriosis vaccine was applied with desirable clinical outcomes in 31 provinces of Iran during the past four decades, since it was well-documented that Ta9 is the only antigen eliciting specific CD8+ T cell responses with high variation in nucleotide and amino acid sequences (Machugh et al., 2008; Hayashida et al., 2013; Hansen et al., 2014; Morrison et al., 2015). Therefore, we purported to perform an investigation on the polymorphism of the Ta9 gene in S15 vaccine strain and the available Iranian *T. annulata* isolates in comparison with the reference registered sequences.

MATERIALS AND METHODS

Theileria samples. There were 14 *Theileria* strains and isolates from Iran, including one *Theileria annulata* schizont-infected cell line S15 vaccine strains and isolates from Alborz, Fars, Golestan, Kordestan, Qazvin, and Qom provinces.

Reference Ta9 gene sequences. the Ta9 gene sequences previously registered in international nucleotide sequence databases were employed for comparative analysis of our local sequences. Twelve *Theileria annulata* Ta9 gene sequences were registered in GeneBank as the following accession numbers: HQ875468, HQ875467, HQ875466, HQ875465, HQ875464, HQ875463, HQ875462, HQ875461, HQ875460, HQ875459, HQ875458, and HQ875457.

DNA isolation. genomic DNA was extracted from individual samples using proteinase K method (Sambrook et al., 1989). First, blood was added to red blood cell (RBC) lysis buffer, after centrifugation the cell pellets were re-suspended in cell lysis solution including sodium dodecyl sulfate (SDS) and proteinase K (20 mg/ml) and incubated at 56 ^{o}C for one hour. Thereafter, the proteins were precipitated and genomic DNA was collected in cold pure ethanol, washed in 70% ethanol, dried, and then dissolved in dH_2O (12). DNA concentration was determined by either 0.8 % gel agarose electrophoresis or UV spectrophotometry.

***T. annulata* identification.** the identity of *T. annulata* samples was confirmed using two specific sets of polymerase chain reaction (PCR) primers, firstly, at the *Theileria* genus level using 18S rRNA primers, then species-specific PCR by the Tams-1 gene specific oligonucleotide primers (Habibi, 2012) (Table 1).

Ta9 specific primers. since the registered Ta9 gene sequences in GenBank were highly variable in sequences, the sequences were aligned and a consensus

DNA sequence was derived. Ultimately, the degenerative primers were designed by the appropriate bioinformatic software (BioEdit and Gene Runner; Table 2)

DNA amplification by PCR. to amplify the 18S rRNA gene and Tams-1 gene sequences, PCR was performed on genomic DNA using specific primers. The applied primers are presented in tables 1 and 2. PCR was carried out for amplification of *Theileria* 18S rRNA gene sequence for the specific fragment of 770 bp. In a semi-nested PCR, Tams-1 gene specific primers were applied to amplify the two fragments of 597 bp and 470 bp. The Ta9 degenerative primers were used for amplification of the two fragments of 1190 bp and 819 bp. Each PCR utilized 2 µl of PCR buffer (10X PCR buffer), 15 mM $MgCl_2$, 0.1 mM dNTPs, 1 U of Taq DNA polymerase in a total reaction volume of 20 µl, and 10 pmole of each primer except for degenerative primers 50-100 pmol of which was employed. For amplification, the cycling program started with initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 1 min (at 54 °C for Ta9 and Tams-1 for 1 min and at 58°C for 18S rRNA for 1 min) and at 72 °C for 1 min, with a final extension at 72 °C for 8 min and a final hold at 4°C by Techgene thermal cycler (Techne, Cambridge, United Kingdom). The positive and negative controls were applied in all the reactions.

Gel agarose electrophoresis. the PCR products were electrophoresed on 1.5% agarose gel in Tris-acetate EDTA (TAE) buffer containing 100 bp DNA size marker. They were marked by in-gel staining using RedSafe™ (iNtRON Biotechnology Inc) and visualized by UV Transillumination (Uvidoc, Gel Documentation System, Cambridge, UK).

PCR product sequencing. the amplicons were examined for expected size and for nucleic acid sequencing. The PCR products were purified by PCR Clean-up Kit and sent for direct sequencing in both directions. Ultimately, the prepared products were sent to Source Bioscience Sequencing center (Cambridge, UK) for DNA sequencing.

Molecular data analysis. the obtained data from DNA sequences were analyzed using "Nucleotide" and "Blast n" online software (http://www.ncbi.nlm.nih.gov /nuccore/, http://blas*T*.ncbi.nlm.nih.gov). Afterwards, the level of homology and identity matrix of the provided sequences were analyzed by BioEdit software (Version 7.0.1). The comparison of sequences to achieve a consensus sequence, analysis of the ability of the translated amino acid sequence to activate B and/or T lymphocytes, and MHC affinity were performed by dint of online Immune Epitope Database software (http://www.iedb.org/).

Statistical analysis. The differences in results between the two methods were compared using paired Student's t-test. P-value less than 0.05 was considered statistically significant (Microsoft Office Excel 2007).

RESULTS

The comparison of the translated amino acids of the two proposed epitope regions in the nine Iranian Ta9 gene sequences including S15 vaccine strain and 12 reference Ta9 gene sequences reflected that there is a wide range of diversity in amino acid sequences.

Identity test. All the available samples were confirmed for *Theileria annulata* infection and were subjected to further Ta9 amplification. The following figure shows Tams-1 gene fragment amplification (Figure 1).

Ta9 gene amplification: Ta9 gene fragment was amplified in all the identified *T. annulata* positive samples first by external primers, and then the semi-nested PCR was performed using another internal primer (Figure 2).

DNA sequencing: DNA samples were amplified, purified, and gel-extracted for DNA sequencing. The nine samples revealed suitable results for DNA sequence analysis as follows: T. annulata S15 vaccine strain, two samples from Boein-Zahra (C1 and C2 from Qazvin province), Khezrian isolate from Qazvin province, single isolate from the provinces of Fars, Golestan, Alborz, and Kordestan and a T. lestoquardi isolate. The obtained DNA sequences were between

Table 1. The oligonucleotide primers used for amplifying 18S rRNA gene and Tams-1 gene sequences for identification of *Theileria annulata* at genus and species levels

Target DNA	Primer sequence	GeneBank Acc. No.	Polymerase chain reaction fragment size (bp)
18S rRNA	F2: CAG ATA CCG TCG TAG TCC R2: CCT TGT TAC GAC TTC TCC	EU083801	770 bp
Tams-1	Tms92F: GAGACAAGGAATATTCTGAGTCC Tms92R: TTAAGTGGCATATAATGACTTAAGC	TAU22888	597
	Tms92nF: CGGCACTGGAAAGAAGTACACC		470

Table 2. The degenerative oligonucleotide primers used for amplifying Ta9 gene sequence

Target DNA	Primer sequence	Reference	PCR Fragment size (bp)
Ta9	Ta9F: CATTCTACCTATCCACTTGTATGG Ta9R: TTTBTTKCCAKBKTTTACCAC	Consensus sequence derived from 12 reference sequences	1190
	Ta9nF: CDTATCMACMACAGACTCCAGG Ta9nR: CCTSCTGATGCACCAYCTCC		819

Abbreviations: Y=C or T, M=A or C, K=G or T, S=C or G, B=C or G or T, D=A or G or T

Table 3. Identity matrix of nine Ta9 gene sequences of Iranian strains and isolates

Sequence	BZ C1 Qazvin	BZ C2 Qazvin	Golestan	Khezrian Qazvin	Kordestan	Alborz	S15 Vaccine	*T. lestoquardi*	Fars
BZ C1 Qazvin	ID	0.965	0.625	0.611	0.963	0.695	0.980	0.500	0.537
BZ C2 Qazvin	0.965	ID	0.635	0.596	0.982	0.680	0.953	0.485	0.522
Golestan	0.625	0.635	ID	0.513	0.636	0.606	0.614	0.449	0.452
Khezrian Qazvin	0.611	0.596	0.513	ID	0.601	0.616	0.620	0.516	0.635
Kordestan	0.963	0.982	0.636	0.601	ID	0.687	0.952	0.490	0.527
Alborz	0.695	0.680	0.606	0.616	0.687	ID	0.685	0.496	0.506
S15 Vaccine	0.980	0.953	0.614	0.620	0.952	0.685	ID	0.505	0.543
T. lestoquardi	0.500	0.485	0.449	0.516	0.490	0.496	0.505	ID	0.529
Fars	0.537	0.522	0.452	0.635	0.527	0.506	0.543	0.529	ID

Abbreviations: BZ=Boein-Zahra region in Qazvin provine, C1 and C2 are two strains isolated and characterized from Boein-Zahra region.

514 and 725 nucleotides long. Nine Iranian Ta9 gene sequences were compared using BioEdit software. The results of DNA alignment demonstrated that there are many highly variable regions along the Ta9 gene. These diversities are prominent in illustrated entropy plot (Figure 3). The identity matrix for the nine Iranian Ta9 sequences showed the most similarity among the four samples of S15 vaccine strain, C1 and C2 Boein-Zahra isolates, and Kordestan (group 1), but the remaining samples including Khezrian, Fars, Golestan, Alborz, and T. lestoquardi (group 2) exhibited high level of variation within and between groups.

Comparison of Iranian Ta9 sequences with reference Ta9 gene sequences: Comparison between Ta9 registered Theileria reference sequences and Iranian Ta9 Theileria sequences exhibited high level of sequence variation.

Phylogenetic analysis: the comparative analysis of Iranian strains and isolates by drawing a phylogenetic tree demonstrated that there are high levels of similarity

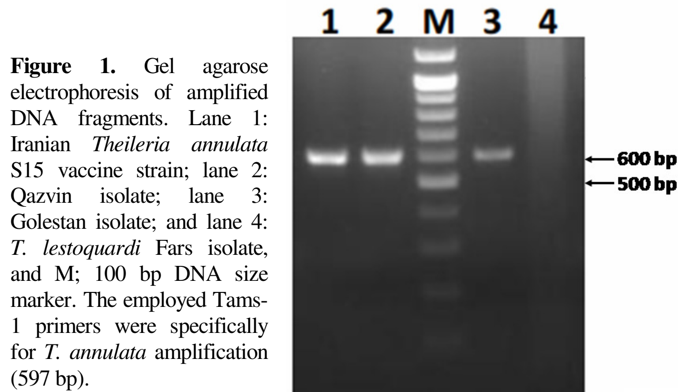

Figure 1. Gel agarose electrophoresis of amplified DNA fragments. Lane 1: Iranian *Theileria annulata* S15 vaccine strain; lane 2: Qazvin isolate; lane 3: Golestan isolate; and lane 4: *T. lestoquardi* Fars isolate, and M; 100 bp DNA size marker. The employed Tams-1 primers were specifically for *T. annulata* amplification (597 bp).

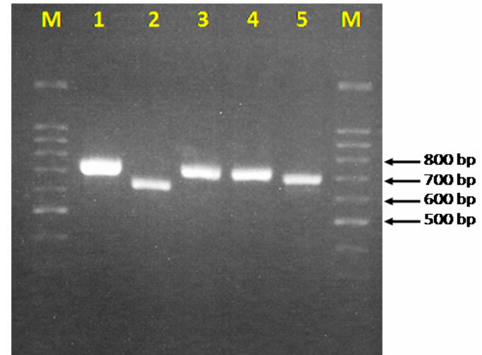

Figure2. Gel agarose electrophoresis of the amplified DNA fragments. Lanes 1 to 5 are the Ta9 gene amplified fragments using Ta9 FnR primers, Iranian S15 vaccine strain, Alborz, three Qazvinn isolates and M, the 100 bp DNA size marker.

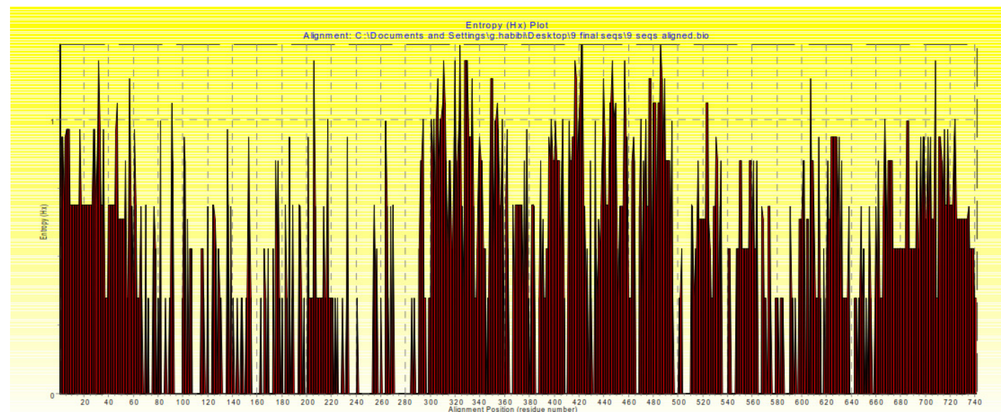

Figure 3. Entropy plot of nine Iranian Ta9 gene aligned sequences. The picks show the level of variation along the compared sequences, as the long picks represent the high degree of variation and vice versa.

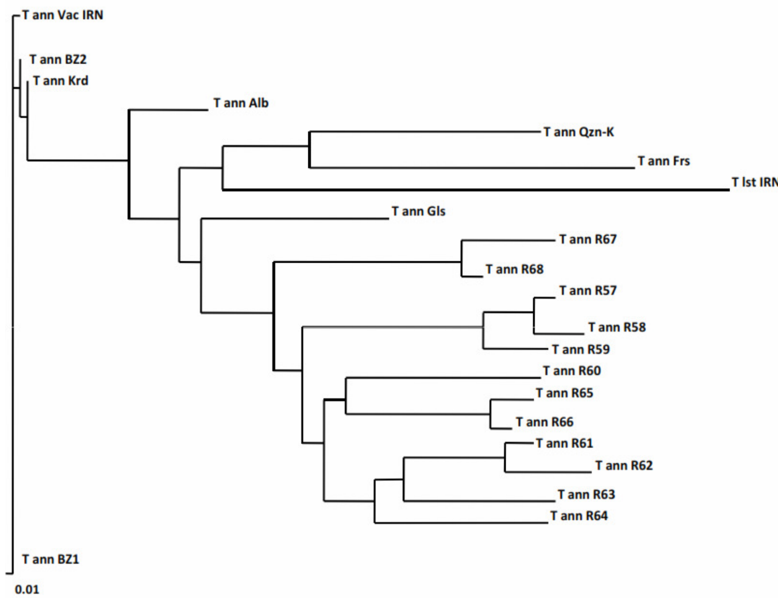

Figure 4. The representation of constructed phylogenetic tree of Ta9 gene sequences. Nine Iranian strains and isolates as well as 12 reference sequences were used for this comparative analysis using BioEdit software. The distance between sequences could be measured by the scale bar at the bottom of the graph. Abbreviation for the samples are: T ann=*Theileria annulata*, Vac IRN=Iranian vaccine strain, BZ1=Boein Zahra strain 1, BZ2=Boein Zahra strain 2, Krd=Kordestan province, Alb=Alborz province, Qzn-K=Qazvin province Khezrian isolate, Frs=Fars province, T lst IRN=*Theileria lestoquardi* Iran strain, Gls=Golestan province, and T ann R57-67=*Theileria annulata* reference sequences registered in GeneBank (for more information see Methods and Materials).

		A10		A14
Reference Ta9 sequences R57-R67		KRSSTFGGPL	GESFSGPYTKKDLD	DKFPGMKSG
		KRSSMFEGGL	GESFVDPYSKEELE	KKFSGMRMG
		QRSPMFGDPL	GSSFSKPYTSEELS	DKFPGLKSG
		QRSPMFEGGL	GXXFSKPYSKEELE	GKFPGLKSG
		QRSPMFGDPL	GSSFSKPYTSEELS	DKFPGLKSG
		QRSPMFGDPL	GSSFSKPYTSEELS	DKFPGLKSG
		KRSSMFEGRL	GESFSGQYTKEDLD	SKFPGLKSG
		KRSSMFEGGL	GGSFVDPYTKEDLS	DKFPGLKSG
		KRSDMFEGGL	GSSFSKPYSKEELE	DKFPGLKSG
		QRSGMFTGPL	GGSFVKPYTKEELS	DKFPGLKSG
		ERSPTFGGPL	GESFSGPYTKKDLD	DKFPGMKSG
		ERSSMFSDPL	GSSFSKPYTKEELS	DKFPGLKSG
Iran Strains and isolates	BZ1	QRSSMFGGPL	GGSFTKPYGKEELE	GKFPGLKSG
	BZ2	QRSSMFGGPL	GGSFTKPYGKEELE	GKFPGLKSG
	Frs	KRSDMFEEGL	GGSFVDPYTKEDLS	NKFPGLKSG
	Gls	ETSGMFTGPL	GGSFVKPYSQEELE	GKFAGMRMG
	Qzn-K	QRSPMFEEGL	GGSFVDPYTKEELD	SKFPKMRMG ← 100% identity for MHC I haplotype A14 CD8 T cell epitope, Iran, Qazvin Khezrian strain
	Krd	QRSSMFGGPL	GGSFTKPYGKEELE	GKFPGLKSG
	Alb	KRSDMFGGPL	GSSFAKPYTKEELD	SKFPGLKSG
	T. lst	ERNPMFDEPL	GSSFVKYYTDDEME	SKFPRLKPR
	Vac S15	QRSSMFGGPL	GGSFTKPYGKEELE	GKFPGLKSG
	C9 Ankara	QRSPMFEGTL	GESFTGGYSKEELE	SKFPKMRMG

Figure 5. The comparison of translated amino acids in two regions of CD8+ T cell epitopes in nine Iranian Ta9 sequences (Iran S15 vaccine strain and eight indigenous isolates), 12 reference sequences, as well as C9 Ankara strain. Two epitopes of A10 and A14 are illustrated at the end row for Ta9 C9 Ankara strain. There are no similarities between C9 epitopes and other strain and isolates except for Khezrian isolate from Qazvin province (Analyzed by BioEdit software).

among four S15 vaccine strains, two Boein-Zahra, and Kordestan isolates, but revealed polymorphism in the remaining isolates and *T. lestoquardi* strains and Ta9 reference sequences (Figure 4).

The results of T cell epitope prediction and MHC binding: the comparison of Iranian Ta9 gene sequences to find a consensus sequence with minimum entropy and further translation for amino acid analysis and evaluation of the obtained sequence for the activation of T- or B- lymphocytes and MHC binding showed that there are two consensus sequences of 242-253 and 271-284. However, the online software of Immune Epitope Database (WWW.iedb.org) did not reveal any antigenic specificity in these regions in all the six translated frames.

Analysis of the derived polypeptide from conserved sequence: all the six translated polypeptides from the conserved sequence were analyzed by the online IEDB software revealed no specific epitope for T/B lymphocytes in cattle according to the available data, and there was no specific binding site for bovine MHC class I (www.iedb.org).

Comparison of two proposed epitopes: comparison of the translated amino acids of the two proposed epitope regions in nine Iranian Ta9 gene sequences and 12 reference Ta9 gene sequences reflected that there is a wide range of diversity in amino acid sequences, as well. The epitope analysis of the corresponding amino acid sequences in all the available data exhibited no epitopes for lymphocyte activation and/or MHC binding except for A14 epitope on isolate of Khezrian from Qazvin province that showed 100% similarity to C9 (Ankara strain; Figure 5).

DISCUSSION

Bovine theileriosis is economically important to cattle farming industry causing severe and often lethal diseases in imported, high-grade cattle and crossbreeds and might lead to morbidity and loss of productivity in the indigenous cattle.

Theileriosis is generally managed by the use of acaricides, but this method is expensive, environmentally hazardous, and can lead to development of tick resistance. Cattle are immunized against tropical theileriosis by means of live, attenuated vaccines, which are produced by prolonged passage of the schizont-infected cells. The main effector cells are considered to be cytotoxic CD8+ T cells and possibly natural killer (NK) cells. However, the nature of the protective schizont target antigens remains unknown.

Immunization of cattle with a specific *Theileria* strain provides efficient long-lasting immunity against the homologous strain, but unpredictable protection against the heterologous strains (Radley et al., 1975; Taracha et al., 1995). In *Theileria parva* infection, variation among animals in the strain specificity of CD8+ T cell responses was shown to be dependent on incomplete cross-protection between parasite strains (Taracha et al., 1995). One of the three identified *T. annulata* CD8+ T cell antigens is the highly dominant target for A10-restricted response. In addition, it was demonstrated that extensive polymorphism of this antigen results in differential recognition of isolates by CD8+ T cells and demonstrated evidence that the T cell epitopes tolerated positive selection for sequence diversity (MacHugh et al., 2011). MacHugh et al. (2011) noted that CD8+ T cells from animals of diverse MHC genotypes tended to recognize different antigens, suggesting differences in the dominance of antigens depending on the presenting MHCs. All the A10-restricted CD8+ T cell clones obtained from the immunized animals were found to be specific for a single epitope in Ta9 antigen, demonstrating that Ta9 is a highly dominant antigen in the context of this MHC haplotype. The failure to detect Ta9 by CD8+ T cell lines restricted by three other MHC class I haplotypes (A15, A18, and A31) confirms that dominance of this antigen is dependent on the host MHC genotype (MacHugh et al., 2011). The sequence analysis of the Ta9 field isolates demonstrated vast diversity along the Ta9 gene sequence, leading to amino acid polymorphism in both A10 and A14-restricted epitopes (MacHugh et al., 2011). The production of effector responses against polymorphic antigens is the prime purpose of vaccination. The live, attenuated bovine theileriosis vaccine tolerates the prolonged passage of *T. annulata*-infected cell lines, which is required to achieve attenuation of live vaccines and results in selection of clonal or near-clonal populations in the cell lines employed for vaccination (Darghouth et al., 1996; Sutherland et al., 1996). Thus, in the Iranian S15 vaccine cell line sub-cultured for over 260 passages, the diversity might be lost and the cell line can be almost entirely colonized. The Ta9 antigen was identified as the only protective and immunogenic *Theileria* antigen for specific CD8+ T lymphocyte response so far, but the effective mechanism of the live vaccine in endemic areas remains to be clarified. Although poor protection against heterologous parasite challenge was reported, compared to homologous challenge, there are some reports for generation of high specific immune responses against polymorphic antigens by schizont infected cell line vaccines (Pipano, 1989; Darghouth et al., 1996). While the immune responses to Ta9 epitopes include MHC class I restricted CD8+ T cell response and extreme degree of Ta9 polymorphism, application of such antigens for the development of a subunit vaccine would present an important challenge. However, analysis of the Iranian strains and isolates is of great importance, particularly for S15 vaccine strain. The obtained results in the above-mentioned nine samples showed that despite the high level (90%) sequence identity for four Ta9 sequences of Iranian vaccine strains, two Boein-Zahra isolates, and one Kordestan isolate, and most strains and isolates from Iran and other countries registered in GenBank showed high level of sequence variation in the nucleotide and amino acids. The results of phylogenetic analysis in the depicted tree also confirmed the close relationship between the four Iranian isolates, but high level of variation among other samples and reference sequences (Figure 4). Nevertheless, an interesting findingwa the expression of A14 epitope sequence of Ta9 gene sequenced in Qazvin isolate. This finding is the reason for MHC-restricted CD8+ T cell response. Our results are congruent with those of MacHugh et al. (2011) demonstrating that there is high level of sequence polymorphism in Ta9 gene from parasites isolated from naturally infected cattle with 12 distinct alleles. Sequence diversity in the defined epitopes indicate differential recognition of the allelic variants by specific CD8 T cells as referred to MHC-restricted cytotoxicity; therefore, some variants can escape from T cell cytotoxicity response induced against the C9

parasite genotype. MacHugh et al. (2011) reported that some specific CD8+ T cell clones can tolerate up to three amino acid exchanges within the epitope sequence, while others fail to recognize a variant epitope with a single substitution. In each case, the level of killing of the cells expressing the variant epitopes was similar to those obtained with the reference C9 epitope. This signifies that the exact epitope sequence might not be essentially required for CD8+ T cell recognition. These findings indicate that at least those allelic variants that are recognized by some of the T cell clones maintain the ability to bind to the N*00201 class I MHC, and the interesting finding is that allelic polymorphism by itself is not necessarily sufficient to impede CD8+ T cell recognition (MacHugh et al., 2011). These outcomes reveal that the overall strain specificity of the CD8+ T cell response due to Ta9 epitope can vary among animals. CD8+ T cell response to the combined cross-reactive T cells by immunization with simultaneous constructs that express two or more alleles of Ta9 is possible. The results of the current and former studies are important for understanding cross-protection between parasite strains and for vaccine development. In spite of the colonized, attenuated *T. annulata*-infected cell line used for vaccination, animals are exposed to heterologous parasites in the field. Accordingly, more antigenic specificity for the CD8+ T cells are available for animals in the field that act as a natural challenge to complete specific immunity against heterologous parasites (Darghouth et al., 1996; Weir et al., 2007). Superantigens (SAg) were suggested to play a critical role in pathogenesis of several diseases, including tropical theileriosis. SAg interaction with T-lymphocytes may lead to inflammation, cytotoxicity, and removal of T-cells and autoimmunity. This antigenic property of *T. annulata* might account for optimum immunity coverage and a possible explanation for Iranian vaccine strain efficacy in the field. However, based on the above-mentioned results, *Theileria annulata* SAg might act as a powerful and non-specific antigen for inducing immune system, thereby, the colonized infected *Theileria annulata* cell line in the Iranian vaccine strains could be sufficient for inducing protective immunity against *T. annulata* heterologous strains (Campbell et al., 1997; Jappe, 2000). Further studies are required to estimate the antigenic specificity of CD8+ T lymphocytes against *T. annulata* in cattle of various MHC genotypes to evaluate the effect of antigens and polymorphism and to determine the potential of the employed antigens inducing immunity in vaccinated animals.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

None declared.

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