A Polymorphism Observed in the Experimentally Successful Peptide Vaccine Sequence Derived from *Theileria sergenti* Piroplasm Major Surface Antigen (p33)

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ABSTRACT. A polymorphism in the experimentally successful peptide vaccine sequence (EVVWKEKKEVKDLDA, amino acids 134–148) derived from the 33 kDa piroplasm major surface antigen (p33) of *Theileria sergenti* was examined. The vaccine sequences obtained by PCR amplification and sequencing of the p33 gene from a total of 15 parasite-infected cattle blood samples collected from 4 prefectures through Hokkaido to Kumamoto revealed the two major sequences (Ikeda and Chitose stock types) either of which was identified in all samples. Since the peptide vaccine develops the parasite species- or stock-specific immunity in the animals, an application of the two major peptide sequences as cocktailed vaccine should be evaluated for a practical use of this strategy to controlling *T. sergenti* infection in Japan. — KEY WORDS: p33, *Theileria sergenti*, vaccine.

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Theileria sergenti is a tick-transmitted protozoan parasite that frequently causes severe anemia due to the presence of intraerythrocytic piroplasms in cattle in Japan [1, 9]. The current available approaches that mainly consist of tick control using acaricides and the treatment of the infected animals with 8-aminoquinoline compounds [2] do not always result in an effective control of the disease. Since *T. sergenti* infection in cattle continually occurs throughout the country, improved strategies for the disease control, including an effective vaccine are required.

We and other have recently reported the potential use of synthetic peptide as a vaccine for reduction of the severe consequences of the experimentally induced parasite infection in cattle [5, 11]. The peptide contained putative erythrocyte-binding motif (Lys-Glu-Lys) of *Plasmodium falciparum* [10], was derived from the 33 kDa piroplasm major surface antigen (p33) of *T. sergenti* Ikeda or Chitose stock [3, 8]. However, the immunity introduced by the vaccination was the parasite species- or stock-specific [5, 11] and this drawback has obstructed a practical application of the vaccine for Japanese pastures, in which high prevalence of mixed parasite population with the different peptide vaccine sequence was expected [6, 8, 12].

In the present report, we surveyed a variation of the peptide vaccine sequence among *T. sergenti* field isolates originated from several pastures in Japan. Based on the results obtained, we proposed a potential combination of the peptide sequences to be cocktailed for a practical application of this experimentally successful synthetic peptide vaccine strategy.

A total of 15 blood samples from *T. sergenti*-infected cattle were collected from 7 pastures in Japan, i.e., 9 samples from 4 pastures in Hokkaido (2–3 samples from each pasture) and 2 samples each from a pasture in Tochigi, Tottori and Kumamoto prefecture, respectively. During collection of blood, ethylenediaminetetraacetic acid (EDTA) was added to the sample to prevent coagulation. The parasitemia of intraerythrocytic piroplasm recorded for the samples were < 1.0–8.8%. The template DNA preparation and PCR amplification of the template DNA were performed under the protocol and the conditions as described previously [4]. The primers used were : 5'-CCA CAG ACT GAA GCA TG-3'; and 5'-ACC TTT CCG GTA CCG AA-3' to amplify the middle part (nt 276–500) of the p33 gene (852 bp) of *T. sergenti* [3, 8]. The PCR product of sizes 225 bp was purified from amplification reaction using QIAquick™ PCR purification Kit (QIAGEN GmbH, Hilden, Germany). A part (2 μl) of each purified product was subjected to one-step cloning with plasmid vector utilizing the TA Cloning® system (Invitrogen Corporation, CA., U.S.A.). A total of 52 clones were randomly selected from 15 cloning experiments, comprising 1–5 clones from each experiment. The inserted DNA of 225 bp in the plasmid clones were sequenced on both strands with Dye Primer Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Japan, Chiba, Japan). The DNA sequence obtained was translated into 74 amino acids sequence encompassed in the central region (amino acids 93–166) of p33 (283 amino acids). The sequence included the 15 residues (amino acids 134–148) that was used as the peptide vaccine sequence [5]. The amino acid sequences predicted were aligned for comparison using genetic information processing program (GENETIX; Software Development Co., Ltd., Japan).

As shown in Fig. 1, the predicted amino acid sequences from the 52 DNA sequences could be fallen into 11 variants. They were differentiated each other by single to maximum of 16 amino acid substitutions that were taken place for a total of 26 residues in the 74 amino acids sequence. Although such polymorphism was observed among those amino acid sequences, almost all substitutions found (23 out of the 26 substitutions) were of that made between similar amino acids. This may imply structural or functional

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TSI  a)  93:HRLKHADDLFFKLNLSHAKPLLFKKKTDKDWVQFSFAQYLDEVVWKEKKEVKDLDASKFA 152
TSC  b)  93:HRLKHAEDLFLKLDLSHAKPLLFKKKSDKEWVQFSFAQYLDEVLWKEKKESKDLDASKFA 152
H 53 c)  93:HRLKHAEDLFLKLDLSHAKPLLFKKKSDKEWVQFSFAQYLDEVLWKEKKESKDLDASKFA 152
H104     93:HRLKHADDLFLKLNLSHAKPLLFKKKTDKDWVQFSFAQYLDEVVWKEKKEVKDLDASKFA 152
H105     93:HRLKHADDLFFKPNLSHAKPLLFKKKTDKDWVQFSFAQYLDEVVWKEKKEVKDLDASKFA 152
H192     93:HRLKHAEDLFLKLDLSQAKPLLFKKKSDKEWVQFSFAQYLDEVLWKEKKESKDLDASKFA 152
C 34 d)  93:HRLKHGDALFFKLDLSHAKPLLFKKKTDKDWVQFNYGQYLEEVLWKDKKELKDLDASKFT 152
C 35     93:HRLKHADDLFFKLNLSHAKPLLFKKKTDKDWVQSSFAQYLDEVVWKEKKEVKDLDASKFA 152
T 23     93:HRLKHAEDLFLELDLSHAKPLLFKKKSDKEWVQFSFAQYLDEVLWKEKKESKDLDASKFA 152
K 43     93:HRLKHAEDLFLKLDLSHAKPLLFKKKSDKEWVQFSFAQYLDEVLWKEKKESKDLDASKFA 152
K 52     93:HRLKHAEDLFLKLDLSHAKPLLLKKKSDKEWVQFSFAQYLDEVLWKEKKESKDLDASKFA 152
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TSI     153:DAGLFAAEAFGTGK (11) e)                                        166
TSC     153:EAGLFAPDAFGTGK (28)                                          166
H 53    153:EAGLFTPDAFGTGK ( 2)                                          166
H104    153:DAGLFAAEAFGTGK ( 2)                                          166
H105    153:DAGLFAAEAFGTGK ( 1)                                          166
H192    153:EAGLFAPDAFGTGK ( 1)                                          166
C 34    153:EAGLFAADSFGTGK ( 2)                                          166
C 35    153:DAGLFAAEAFGTGK ( 1)                                          166
T 23    153:EAGLFAPDAFGTGK ( 1)                                          166
K 43    153:EVGLFAPDAFGTGK ( 2)                                          166
K 52    153:EVGLFAPDAFGTGK ( 1)                                          166
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Fig. 1. Polymorphism observed in the central region (amino acids 93–166) and the peptide vaccine sequence (amino acids 134–148) of *Theileria sergenti* piroplasm major surface antigen (p33). The asterisks or the dots under the sequence alignment denote identical or similar amino acids, respectively.; a) *T. sergenti* Ikeda stock (TSI) type [3].; b) *T. sergenti* Chitose stock (TSC) type [8]. Solid underlines mark the peptide vaccine sequences that are identical to the TSC.; c) (H)okkaido, To(C)higi, (T)ottori or (K)umamoto indicates the prefecture from which the sequence variant was originated.; d) The variant with a particular peptide vaccine sequence (see the text). Broken underline marks the vaccine sequence which differs from both of TSI and TSC.; e) Number of the sequence identified.

conservation of the central region (amino acids 93–166) of the p33 among the compared *T. sergenti* field isolates.

Interestingly, 9 out of 15 blood samples tested beard more than two variants for the central region of p33 (data not shown), indicates high prevalence of a mixed parasite population [6, 8, 12] in *T. sergenti*-infected animals.

On the other hand, the polymorphism observed for the peptide vaccine sequences (amino acids 134–148) among the tested clones was lower, as compared with that observed for the central region (amino acids 93–166) of p33. The peptide vaccine sequences found were classified into 3 variants including Ikeda, Chitose and Tochigi C 34 (Fig. 1). These three sequences contained either of the putative erythrocyte-binding motif [10], Lys-Glu-Lys (amino acids 138–140) or Lys-Glu-Leu (amino acids 141–143). Among the 52 clones examined, Ikeda or Chitose type of the peptide vaccine sequence was conserved in 15 clones (29%) or 35 clones (67%), respectively. Other two clones with Tochigi C 34 type of the peptide vaccine sequence were each derived from two separate blood samples, in both cases together with clones retaining Ikeda type of the vaccine sequence (data not shown). These results indicates the fact that all animals examined were infected with the parasites which were associated with either Ikeda or Chitose type of the peptide vaccine sequence.

Practical application of the synthetic peptide vaccine for *T. sergenti* infection has been hampered by two major obstacles, i.e., species or stock specificity of the immunity developed by the synthetic peptide sequence, and employment of Freund's complete adjuvant as an immune potentiator to achieve a reliable host-protective immune response with the peptide [5, 11]. The results obtained in this study may pave the way to circumvent the first drawback, by cocktailing Ikeda and Chitose types of the vaccine sequences. An application of the two major peptide sequences as cocktailed vaccine should be evaluated against the simultaneous, experimentally induced infection of cattle with *T. sergenti* Ikeda and Chitose stocks. In addition, further investigations, especially a research endeavors to develop an acceptable adjuvant for the field application should be conducted. Imperative investigations for such immune potentiator including cytokines [7] which enables firm and stable immunity against the synthetic peptide will facilitate an establishment of practical application of the synthetic peptide vaccine as an alternative way to controlling *T. sergenti* infection in cattle.

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