ORIENTIA TSUTSUGAMUSHI TRUNCATED RECOMBINANT OUTER MEMBRANE PROTEIN (r47) AND (r56) VACCINES DIAGNOSTICS AND THERAPEUTICS FOR SCRUB TYPHUS AND HIV INFECTION

FIELD OF THE INVENTION

The present invention relates to a 47kDA and a 56kDA surface protein antigen of Orientia tsutsugamushi which are useful as diagnostic antigens as well as immunizing agents. The invention further relates to a therapeutic method of reducing the HIV viral load in a patient.

DESCRIPTION OF THE PRIOR ART

Scrub typhus infection is caused by the obligated intracellular, gram-negative bacterium O. tsutsugamushi. It accounts for up to 23% of all febrile episodes in areas of the Asia-Pacific region where scrub typhus is endemic and can cause up to a third of mortality if left untreated. Symptoms may include pneumonitis, meningitis, rash, and headache. Differentiating scrub typhus from other acute febrile illness, such as leptospirosis, murine typhus, malaria, can be difficult because of the similarities in signs and symptoms. Sera from 95-99% of patients with scrub typhus recognize a 56-kDa protein of O. tsutsugamushi which comprises 10-15% of the total rickettsial cellular protein content. This 56-kDa protein has both conserved

and unique sequences among different strains, has been developed and used for early diagnosis and a potential vaccine candidate.

Whole-organism vaccines have been previously developed and their protections have been short-lived and lack of cross strain protection. The major surface protein antigen, the variable 56-kDa protein which account for the antigenic variation, has been shown to induce protective immunity against the homologous strain but not the heterologous strains. The fact that other antigens, such as 110, 47 and 22 kDa have also been identified with high seroreactivity suggests that a combination of several of these antigens may provide better protection against various stains of O. tsutsugamushi infection. Recently, it has been demonstrated that the infusion of plasma from scrub typhus infected patients into HIV infected patients led to the decrease of HIV virus load burden determined 2 months after the initial infusion, suggesting the pharmaceutical effect of the scrub typhus patients plasma.

SUMMARY OF THE INVENTION

In this report, we have demonstrated the cloning of 47-kDa protein gene, NCBI accession number L31934.1, and the attempt to obtain pure r47, in an effort leading to the production of sufficient quantity and high purity recombinant 47-kDa protein to use in combination with r56 protein as a vaccine candidate to afford broader protection. In addition, the sequence search of this outer membrane protein showed similarity with serine

proteases, suggesting r47 protein itself may function as a protease.

Western blot results revealed that plasma from scrub typhus patients showed strong antigenic response to the r47 protein, suggesting a potential protective role of r47 against HIV infection. It is possible that this inhibitory effect was due to the antibody response toward r47 protein. Alternatively, the homology between serine protease and r47 protein indicating a potential protease activity associated with r47 which may result in interference with HIV processing.

A search of the NCBI protein database for short, nearly exact matches revealed a 10-amino-acid match (8 identical and 2 conserved) between the O. tsutsugamushi Karp strain 47kDa antigen (accession number gi/1220501) 47 kDa and HIV envelope protein (accession number gi/2250974) HIVgp120.

Pattern: TLR+IVTN+K

Accordingly, the present inventive subject matter is directed to a scrub typhus immunogenic composition comprising an r47 truncated protein of SEQ ID No.1, an r56 truncated protein selected from the group consisting of SEQ ID No. 2, SEQ ID No. 3, and SEQ ID No. 4 and a pharmaceutically acceptable carrier.

The present inventive subject matter is further still directed to a method of treating a patient suffering from scrub typhus, the method comprising administering a pharmaceutically effective dose of antibodies raised against the combination of the

truncated r47 protein of SEQ ID No. 1 and the truncated r56 protein selected from the group consisting of SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 4 and a pharmaceutically acceptable carrier. Alternatively, the method may be comprised of the administration of antibodies raised against either the truncated r47 or the truncated r56 protein.

The present inventive subject matter further contemplates a method for immunizing a subject against scrub typhus comprising administering a pharmaceutically effective amount of a scrub typhus vaccine.

The present inventive subject matter also contemplates an assay for detecting antibodies to scrub typhus comprising obtaining a sample from a subject, exposing the sample to r47 protein as a complement to an r56 assay in assay equipment selected from the group consisting of ELISA plates, dot-blot matrices, and hand held chromatographic and flow through assay devices.

The present inventive subject matter further contemplates a treatment of an HIV patient which comprises a truncated r47 protein of SEQ ID No.1 and a pharmaceutically acceptable carrier which results in a reduction of the HIV viral load of an HIV patient.

The present inventive subject also contemplates a treatment of an HIV patient which comprises an r56 protein selected from the group of SEQ ID No. 2, SEQ ID No. 3, SEQ and SEQ ID No. 4.

The present inventive subject matter further contemplates an HIV vaccine comprising a potential HIV vaccine and a truncated r47 or r56 protein or protein combinations thereof.

The foregoing and other features and advantages will become further apparent from the following detailed description of the presently preferred embodiments, when read in conjunction with the accompanying examples and made with reference to the accompanying drawings. It should be understood that the detailed description and examples are illustrative rather than limitative, the scope of the present invention being defined by the appended claims and equivalents thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is an SDS-PAGE showing the PCR product of DNA coding for the truncated 47-kDa protein and digested plasmids;

Fig. 2 is An SDS-PAGE showing the induction of r47 protein with IPTG and fractionation of r47 from crude extract;

Fig. 3 is an SDS-PAGE showing fractionation of r47 protein on DEAE anion exchange column (pH 8.0);

Fig. 4 is an SDS-PAGE showing fractionation of r47 protein DEAE anion exchange column (pH 7.0);

Fig. 5 is a Blast Search of r47 protein sequence; and

Fig. 6 is a chart comparing conserved domains among r47 and serine proteases.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Scrub typhus of tsutsugamushi disease is an acute, febrile disease caused by infection with Orientia tsutsugamushi. O. tsutsugamushi is a gram-negative bacterium but has neither lipopolysaccharide nor a peptidoglycan layer. Orientia isolates are highly variable in their antigenic properties. The major surface protein of O. tsutsugamushi is the variable 56-kDA protein. Serotype-specific monoclonal antibodies to Orientia react with the 56-kDA protein. Although sera from most patients with scrub typhus recognize this protein, another 47-kDA protein is also recognized, suggesting that both 56-kDA and 47-kDA proteins are good candidates for use as diagnostic antigens and that the combination of these two proteins has potential use as an immunogenic composition.

In accordance with the present invention, there is generally provided a scrub typhus immunogenic composition comprising an r47 truncated protein of SEQ ID No.1, an r56 truncated protein; and a pharmaceutically acceptable carrier.

The 47 kDa antigen is a membrane protein. In order to produce a properly folded r47 protein in aqueous solution, its n-terminus which contains the hydrophobic domain was truncated. Forward primer SEQ ID NO. 5 and reverse primer, SEQ ID NO. 6, were designed to amplify the gene segment of nucleotides 174-1481, SEQ ID NO. 7. The amplified gene segment was cloned into pET24a vector. The expressed protein sequence is in SEQ ID NO.1.

In embodiments of the inventive subject matter, the scrub typhus immunogenic composition may comprise the r47 truncated protein in combination with one of the following r56 truncated proteins: SEQ ID No. 2, SEQ ID No. 3, or SEQ ID No. 4 and a pharmaceutically acceptable carrier.

DOSING AND ADMINISTRATION

The optimum dose of the immunogenic composition is contemplated to be .5-20 mg for humans and .5-2.5 mg for primates. Administration of the immunogenic composition is done subcutanously.

The inventive subject matter also provides an assay for detecting antibody to scrub typhus comprising obtaining a sample from a subject, exposing the sample to a truncated r47 protein as represented in SEQ ID No. 1 in assay equipment. Such assay equipment may be selected from the group consisting of ELISA plates, dot-blot matrices, and hand held chromatographic and flow through assay devices. Samples suitable for such assay methods include blood, oral fluids and urine from which antibodies could be detected and from which antigens could be detected by way of laboratory generated antibodies.

The inventive subject matter further comprise a method of treating a patient suffering from scrub typhus, the method comprising administering a pharmaceutically effective dose of antibodies raised against the combination of truncated proteins of SEQ ID No. 1 and the truncated r56 protein selected from the group

consisting of SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 4 and a pharmaceutically acceptable carrier.

The inventive subject matter also contemplates a pharmaceutical composition for treatment of an HIV patient comprising antibodies raised against a truncated r56 protein and a pharmaceutically acceptable carrier wherein administration of the composition reduces the patients viral load. Such truncated proteins may be selected from the group consisting of SEQ ID Nos. 2-4 or combinations thereof. The dosing and administration are the same as stated above for the immunogenic compositions.

The present inventive subject matter further contemplates a treatment of an HIV patient which comprises antibodies raised against a truncated r47 protein selected from the group of SEQ ID No.1 and a pharmaceutically acceptable carrier which results in a reduction of the HIV viral load of an HIV patient. Dosing and administration are also as stated above for this embodiment.

The inventive subject matter also contemplates treatment of an HIV patient which comprises administering a pharmaceutical composition comprising antibodies raised against a combination of an r47 truncated protein and a truncated r56 protein and a pharmaceutically acceptable carrier to reduce the patients HIV viral load. Dosing and administration are also as stated above for this embodiment.

The inventive subject matter also contemplates an immunogenic composition against HIV infection comprising a pharmaceutical

effective dose of antibodies raised against a truncated r47 protein and a truncated r56 protein of SEQ ID Nos. 2-4 and combinations thereof. Dosing and administration are as stated above for this embodiment.

The following examples are illustrative of preferred embodiments of the invention and are not to be construed as limiting the invention thereto.

EXAMPLES

EXAMPLE 1

Forward and reverse DNA primers (SEQ ID No. 5-6, respectively) specific for 47-kDa (accession no. L31934.1) were designed with BamH1 and NcoI (both from NEB) restriction sites and used for PCR to amplify the gene coding for a truncated 47-kDa protein using DNA purified from karp strain of O. tsutsugamushi. The PCR product (insert) was ligated to plasmid pET24d (Novagen) digested with BamH1 and NcoI. The sequence of insert was confirmed and the ligated plasmid was transformed into BL21 (DE3) cells (Invitrogen). Colony containing ligated plasmid was grown 2YT medium and induced with 1mM of IPTG at 37°C for 3 h for protein expression. To purify this expressed protein, 600 ml of Bl21(DE3) cells were grown, induced, centrifuged and cell pellet was lysed with 20 mM Tris-HCL(pH 8.0) containing 5 mM EDTA and 1 mM PMSF according to the method developed by Ching et al., Clin. Diag. Lab. Immuno. **5**, 519-526, 1998. After ultrasonic disruption and centrifugation, the expressed protein was mainly associated with

supernatant rather than inclusion body. This fraction was further purified with a 30,000 MW cutoff membrane follow by DEAE anion exchange using 20 mM Tris pH 8.0 and 2M NaCl salt gradient (Agilent 1100 HPLC). Alternatively, the protein was separated on a DEAE anion exchange using 20 mM Tris pH 7.0 and 2M NaCl salt gradient. The protein eluted with salt gradient was collected, analyzed with SDS-PAGE, and stained with coomassie blue to identify the purity of each fraction. In some cases, different fractions were poured together for the SDS-PAGE analysis.

The sequence homology of 47-kDa protein with other known proteins was searched with standard BLAST method and the conserved domain analysis of 47-kDa protein was analyzed using NCBI CDD algorithm. (Figs. 5-6) In addition, proteins with similar domains as 47-kDa protein were aligned together to analyze the conserved amino acid sequence within the family.

RESULTS

The DNA coding for 47-kDa protein was successfully PCR amplified using DNA purified from O. tsutsugamushi karp strain as template and primers designed specifically for this genes (Fig. 1). The cloning of this PCR product into Pet24d(+) plasmid was confirmed after digestion with BamH1 and NcoI (Fig. 1) and DNA sequence was verified with a DNA sequencer.

Expression of the 47-kDa protein was observed after 3 h of 1mM IPTG induction at 37°C (Fig. 2). The sequence of this

expressed protein was confirmed with protein sequencer. (After sonication and centrifugation, the expressed protein was mainly associated with supernatant after the first centrifugation (Fig. 2) rather than associated with inclusion body as the r56 protein reported by Ching et al. The expressed protein may be associated with the membrane fraction and the addition of detergent (1% of NP40) appears to increase the yield during purification procedure (data not shown). Part of the supernatant containing r47 was first purified with DEAE anion exchange on a HPLC using a Tris buffer at pH 8.0 and protein was eluted with a NaCl gradient (up to 0.8 mM) and the fractions were collected and analyzed with SDS-PAGE (Fig. 3). The other part of the supernatant was purified with HPLC using a Tris buffer at pH 7.0 and eluted with a NaCl gradient (up to 2 M). The resulted fractions were poured and analyzed for r47 (Fig. 4).

The search of similarity of r47 with standard BLAST resulted in homologues with various serine proteases (Fig. 5). When r47 was searched against protein conserved domain database maintained by NCBI, a trypsin-like (77.7% of the conserved domain region (217 residues) was aligned) and PDZ domain (73.3% of the conserved domain region (86 residues) was aligned) were identified. These two domains were also associate with different serine proteases (Fig. 6).

EXAMPLE 2

ASSESSMENT OF HOMOLOGOUS PROTECTION INDUCED BY RECOMBINANT 56 kDa PROTEIN FROM ORIENTIA TSUTSUGAMUSHI IN COMBINATION WITH 47 kDa SCRUB TYPHUS ANTIGENS

In order to assess the protective effect of r56 and r47 proteins against infection, mice will receive varying doses of r56 and r47 protein components from the Karp strain. Since the immunization with 25 ug or 2 ug of r56 in mice showed a protective role in our previous experiments (Annual Report J2_00_NMRC), we will use the same doses for testing r56 in the present study. For the r47 proteins, we do not know the optimum doses, but we will start with two dosages of 5 ug and 25 ug at this time. Alum and CpG will be used as adjuvants at the ratio of protein: Al +3: CpG =1: 25: 10. Alum has been approved for human use for a long time. Vaccines prepared by antigens adsorbed onto alhydrogel have several advantages. The antigenic content is easier to standardize and the vaccine has good stability. Alhydogel adsorbed antigens are simple and more convenient to use. In addition, the degree of local reaction following immunization is reduced. CpG induces a Th1-like pattern of cytokine production dominated by IL-12 and IFN-gamma with little secretion of Th2 cytokines.

Female CD1 mice, 4 to 5 weeks old, 13 to 15 grams in weight, will be purchased from Charles River Laboratories (Wilmington, MA.). Each Study includes 5-7 groups of mice. A minimum of 15-20 mice is needed to down select the 20% difference in protective efficacy at

80-85% confidence level. Each group of 20 mice will be immunized i.m. with the 56 kDa antigen alone or in combination with r47 antigen except for the negative control groups. 15 mice will be challenged 4 weeks later and 5 mice will be sacrificed for measuring T-cell immune responses right before challenge.

Detailed experimental designs:

Study 1(5 groups):

1. Negative control: PBS only
2. 25 ug of r56 only
3. 25 ug of 47 kDa only

Study 2 (7 groups):

1. Negative controls: the same as the group 1 in Study 1
2. 25 ug of r56, plus 5 ug of 47 kDa
3. 25 ug of r56, plus 25 ug of 47 kDa

Study 3 (5 groups):

1. Negative control: PBS only
2. 2 ug of r56 only
4. 3. 4.2 ug of 47 kDa only

Study 4 (7 groups):

1. Negative controls: the same as the group 1 in Study 1
2. 2 ug of r56, plus 5 ug of 47 kDa
3. 2 ug of r56, plus 2 ug of 47 kDa

3) Monitoring the Immune Response:

(a) Antibody Detection: Mouse sera will be drawn immediately before immunization and before challenge. Each serum sample will be assayed in duplicate by enzyme-linked immunosorbent assay (ELISA) for antibodies against each antigen. Anti-mouse IgG and IgM peroxidase-labeled conjugate (Boehringer-Mannheim,

indianapolis, IN) will be tested.

(b) Cytokine detection in serum: Cytokines have been studied physiologically and pathologically with a focus on the roles in immune regulation during inflammation. IFN gamma and IL-4 are cytokines for Th1 and Th2 cells, respectively. For many intracellular infections, IL-12 may induce Th1 response, i.e. IFN-gamma production. The levels of IFN gamma and IL-2 were elevated in spleen mononuclear cells from 56 kDa immunized mice. Increased IFN was also detected in spleen cells and lymph nodes cells from mice chronically infected with Orientia. It was noted that Orientia was susceptible to INF gamma in cultured mouse cells. In the sera of patients with scrub typhus infection, the levels of G-CSF, M-CSF, IFN gamma, and TNF alpha were up regulated. Mice blood will be drawn in heparin before immunization, 2, 4, 6, 8,10, 15,and 30 days after immunization. Sera samples will be assayed in duplicate for cytokine levels of IFN gamma, TNF alpha, IL-4, IL-12, G-CSF,and M-CSF.

(c) Cytokine production by antigen specific T-cells: Spleen mononuclear cells (SMNC), stimulated by recombinant antigens, will be monitored for the productions of IFN gamma and IL-2 at day 1, 2, and 3 post stimulation. The cells will be isolated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. After washing, the cells will be resuspended to a concentration of 5x 10E6 per ml in warm RPMI-10, and 100 ul will be distributed in each well of a 96-well plate as described. Following incubation with corresponding recombinant antigens, culture supernatants will be collected and used in cytokine assays. The supernatants from triplicate SMNC cultures will be pooled, and all samples will be assayed in triplicate. IL-2 activity in the supernatants from antigen-stimulated SMNC will be tested for its ability to support growth of IL-2-dependent CTLL-2

cells in the presence of anti-IL-4 antibody (11B11; 10 ug/ml). For INF-gamma and IL-4, the levels will be measured by enzyme-linked immunosorbent assay kit (Genzyme, Boston, Mass.) according to the manufacturer's instructions. CD4 and CD8 cells will be selected to determine which cells are producing the cytokines using MACS direct MicroBeads (Miltenyi Biotec, Auburn CA).

(d) Proliferative response of spleen cells: SMNC will be prepared as described above. After the stimulation by different amounts of recombinant protein or Orientia Karp, the cultures will be incubated at 37C for 72 h under a humidified atmosphere of 5% CO2. A total of 1 uCi of [3H] thymidine will be added to each well for the another 18 h of culture. Cells will be harvested onto glass fiber filter strips, and incorporation of [3H] thymidine will be counted in a liquid scintillation counter.

PROPHETIC EXAMPLE 3

Prophetic examples for the treatment of HIV patient to reduce the virus load with immunoglobulins from subjects immunized by the immunogenic composition.

There is no animal model for HIV infection at the present time. However, transgenic mice carrying the human CD-4 gene can be infected by HIV. This type of mice will be used in the following experiments:

Mice will be infected with HIV. Two months later, one group of mice will be treated with hyper immune plasma from the same type of mice immunized by the said immunogenic composition (group 1). Another group of mice will be treated with normal plasma to serve as the control group (group 2). Blood will be drawn from both groups at one week interval for four times. The HIV load in the

blood will be quantitated by real time PCR. Results from group 1 will be compared to group 2 to demonstrate the dereased virus load by the hyper immune plasma.

CONCLUSIONS

The truncated 47-kDa protein was successfully cloned and overexpressed in BL21 cells. The over expressed protein was not in the inclusion body making the purification procedure complicated. The addition of detergent increased the amount of r47 suggesting the association of protein with membrane fraction. Although dialysis of crude extract with 25,000 MW cutoff offered some purification and further purification using pH 8.0 or pH 7.0 buffer and salt gradient on an anion exchange column resulted in further purification, the protein was not yet fully purified. The pI of r47 was predicted to be 8.2 and suggested a cation exchange is more suitable for purification. Experiments will continue to purify r47 with cation exchange followed by gel filtration to achieve desired purity.

SEQUENCE LISTING

SEQ ID No. 1: Truncated Protein Sequence of r47 Protein
Expressed, truncated protein sequence (translated from 184-1458 of DNA sequence, bolded sequence was confirmed by N-terminal protein sequencing)

MVNSLSDIVEPLISTVVSIYAVDTNIGISFNNKVSKYQQEVFLGSGVIIDSSGYIVTNENVIA
GAENIKVKLHDGSELIAELVGSDNKINIALLKINSPAALSYATFGDSNQSRVGDQVIAIGSPF
GLRGTVTNGIISSKGPDMGNGIVTDFIQTNAAIHMGSFGGPMFNLEGKIIGINSIHVSYSGISF
AIPSNTVLEAVECLKKGEKIRRGMLNVMLNELTPELNENLGLKQDQNGVLITEVIKEGSAA
QCGIAPGDVITKFHDKEIKTGRDLQVAVSSTMLNSEREVELLRNGKSMTLKCKIIANKGEDS
EQQSNDQSLVVNGVKFVDLTPDLVKKYNITSANNNGLFVLEVSPNSSWGRYGLKMGLRPR
DIILSVKRDDNKKDISVKTLREIVTNIKHNEIFFTV QRGDRMLYIALPNINK

Protein sequence data output (only N-terminal):

MVNSLSDIVEPLISTVV

SEQ ID No. 2: r56 Protein Karp (Ching)

The confirmed sequence for r56 by Ching (amino acid 80-456). The corrected sequence is highlighted and underlined

MTIAPGFRAEIGVMYLTNITAQVEEGKVKADSVGETKADSVGGK
DAPIRKRFKLTPPQPTIMPISIAVRDFGIDIPN**IPQQQAQAAQPQ**LNDE
QRAAARIAWLKNCAGIDYRVKNPNDPNGPMVINPILLNIPQGNP
NPVGNPPQRANPPAGFAIHNHEQWRHLVVGLAALSNANKPSASP
VKVLSDKITQIYSDIKHLADIAGIDVPDTSLPNSASVEQIQNKMQ
ELNDLLEELRESFDGYLGGNAFANQIQLNFVMPQQAQQQGQGQ
QQQAQATAQEAVAAAAVRLLNGNDQIAQLYKDLVKLQRHAGIK
KAMEKLAAQQEEDAKNQGEGDCKQQQGTSEKSKKGKDKEAEFD
LSMIVGQVKLYADVMITESVSI

PI = 5.8,
MW 40903, 377 a.a.
Extinction coefficient A_{280}=0.754 (1 mg/ml)

SEQ ID No. 3: r56 Protein Sequence (Kato)

>Kato sequence number 21 529 aa

MKKIMLIASA MSALSLPFSA SAIELGDEGG LECGPYAKVG VVGGMITGVE STRLDPADAG GKKQLPLTTS

MPFGGTLAAG **MTIAPGFRAE LGVMYLANVK AEVESGKTGS DADIRSGADS PMPQRYKLTP PQPTIMPISI**

ADRDLGVDIP NVPQGGANHL GDNLGANDIR RADDRITWLK NYAGVDYMVP DPNNPQARIV NPVLLNIPQG

PPNANPRQAM QPCSILNHDH WRHLVVGITA MSNANKPSVS PIKVLSEKIV QIYRDVKPFA RVAGIEVPSD

PLPNSASVEQ IQNKMQELND ILDEIRDSFD GCIGGNAFAN QIQLNFRIPQ AQQQGQGQQQ QQAQATAQEA

AAAAAVRVLN NNDQIIKLYK DLVKLKRHAG IKKAMEELAA QDGGCNGGGD NKKKRGASED SDAGGASKGG

KGKETKETEF DLSMIVGQVK LYADLFTTES FSIYAGLGAG LAYTSGKIDG VDIKANTGMV ASGALGVAIN

AAEGVYVDIE GSYMHSFSKI EEKYSINPLM ASFGVRYNF

SEQ ID No. 4: r56 Protein (Gilliam)

>Gilliam sequence number 25 524 aa

MKKIMLIASA MSALSLPFSA SAIELGEEGG LECGPYGKVG IVGGMITGAE STRLDSTDSE GKKHLSLTTG

LPFGGTLAAG **MTIAPGFRAE LGVMYLRNIS AEVEVGKGKV DSKGEIKADS GGGTDTPIRK RFKLTPPQPT**

IMPISIADRD VGVDTDILAQ AAAGQPQLTV EQRAADRIAW LKNYAGIDYM VPDPQNPNAR VINPVLLNIT

QGPPNVQPRP RQNLDILDHG QWRHLVVGVT ALSHANKPSV TPVKVLSDKI TKIYSDIKPF ADIAGIDVPD

TGLPNSASVE QIQSKMQELN DVLEDLRDSF DGYMGNAFAN QIQLNFVMPQ QAQQQQGQGQ QQQAQATAQE

AVAAAAVRLL NGNDQIAQLY KDLVKLQRHA GVKKAMEKLA AQQEEDAKNQ GEGDCKQQQG ASEKSKEGKG

KETEFDLSMI VGQVKLYADL FTTESFSIYA GVGAGLAHTY GKIDDKDIKG HTGMVASGAL GVAINAAEGV

YVDLEGSYMH SFSKIEEKYS INPLMASVGV RYNF

Primer sets

a. Forward primers:

Gene (125-174 as target gene sequence)

5'-tatgcat aCCATGGtaa atagtttatc tgat-3' (SEQ ID No. 5)
Nco I site

b. Reverse Primers:

Gene sequence of 1443-1477 as target, 35 bp
5'-cgcctGGATCC tagatttacttattaatattaggt-3'
BamH1 site

(SEQ ID No. 6)

SEQ ID NO. 7: PCR amplified sequence: 174-1481 nt. Expressed DNA sequence:184-1458 nt, aa 42-466. the coding region is bold.

174 tatgcat
181 att **atg gtaa atagtttatc tgatatagtt gagccattaa tatctacagt agtaagtatt**
241 **tatgctgtag atactaacat tggtattagt tttaataata aggtatctaa gtatcagcaa**
301 **gaagtgttct taggttctgg ggttatcatt gatagttctg ggtatattgt tactaatgag**
361 **aatgttatag caggagctga aaatataaaa gtaaagttgc atgatggttc agaactcata**
421 **gcagaattag ttggtagtga caataaaatt aatatagctt tattaaaaat taattctcca**
481 **gcagcattat cttatgcgac ttttggcgac tcaaatcagt ctagagtagg agatcaggtt**
541 **attgcaatag gaagtccttt tggtttaaga ggaacagtaa caaatggcat tatttcttct**
601 **aaaggaccag atatgggtaa cggcatagta actgatttta ttcaaactaa tgctgctatt**
661 **catatgggta gctttggtgg accgatgttt aatcttgaag gaaaaattat tggaattaat**
721 **tccattcacg tatcttactc aggcataagt tttgctattc catctaatac tgtacttgaa**
781 **gcagttgaat gcttaaaaaa aggagaaaaa attcgtcgtg gtatgttaaa tgttatgctt**
841 **aatgaattaa ctccagaatt aaatgagaat ttaggactta aacaagatca aaatggagtt**
901 **ctaataactg aagttataaa agaaggatct gcagcacaat gtggaattgc tcctggagat**
961 **gtaattacta aatttcatga taaagagatc aaaacaggga gagatttaca ggtagctgta**
1021 **tcttcaacta tgcttaattc tgaaagagaa gttgagcttt tacgtaatgg taagtcgatg**
1081 **actctaaaat gtaaaattat tgccaacaaa ggtgaggata gtgagcaaca aagtaatgat**
1141 **caaagccttg tggttaatgg tgtaaaattt gttgatctta cacctgattt agtgaagaaa**
1201 **tataatatta cttcagctaa taataatggg ttatttgtcc ttgaagtttc gcctaactct**
1261 **tcttggggga gatatggttt aaaaatgggg ctaagaccta gagatataat tttatcagtt**
1321 **aaacgtgatg ataataaaaa agatatttct gttaaaactc taagagaaat agtgacaaat**
1381 **ataaagcata atgaaatttt ctttacagtg caaagaggag atagaatgct ttacattgct**
1441 **ttacctaata ttaataag** ta aatctagttt aaaggcgatt t

The inventive subject matter being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the inventive subject matter, and all such modifications are intended to be within the scope of the following claims.

Claims:

1. A scrub typhus immunogenic composition comprising:

   an r47 truncated protein;

   an r56 truncated protein; and

   a pharmaceutically acceptable carrier.

2. The composition of claim 1, wherein said r47 truncated protein is SEQ ID NO.1.

3. The composition of claim 1, wherein said r56 truncated protein is selected from the group consisting of SEQ ID NOs. 2-4.

4. The composition of claim 1, wherein said r56 truncated protein is any two proteins selected from the group consisting of SEQ ID NOs. 2-4.

5. The vaccine of claim 1, wherein said r56 truncated protein comprises SEQ ID NOs. 2-4.

6. A use a pharmaceutically effective dose of antibodies raised against the combination of a truncated r47 protein and a truncated r56 protein in the manufacture of a medicament for treating a patient suffering from scrub typhus.

7. The use of claim 6, wherein said dose is suitable for subcutaneous administration.

8. The use of claim 6, wherein said r47 truncated protein is SEQ ID NO.1.

9. The use of claim 6, wherein said r56 truncated protein is selected from the group consisting of SEQ ID Nos. 2-4.

10. The use of claim 6, wherein said r56 truncated protein is any two selected from the group consisting of SEQ ID Nos. 2-4.

11. The use of claim 6, wherein said r56 truncated protein comprises SEQ ID Nos. 2-4.

12. An assay method for detecting scrub typhus comprising:

a. obtaining a sample from a subject;

exposing said sample to a combination of a truncated r47 protein plus a truncated r56 protein or a laboratory produced antibodies to said combination in assay equipment selected from the group consisting of ELISA plates, dot-blot matrices, and hand held chromatographic and flow through assay devices; and

b. detecting antibodies to said protein combination in said sample or

detecting an antigen to said laboratory antibodies in said sample in said assay equipment.

13. The assay method of claim 12, wherein said sample comprises blood, urine or oral fluids.

14. A pharmaceutical composition for treating an HIV patient comprising antibodies raised against a truncated r47 protein and a truncated r56 protein selected from the group consisting of SEQ ID NOs. 2-4 and combinations thereof and a pharmaceutically

acceptable carrier, wherein administration of said composition reduces said patients HIV viral load.

15. The pharmaceutical composition of claim 14, wherein said truncated r47 protein is SEQ ID NO. 1.

16. An immunogenic composition against HIV infection comprising a pharmaceutically effective dose of a truncated r47 protein and a truncated r56 protein selected from the group consisting of SEQ ID NOs. 2-4 and combinations thereof, and a pharmaceutically acceptable carrier.

17. The composition of claim 16, wherein said truncated r47 protein is SEQ ID NO.1.

18. A pharmaceutical composition for treatment of HIV patient comprising a truncated r56 protein and a pharmaceutically acceptable carrier wherein administration of said composition reduces said patients viral load.

19. The composition of claim 18, wherein said r56 protein is selected from the group consisting of SEQ ID NOs. 2-4.

20. The composition of claim 18, wherein said r56 protein is any two selected from the group consisting of SEQ ID NOs. 2-4.

21. The composition of claim 18 comprising SEQ ID NOs. 2-4.

22. A use of a pharmaceutically effective dose of a truncated r47 protein and a truncated r56 protein and a pharmaceutically acceptable carrier in the manufacture of a medicament for treating an HIV patient wherein said protein reduces said patients viral load.

23. The use of claim 22, wherein said truncated r56 protein is selected from the group consisting of SEQ ID NOs. 2-4.

24. The use of claim 22, wherein said truncated r56 protein is any two selected from the group consisting of SEQ ID NOs. 2-4.

25. The use of claim 22, wherein said truncated r56 protein comprises SEQ ID NOs. 2-4.