We evaluated the immunogenicity and efficacy of a candidate vaccine comprising the major outer membrane protein (MOMP) multi-epitope of \textit{Chlamydia trachomatis}. A short gene of multi-epitope derived from MOMP containing multiple T- and B-cell epitopes was artificially synthesized. The recombinant plasmid pET32a(+) containing codon optimized MOMP multi-epitope gene was constructed. Expression of the fusion protein Trx-His-MOMP multi-epitope in \textit{Escherichia coli} was confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and western blot analysis. Balb/c mice were inoculated with the purified fusion protein subcutaneously three times with 2-week intervals. Results showed that the MOMP multi-epitope elicited not only strong humoral immune responses to \textit{C. trachomatis} by generating significantly high levels of specific antibodies (IgG1 and IgG2a), but also a cellular immune response by inducing robust cytotoxic T lymphocyte responses in mice. Furthermore, the MOMP multi-epitope substantially primed secretion of IFN-\(\gamma\), revealing that this vaccine could induce a strong Th1 response. Finally, the mice vaccinated with the MOMP multi-epitope displayed a reduction of \textit{C. trachomatis} shedding upon a chlamydial challenge and an accelerated clearance of the infected \textit{C. trachomatis}. In conclusion, the MOMP multi-epitope vaccine may have the potentiality for the development of effective prophylactic and therapeutic vaccines against the \textit{C. trachomatis} infection.

\textbf{Keywords} \textit{Chlamydia trachomatis}; vaccine; major outer membrane protein; multi-epitope; humoral immune response; cytotoxic T lymphocyte (CTL) response

\textbf{Introduction}

\textit{Chlamydia trachomatis}, an obligate intracellular bacterium, is the most common sexually transmitted pathogen worldwide [1,2]. The majority of genital chlamydial infection may lead to severe complications such as pelvic inflammatory disease, ectopic pregnancy, and infertility if the infection is not treated [3,4]. Chlamydial infection is also associated with an increased risk of human immunodeficiency virus-related acquired immunodeficiency syndrome and cervical dysplasia [5,6]. Despite the availability of effective antimicrobial therapy, a distinct increase in the number of \textit{C. trachomatis} infections has been observed over the last decade because of the high recurrence rate and drug resistance [7,8]. Vaccines have been considered to be the most effective means for preventing \textit{C. trachomatis} infections and associated diseases [1,2,9], such as autoimmune diseases directly or indirectly caused by \textit{C. trachomatis} infection [10–12]. Although efforts have been made throughout the years to develop a protective vaccine, there are no vaccines available which effectively prevent \textit{C. trachomatis} genital infections. Therefore, developing an effective vaccine capable of preventing and controlling \textit{C. trachomatis} genital tract infections is urgently required [13,14].

Current challenges in the field of chlamydial vaccine design and development are the selection of appropriate candidate antigens and its delivery system [15]. Several surface-associated proteins highly conserved among the different serotypes of \textit{C. trachomatis} [16,17] may represent potential candidates for the development of novel vaccine formulations. Among them, the most suitable candidate for a \textit{C. trachomatis} vaccine is the cysteine-rich major outer membrane protein (MOMP), which makes up \(\sim 60\%\) of the total...
outer membrane and contains both T- and B-cell epitopes that can induce specific anti-Chlamydia immune responses. Whereas previous studies showed that it is difficult to produce recombinant MOMP in a native form with intact, conformationally relevant epitopes and on a scale large enough to be commercially viable [18]. At present, a multi-epitope of MOMP with T- and B-cell epitope-rich clusters was selected as research subject.

An epitope-based peptide vaccine with a unique design can incorporate not only T- and B-cell epitopes, but also other epitopes such as Th-cell epitopes by different combinations to develop a chimeric vaccine against a variety of pathogens or tumors [14,19–22]. A MOMP-based subunit vaccine is also considered to be safer and can avoid the immunopathology or inflammatory damage induced by whole Chlamydia organism vaccines [23]. Apparently, a broad immune response simultaneously against multiple cytotoxic T lymphocyte (CTL) epitopes that spread over several antigenic proteins is necessary for developing an effective vaccine against multiple pathogens. Considering that MOMP as the primary candidate for a subunit vaccine has only had a partial protection against genital Chlamydia challenge, we designed a peptide-based MOMP multi-epitope vaccine to investigate its immunogenicity and protective immunity against Chlamydia infection in mice.

Materials and Methods

Construction of pET32a(+)MOMP multi-epitope plasmid
SYFPEITHI program (http://www.syfpeithi.de/Scripts/MHC Server.dll/EpitopePrediction.htm) was used to predict and screen the CTL (human HLA-A2-restricted and mouse H2-Kd-restricted epitopes), B-cell, and Th epitopes within the sequence of C. trachomatis MOMP. Two strings of amino acid MOMP160–187 (GDENQSTVKTNSVPNMSLDQSV VELYT) and MOMP271–290 (WQASLALSYRLNMFTPY IGV) were selected from the epitope-rich immunodominant region and sequentially connected to form a target MOMP multi-epitope, which contained 48 amino acids (Figs. 1 and 2A). The target multi-epitope gene was artificially synthesized after codon optimization and then inserted into pET32a(+) after HindIII/Xhol digestion. The construct was verified by PCR and sequencing.

Expression and purification of the Trx-His-MOMP multi-epitope fusion protein
Escherichia coli (DE3) was transformed with either a pET32a(+)MOMP multi-epitope plasmid or a pET32a(+) vector to express multi-epitope fusion protein or Trx-His protein, respectively, induced by isopropyl-β-D-thiogalactoside at a final concentration of 0.2 mM for 4 h at 37°C. The fusion proteins were purified using Ni-NTA agarose (Qiagen, Frankfurt, Germany) according to the elution protocol listed in the Ni-NTA Superflow Cartridge Handbook (March 2007), and quantified using a DNA/protein analyzer (Beckman CoulterTM, Brea, USA). The purified fusion proteins were characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and western blot analysis with antibodies against C. trachomatis and His-tag.

Preparations of C. trachomatis stock
HeLa 229 cells infected with C. trachomatis serovar E strain (VR-348B; ATCC, Manassas, USA) were grown in Eagle’s minimal essential medium (Invitrogen, Carlsbad, USA) supplemented with 10% FCS and 1.5 μg/ml cycloheximide (Invitrogen) at 37°C for 2 days. The C. trachomatis-infected cells were harvested, sonicated on ice for 1 min, and then centrifuged at 1000 g for 10 min at 4°C. The chlamydial elementary bodies (EBs) in the supernatant were pelleted at 12,000 g for 30 min at 4°C. The EB pellet was resuspended in SPG buffer (0.25 M sucrose, 10 mM sodium phosphate, 5 mM glutamic acid, pH 7.2) and purified by 50% sucrose gradient centrifugation, 8000 g for 1 h at 4°C to produce C. trachomatis EB stocks. The C. trachomatis EB stocks in SPG buffer were stored at −70°C.

Immunization
Female Balb/c mice (6–8 weeks old) were provided by Shanghai Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China), Animal Production License No.: SCXK (Shanghai) 2007-00005. Animal care and monitoring were carried out in accordance with strict guidelines issued by the PR China legislation. All animal procedures and treatments were approved by the Animal Care and Ethics Committee of Wenzhou Medical University (Wenzhou, China). A total of three groups of mice with 15 in each group were immunized subcutaneously with multi-
epitope fusion protein (A), phosphate buffered saline (PBS; B, negative control), and Trx-His protein (C, pET32a(+) basal vector protein control), respectively. Before immunization, the fusion protein was mixed with 100 μl of complete Freund’s adjuvant in the initial immunization (day 0) and 100 μl incomplete Freund’s adjuvant in the second and third immunization (days 14 and 28). Each mouse was immunized with 100 μg of fusion protein in 100 μl sterile PBS or 100 μl sterile PBS only. Immunizations were administered on day 0, and boosted on days 14 and 28, respectively. Fourteen days after the final immunization, serum and genital fluid samples were collected from the immunized mice. Genital fluid samples were collected by flushing the vagina with 50 μl sterile PBS. Both serum samples separated by centrifugation and genital fluid samples were frozen at −70°C until use.

Levels of different types of antibodies and IgG subtypes
The levels of *C. trachomatis*-specific IgG, IgA, IgM, and IgG subtype antibodies in serum and IgA in genital fluid were detected by the enzyme-linked immunosorbent assay (ELISA). Falcon 96-well flexiplates were coated with inactivated EBs (1 × 10^5 inclusion-forming units, IFU/ml) in a sodium bicarbonate buffer (pH 9.5) at 4°C overnight and washed with 0.05% Tween 20 in PBS (PBST) three times. The plates were then blocked with 3% bovine serum albumin (200 μl/well) in PBST at 37°C for 1 h and washed further with PBST three times. A total of 50 μl of 1:10 dilutions of serum or 1:10 dilutions of vaginal lavage fluid was added to each well at 37°C for 1 h, respectively. After washing with PBST, 50 μl of horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG, IgA, IgM, IgG1, IgG2a, IgG2b, and IgG3 antibodies (SBA Clonotyping TM System/HRP, Birmingham, USA) was added to each serum well, respectively, for 1 h. The genital fluid sample wells were added with an HRP-conjugated goat anti-mouse IgA antibody. After washing again, the substrate, 3,3',5,5'-tetramethylbenzidine, was then added for developing color and the absorbance was measured at 450 nm using a microplate reader (Bio-Tek, Winooski, USA).

Measurement of serum IL-4 and IFN-γ
In order to assess the Th cell response in immunized mice, blood samples were collected on day 14 after the final immunization, and were immediately centrifuged at 2000 g and 4°C for 15 min after collection. The concentrations of IL-4 and IFN-γ were assessed by using a commercial ELISA kit (QuantoBio, Inc., Beijing, China) according to the manufacturer’s instructions. The absorbance was measured at 450 nm using the microplate reader. All the serum samples collected from the three immunized mice groups were assayed in duplicate. The concentrations of the two cytokines at pg/ml were calculated from the established standard curves. The minimum detectable doses of mouse IL-4 and IFN-γ were <2.2 and <10 pg/ml.

CTL function assays
To assess CTL functions, *in vitro* restimulated spleen cells were used in a lactate dehydrogenase (LDH) release assay (Roche, Basel, Switzerland) by incubation with target cells. All steps were performed following the manufacturer’s instructions. Splenocytes (2 × 10^6 cells/ml) isolated from the immunized mice on day 14 after the final immunization were co-cultured with 2 × 10^5 inactivated EBs in the presence of 20 U/ml mouse IL-2 at 37°C for 5 days. The stimulated T cells were isolated and used as effector cells, P815 mouse mastocytoma cells pulsed with CTL epitope peptide (LYTWQASLA) were used as target cells. Target cells were dispensed into 96-well round-bottomed plates (10^4 cells/well) and then serial dilutions of CTLs were added to the wells. The plates were centrifuged once at 339 g for 10 min. After incubation in 5% CO_2 at 37°C for 6 h, the plates were centrifuged again. Fifty microliters of the supernatant was transferred to another plate and mixed with the substrate mix provided by the test kits for CTL reaction. The reaction was stopped after 30 min and the absorbance was measured at 490 nm. Spontaneous release was determined in wells with target cells without CTL. Maximum release was determined by addition of detergent to wells containing target cells. Specific lysis was determined according to the following formula:

\[
\text{Specific lysis} (%) = 100 \times \left[\frac{\text{release by CTL} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}}\right].
\]

In all experiments, spontaneous release was <30% of maximal release by detergent.

Vaginal *C. trachomatis* challenge and determination of bacterial shedding
On day 14 after the last vaccination, mice were intravaginally challenged with 10^6 IFU of *C. trachomatis* EBs in 20 μl SPG buffer. To synchronize the menstrual cycle and increase the susceptibility of mouse to *C. trachomatis* infection, the vaccinated mouse was subcutaneously injected with 2.5 mg Depo-Provera. To monitor *C. trachomatis* shedding, vaginal swabs of the challenged mice were collected at 5-day intervals, and the swab material was plated and incubated for 28 h on HeLa 229 cell monolayers grown on culture coverslips per animal. Chlamydial inclusions were detected by indirect immunofluorescence labeling using a rabbit anti-*C. trachomatis* serum antibody. The average number of inclusions in five random microscopic fields was calculated for each animal. The bacterial shedding was calculated and
expressed as the number of IFUs. All the experiments were repeated twice.

Statistical analysis
The differences in antibody and CTL responses among these groups were calculated by one-way analysis of variance for three independent groups or by Student’s t-test for two independent groups. The Kruskal–Wallis test was used to determine the differences in vaginal chlamydial shedding between the experimental groups. The chlamydial infection clearance over time among the groups was compared using the Kaplan–Meier test. The criterion for statistical significance was set at \( P < 0.05 \), and all calculations were done with SPSS 13.0 software.

Results

Construction and expression of the MOMP multi-epitope
The MOMP multi-epitope composed of 48 amino acid residues comprises at least seven overlapping human HLA-A2-restricted CTL epitopes, two mouse H2-Kd-restricted CTL epitopes, one Th epitope, and one B-cell epitope (Table 1) [24,25]. The codon-optimized multi-epitope gene was used to construct a recombinant pET32a(+) MOMP multi-epitope plasmid (Fig. 2A). The construct was identified by PCR (Fig. 2B), restriction enzyme digestion (Fig. 2B), and sequencing analysis (data not shown). This construct was transformed into E. coli and expressed a Trx-His-MOMP multi-epitope fusion protein with a predicted size of 25 kDa, which was confirmed by SDS–PAGE (Fig. 2C) and western blot analysis (Fig. 2D and E). The fusion proteins reacted with a specific anti-His tag antibody (Fig. 2D), as well as with an anti-C. trachomatis antibody (Fig. 2E). Furthermore, a Trx-His fusion protein at a size of 19 kDa was also identified by SDS–PAGE (Fig. 2C) and western blot analysis (Fig. 2D, E).

MOMP multi-epitope induced humoral immune response
Previous studies [26] indicated that both IgG and secretory IgA play a protective role during genital chlamydial infection. Therefore, the levels of these antibodies in the serum

<table>
<thead>
<tr>
<th>Epitope source</th>
<th>Predicted sequences</th>
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<tbody>
<tr>
<td>Human HLA-A2 restricted CTL</td>
<td>GDNENQSTV, NMSLDQSVV, SLQSVVEL, ALSYRLNMF, RNMFIPYI, NMIPYIGV</td>
</tr>
<tr>
<td>Mouse H2-Kd restricted CTL</td>
<td>LYTWQASLA, ALDQSVVEL</td>
</tr>
<tr>
<td>Th</td>
<td>VKTNSVPNMSLDQSVVEL</td>
</tr>
<tr>
<td>B cell</td>
<td>NQSTVKT</td>
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Table 1. predicted sequences of 11 epitopes in the short C. trachomatis MOMP

Figure 2. Construction and expression of pET32a(+) MOMP multi-epitope plasmid  (A) Diagram of the pET32a(+) MOMP multi-epitope plasmid. (B) PCR and enzyme restriction analysis of the constructed pET32a(+) MOMP multi-epitope plasmid. M1 and M2: DNA ladders; lane 1: MOMP multi-epitope PCR product; lane 2: HindIII/HindIII restriction of the pET32a(+) MOMP multi-epitope plasmid; lane 3: HindIII restriction of the pET32a(+) basal plasmid; lane 4: pET32a(+) MOMP multi-epitope plasmid without enzyme restriction. (C) Comassie blue-stained SDS-gel of the purified Trx-His-MOMP multi-epitope fusion protein at 25 kDa (lane 1) and Trx-His protein at 19 kDa (lane 2). (D) Western blotting assay using a monoclonal antibody against His-tag. (E) Immunoblotting assay using a polyclonal antibody against C. trachomatis. Both lanes 1 and 2 in (D) and (E) are the same as those described in (C).
and vaginal secretion samples of immunized mice were determined by ELISA. It is very clear that the MOMP multi-epitope immunization induced significantly higher levels of IgG and IgM antibodies than the Trx-His immunization (P < 0.05). Although the levels of serum IgA antibody among the three immunized mice groups were not significantly different, the level of IgA antibody in genital fluid samples collected from the multi-epitope-immunized mice was significantly higher than that from the Trx-His-immunized mice (P < 0.05) (Fig. 3A).

We then determined the levels of the serum IgG subclasses in multi-epitope-immunized mice and compared with those in mice immunized by Trx-His and PBS. As shown in Fig. 3B, the levels of IgG1, IgG2a, IgG2b, and IgG3 antibodies induced by the multi-epitope immunization were significantly higher than those induced by either Trx-His or PBS immunization.

**Induction of serum IL-4 and IFN-γ in immunized mice**

Next, the levels of both IL-4 and IFN-γ in sera collected from the immunized mice on day 14 after the last immunization were detected by ELISA. The level of IL-4 in sera from the mice immunized with the multi-epitope was not significantly different from that in sera from the mice immunized with either Trx-His or PBS (P > 0.05) (Fig. 4A). However, multi-epitope immunization produced significantly a higher level of the serum IFN-γ than Trx-His immunization (P < 0.05), suggesting that the multi-epitope induced strong Th1-type cell reactions in mice (Fig. 4B).

**MOMP multi-epitope induced CTL response**

As shown in Table 1, the MOMP multi-epitope protein contains nine predicted human HLA-A2- or mouse H2-Kd-restricted CTL epitopes. To investigate the cellular immune response of mice immunized with the fusion proteins, splenocytes were isolated for preparing T lymphocytes. Subsequently, CTL activity of each group was determined by measuring the release of LDH, a stable cytoplasmic enzyme, in the culture supernatants. As shown in Fig. 5, at any of effector/target cell (E : T) ratio, T cells derived from the mice immunized with multi-epitope fusion protein exhibited
significantly higher cytotoxicity than Trx-His and PBS control groups \((P < 0.05)\). While there was no significant difference between groups Trx-His and PBS \((P > 0.05)\).

### Chlamydial clearance after genital challenge in vaccinated mice

We next determined the protective efficacy of the MOMP multi-epitope in immunized mice by monitoring vaginal chlamydial shedding at 5-day intervals following the challenge with \(10^6\) IFU of *C. trachomatis* via the intravaginal route (Fig. 6). Chlamydial shedding was assessed by isolation of chlamydiae from vaginal swabs of the vaccinated mice at different time points. The result showed that the mice immunized with the MOMP multi-epitope showed significantly a lower IFU number than those immunized with PBS or Trx-His protein \((P < 0.05)\) on days 20, 25, and 30 post-challenge (Fig. 6). Furthermore, immunization with the multi-epitope protein shortened the infection time course to 25 days with a significant reduction in the infectious titers from the vaginal swabs after infection (Fig. 6). These data suggest that the MOMP multi-epitope can provide partial protection immunity to *C. trachomatis* genital infection in mice.

### Discussion

MOMP is an \(~40\)-kDa highly disulfide cross-linked surface-exposed protein that comprises \(~60\%) of the outer membrane of chlamydiae. MOMP is an immunodominant antigen in both humans and animals and contains multiple B- and T-cell epitopes, eliciting both neutralizing antibody and T-cell immunity [7]. Therefore, it is regarded as the best antigen in potential subunit vaccine candidates [27,28]. However, it is difficult to obtain recombinant MOMP in native form. Safety concerns have led to other strategies for vaccine development that focus on epitope vaccines. Taha et al. [29] selected a small gene fragment from MOMP containing T-cell epitopes (aa 278–370) and generated a recombinant MOMP peptide (rMOMP-278, 93 aa). They used rMOMP-278 to immunize mice and found that the rMOMP-278 induced higher responses for antigen-specific antibodies. Based on published studies [30,31], an ideal peptide vaccine should contain both B- and T-cell epitopes. The multi-epitope MOMP from *C. trachomatis* serovar E as the research subject, which contained not only Th epitopes, but also CTL and B-cell epitopes. Considering the *C. trachomatis* serovars D and E were the most predominant serovars prevalent worldwide [32–34], therefore the amino acid sequence alignment of the multi-epitope MOMP was analyzed and the results showed a high degree homology among serovars E and D (Fig. 1).

Proteins with His-tag can be purified and easily detected. Therefore, the multi-epitope gene fused with Trx-His in the pET32a(+) plasmid to express a 25-kDa Trx-His-MOMP fusion protein, which showed strong immunogenicity due to its ability to produce high levels of antibodies and cellular immune responses in vaccinated mice. Apparently, our results suggest that the MOMP multi-epitope has the potentiality for developing a *Chlamydia* vaccine although it remains unclear what type of immune response would be sufficient to prevent chlamydial infection. The role of specific antibody in
murine chlamydial infections has evolved from being the focus of vaccine development and immune protection [9,35,36]. According to the detection of the antigen-specific isotype antibodies with an order of IgG2b > IgG2a > IgG1, Taha et al. [29] proposed that rMOMP-278 immunization induced a mixed Th1/Th2 response, but with a bias to Th1 response, in contrast to that rMOMP predominantly induced Th2 response with the order of specific antibody production of IgG1 > IgG2b > IgG2a in immunized mice. Here, we confirmed that the MOMP multi-epitope fusion protein induced a mixed Th1/Th2 response, but having a bias to induce Th2 response with the antibody order of IgG1 > IgG2a > IgG2b in mice. Our results are consistent with a previous study that specificity of the IgG subclass production induces IgG2a expression and Th2 response facilitates IgG1 production [37]. It has also been reported that the activated Th1 cells enhance the secretion of IL-2 and IFN-γ to induce IgG2a production, while the activated Th2 cells increase the secretion of cytokines such as IL-4 and IL-5 to stimulate IgG1 generation [37–42]. Based on the detection of IFN-γ and IL-4 in serum, our data support that the MOMP multi-epitope vaccine is biased to induce a Th1-type reaction, because this vaccine induced high IFN-γ level.

In this study, it was clear that the MOMP multi-epitope vaccine substantially induced in vivo CTL responses and provided a partial protection based on the analysis of C. trachomatis shedding and vaginal tract tissue pathologies in mice. These data further suggested that the MOMP multi-epitope vaccine strongly induces Th cell immune responses to C. trachomatis EB infection, consistent with the previously published studies that Th cell immune response, especially Th1 immune response, is required for protective immunity to clear infection of C. trachomatis and resist to its reinfection in mouse models. However, it has gradually attracted the attention of researchers that Th2 immune response may also play an important role in controlling genital C. trachomatis infection [43].

In this study, the level of IgA antibody in vaginal lavage fluid was distinctly increased in mice immunized with both MOMP multi-epitopes. Previously published studies in experimental animals and humans have shown that the IgA plays an important role in the mucosal immunity, preventing C. trachomatis infection [26]. This knowledge has led to the practical approaches for vaccine construction and delivery into mucosal inductive sites in an effort to elicit host protection at mucosal surfaces. It has been reported that C. trachomatis infection resistance correlates with the presence of neutralizing serovar-specific IgA antibodies in tears, which suggested that secretory antibody plays a critical role in protective immunity, although the immune mechanism(s) that functions in protection is still unknown [13,14]. Thus, further experiments are required to focus on whether the current MOMP multi-epitope vaccine, or with some modifications, can elicit a strong mucosal IgA response by providing protective immunity against C. trachomatis infections in mouse models.

It has been reported that the route of vaccine administration influences the strength and nature of immune responses [28,44–47]. Therefore, different delivery systems for the C. trachomatis vaccine such as halobacterium gas vesicles, nanoparticles, live-attenuated influenza viruses, and Vibrio cholerae ghosts have been investigated in mice and non-human primates. Some of the vaccine delivery methods could significantly enhance immunogenicity and protective immunity of the target vaccines.

In conclusion, the MOMP multi-epitope vaccine induced not only strong humoral and cellular immune responses to C. trachomatis, but also a robust mixed Th1/Th2 response in mice. This vaccine prevented the pathogenesis of the genital chlamydial infection by reducing C. trachomatis shedding upon a chlamydial challenge and clearing the infected C. trachomatis in mice. Thus, the MOMP multi-epitope vaccine containing both T- and B-cell epitopes may represent an effective strategy to increase the efficacy of the polypeptide-based vaccines against the C. trachomatis infection.

Funding

This work was supported in part by the grants from the National Natural Science Foundation of China (30972669), the Zhejiang Provincial Natural Science Foundation of China (Y2100611), and the Wenzhou Science and Technology Bureau (Y20100014, and Y20100090).

References

8. Bhengraj AR, Vardhan H, Srivastava P, Salhan S and Mittal A. Decreased susceptibility to azithromycin and doxycycline in clinical isolates of...
A multi-epitopes vaccine of Chlamydia trachomatis MOMP


