Title: RECOMBINANT CHLAMYDIA VACCINE

Abstract: The present invention provides vaccines and methods for making the vaccines that actively or passively protect a human or animal against Chlamydia infection. In particular, the present invention provides a vaccine that provides active immunity which comprises a polypeptide or DNA vaccine that contains or expresses at least one epitope of polypeptide that has an amino acid sequence that is substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis. The present invention further provides a vaccine that provides passive immunity to Chlamydia comprising polyclonal or monoclonal antibodies against at least one epitope of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis. Further still, the present invention provides a method for preventing an inflammatory reaction, in particular, in a skin graft, by providing a polypeptide that is substantially similar to a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis.
RECOMBINANT CHLAMYDIA VACCINE

CROSS-REFERENCE TO RELATED APPLICATION

None

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

None.

BACKGROUND OF THE INVENTION

(1) Field of the Invention

The present invention relates to vaccines and methods for making the vaccines that actively or passively protect a human or animal against Chlamydia infection. In particular, the present invention relates to vaccines that provide active immunity which comprise a polypeptide or DNA vaccine that contains or expresses at least one epitope of a polypeptide that has an amino acid sequence substantially similar to a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis. The present invention further relates to a vaccine that provides passive immunity to Chlamydia comprising polyclonal or monoclonal antibodies against at least one epitope of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis. Further still, the present invention relates to a method for preventing an inflammatory reaction, in particular in a skin graft, by providing a polypeptide that is substantially similar to a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis.

(2) Background of the Invention

Chlamydia species cause a variety of severe,
acute and chronic infections in humans, animals, and birds. Of particular importance to humans are Chlamydia trachomatis and Chlamydia psittaci. Particular serotypes of Chlamydia trachomatis have been shown to be the etiological agent of trachoma, nongonococcal urethritis, or lymphogranuloma venereum whereas Chlamydia psittaci causes psittacosis, a form of pneumonia. Nongonococcal urethritis is one of the most common sexually transmitted diseases in the United States which has made development of a vaccine for controlling the disease an important public health objective. However, even though considerable effort has been directed towards developing a vaccine to control nongonococcal urethritis, a vaccine that effectively controls nongonococcal urethritis has remained elusive. Recently, a third species of Chlamydia, pneumoniae, has been identified and has been implicated as causing a variety of disorders in humans including pneumonia, arthritis, and even degeneration associated with Alzheimer's disease.

While an effective vaccine for controlling Chlamydia-mediated diseases such as nongonococcal urethritis are not yet commercially available, several U.S. patents have disclosed Chlamydia vaccines. U.S. Patent No. 5,725,863 to Daniels et al discloses a vaccine for veterinary uses comprising essentially pure 40 to 140 kDa polypeptides of Chlamydia psittaci isolated from detergent solubilized membrane preparations. U.S. Patent Nos. 5,770,714 and 5,821,055 to Agabian et al disclose a vaccine consisting of the recombinant major outer membrane protein of Chlamydia trachomatis.

While the above vaccines are known, it would be desirable to have a more efficacious vaccine that is
easily produced. For example, *Yersinia pestis* which is unrelated to *Chlamydia* spp., encodes a polypeptide, LcrV, which when incorporated into a vaccine provided an efficacious vaccine against bubonic plague (Motin et al., Vaccines 97, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. pp. 185-190 (1997); Motin et al., Infect. Immunity 64: 4313-4318 (1996)). More recent research has shown that *Pseudomonas aeruginosa*, which is unrelated to both *Chlamydia* spp. and to *Yersinia* spp., encodes an LcrV analog, PcrV, which is able to provide a vaccine that is efficacious against virulent hospital (nosocomial) strains of *Pseudomonas aeruginosa*.

Therefore, it would be desirable to identify a polypeptide of *Chlamydia* spp. which could be used as a vaccine that is as efficacious against *Chlamydia* spp. as the above vaccines against *Y. pestis* and *P. aeruginosa*.

**SUMMARY OF THE INVENTION**

The present invention provides vaccines and methods for making the vaccines that protect a human or animal host against *Chlamydia* infection. In particular, the present invention provides a vaccine that elicits active immunity against *Chlamydia* which contains at least one epitope of a polypeptide that has an amino acid sequence that is substantially similar to a polypeptide encoded by open reading frame CT863 of *Chlamydia trachomatis*. The present invention further provides a DNA vaccine that elicits active immunity against *Chlamydia* comprising a DNA encoding at least one epitope of a polypeptide that is substantially similar to the polypeptide encodes by open reading frame CT863 of *Chlamydia trachomatis*. The present invention further
provides a vaccine that provides passive immunity to Chlamydia comprising polyclonal or monoclonal antibodies against at least one epitope of a polypeptide that is substantially similar to the polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis. Further still, the present invention provides a method for preventing an inflammatory reaction in a host by providing a polypeptide that is substantially similar to a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis. Thus, the present invention provides methods for using polypeptides that are substantially similar to the V antigen of Chlamydia trachomatis.

The present invention provides a vaccine for providing passive immunity to a Chlamydia infection comprising antibodies which are against at least one epitope of a V antigen that corresponds to a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis. In particular, a vaccine wherein the antibodies are selected from the group consisting of polyclonal antibodies and monoclonal antibodies against a V antigen wherein the V antigen has an amino acid sequence which substantially similar to the amino acid sequence set forth in SEQ ID NO.1. In a particular embodiment of the vaccine, the antibodies are against the epitope in a carboxyl terminus region of the V antigen. In a preferred embodiment of the vaccine, the vaccine is provided in a pharmaceutically accepted carrier.

Further, the present invention further provides a vaccine for active immunization of an individual against a Chlamydia infection comprising a polypeptide containing at least one epitope of a V antigen that corresponds to an epitope encoded by open reading frame CT863 of Chlamydia trachomatis. In
particular, the V antigen has an amino acid substantially similar to the amino acid sequence set forth in SEQ ID NO.1 which is encoded by a DNA sequence that is substantially similar to the DNA sequence set forth in SEQ ID NO.2. In one embodiment of the present invention, the V antigen is a recombinant polypeptide produced in a plasmid in a microorganism other than Chlamydia, preferably, in an E. coli. In a preferred embodiment, the vaccine is provided in a pharmaceutically accepted carrier.

Further, the present invention provides for a vaccine wherein the V antigen is provided as a fusion polypeptide wherein an amino end and/or a carboxyl end of the V antigen is fused to all or a portion of a polypeptide that facilitates the isolation of the V antigen from the microorganism in which the V antigen is produced. In a preferred embodiment, the polypeptide is selected from the group consisting of glutathione S-transferase, protein A, maltose binding protein, and polyhistidine.

The present invention also provides a vaccine for protecting an individual from a Chlamydia infection comprising a DNA that encodes at least one epitope of a polypeptide that has an amino acid sequence similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis. In particular, wherein the DNA encodes a polypeptide which has an amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1 which is encoded by a DNA that has a DNA sequence substantially similar to the DNA sequence set forth in SEQ ID NO.2. In a preferred embodiment, the DNA is operably linked to a promoter to enable transcription of the DNA in the cell of an individual. Preferably, the vaccine is provided in a pharmaceutically accepted carrier.
The present invention further provides a method for vaccinating an individual against a Chlamydia infection comprising: (a) providing a recombinant V antigen of Chlamydia produced from a microorganism culture wherein the microorganism contains a DNA that encodes a V antigen that has an amino acid sequence substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis; and (b) vaccinating the individual. Preferably, the vaccine is in a pharmaceutically accepted carrier. In a preferred embodiment, the DNA sequence is the DNA sequence set forth in SEQ ID NO.2.

In a preferred embodiment of the method, the recombinant V antigen is a fusion polypeptide which is fused at the amino terminus and/or carboxyl terminus to a polypeptide that facilitates the isolation of the recombinant V antigen. In particular, the polypeptide is all or a portion of the polypeptide selected from the group consisting of glutathione S-transferase, protein A, maltose binding protein, and polyhistidine. Further, the method includes producing the V antigen from a DNA which is in a plasmid in a microorganism wherein the DNA is operably linked to a promoter which enables transcription of the DNA to produce the recombinant V antigen for the vaccine.

The present invention further provides a method for vaccinating an individual against a Chlamydia infection comprising: (a) providing in a carrier solution a DNA in a plasmid which encodes at least on epitope of a polypeptide which has an amino acid sequence that is substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis; and (b) vaccinating the individual with the DNA in the carrier solution. In
particular, the DNA vaccine wherein the DNA has a DNA sequence that is substantially similar to the DNA sequence set forth in SEQ ID NO.2. Preferably, the DNA is in a carrier solution that accepted pharmaceutically for DNA vaccines. In a preferred embodiment, the DNA is operably linked to a promoter to enable transcription of the DNA in a cell of the individual.

The present invention further provides a method for providing passive immunity to a Chlamydia infection in an individual comprising: (a) providing antibodies selected from the group consisting of polyclonal antibodies and monoclonal antibodies which are against at least one epitope of a V antigen that has an amino acid sequence that is substantially similar to the amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis; and (b) inoculating the individual. Preferably, the antibodies are provided in a pharmaceutically accepted carrier. In a preferred embodiment the V antigen used to make the antibodies has an amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1.

Further still, the present invention provides a method for producing a polypeptide comprising: (a) providing a microorganism in a culture containing a DNA encoding a fusion polypeptide comprising at least one epitope of a V antigen that has an amino acid sequence substantially similar to the amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis and a polypeptide that facilitates isolation of the fusion polypeptide; (b) culturing the microorganism in a culture to produce the fusion polypeptide; and (c) isolating the fusion polypeptide. In particular, the V antigen portion of the fusion polypeptide has an amino acid sequence substantially
similar to the amino acid sequence set forth in SEQ ID NO.1. In one embodiment, the fusion polypeptide is isolated by affinity chromatography which can be affinity chromatography that comprises an IgG-linked resin when the polypeptide consists of all or a portion of protein A, an Ni²⁺ resin when the polypeptide is polyhistidine, amylose resin when the polypeptide is all or part of the maltose binding protein, or glutathione Sepharose 4B resin when the polypeptide is all or part of glutathione S-transferase.

Further still, the present invention provides a method for producing an antibody comprising: (a) providing a microorganism in a culture containing a DNA encoding a fusion polypeptide comprising at least one epitope of a V antigen that has an amino acid sequence that is substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis linked to a polypeptide that facilitates isolation of the fusion polypeptide; (b) culturing the microorganism in a culture to produce the fusion polypeptide; (c) isolating the fusion polypeptide; (d) producing the antibody from the polypeptide. In particular, the fusion polypeptide has an amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1. In a preferred embodiment, the polypeptide is removed from the V antigen portion of the fusion polypeptide.

And further still, the present invention provides a method for producing a monoclonal antibody comprising: (a) providing a microorganism in a culture containing a DNA encoding a fusion polypeptide comprising at least one epitope of a V antigen that has an amino acid sequence that is substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis linked to
a polypeptide that facilitates isolation of the fusion polypeptide; (b) culturing the microorganism in a culture to produce the fusion polypeptide; (c) isolating the fusion polypeptide; and (d) producing the monoclonal antibody from the polypeptide. In particular, the V antigen portion of the fusion polypeptide has an amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1. Preferably, the polypeptide is removed from the V antigen portion of the fusion polypeptide.

The present invention also provides a method for delaying an inflammatory reaction at an injury in an individual by administering to the injury a composition comprising a polypeptide comprising all or a portion of a V antigen that has an amino acid sequence that is substantially similar to the amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis. In a particular embodiment, the injury is a skin graft. In the method, the V antigen has an amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1. Preferably, the V antigen is in a pharmaceutically accepted carrier. In certain embodiments, the V antigen is a fusion protein which comprises all or a portion of a polypeptide selected from the group consisting of glutathione S-transferase, protein A, maltose binding protein, and polyhistidine.

Finally, the present invention provides a method for delaying or ameliorating an inflammatory reaction at an injury in an individual by administering to the injury a composition comprising a DNA encoding all or a portion of a V antigen that has an amino acid sequence substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis. In a particular embodiment, the
injury is a skin graft. In the method, the V antigen has an amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1. Preferably, the V antigen is in a pharmaceutically accepted carrier. Further, it is preferable that the DNA is operably linked to a promoter to enable the DNA to be transcribed in the cell of the individual.

Therefore, it is an object of the present invention to provide a vaccine that produces an active immunity in a vaccinee against a Chlamydia spp. infection.

It is also an object of the present invention to provide a vaccine that affords a vaccinee passive immunity against a Chlamydia spp. infection.

It is further still an object of the present invention to provide a method for delaying or ameliorating an inflammatory reaction at an injury in an individual.

DESCRIPTION OF THE DRAWINGS

Figure 1A is a genetic map of the relevant region of the Chlamydia trachomatis genome that has open reading frame CT863. The region shown is 10,000 bp and represents the region between nucleotides 1010775 and 1020775.

Figure 1B is a genetic map of the relevant portion of the Chlamydia trachomatis genome that has open reading frame CT863 and further shows the open reading frames ccrH and newly identified ccrG. The region shown is a 3,000 bp region contained within the 10,000 bp region shown in Figure 1A.

Figure 2 shows the alignment of the C-terminal part of LcrG from Yersinia enterocolitica and the identified portion of CcrG from Chlamydia trachomatis, serovar D. The alignment was performed using the
program CLUSTAL W (1.74), and the perfect matches, high similarity matches, and low similarity matches are designated as (*) , (:) , and (.), respectively. The heparin-binding motifs XBBXBX and BX, B (wherein B is a basic residue) that were identified within LcrG or were found in CcrG are shown above the LcrG and below the CcrG sequences. In the figure A is alanine, R is arginine, N is asparagine, D is aspartic acid, C is cysteine, Q is glutamine, E is glutamic acid, G is glycine, H is histidine, I is isoleucine, L is leucine, K is lysine, M is methionine, F is phenylalanine, P is proline, S is serine, T is threonine, W is Tryptophane, Y is tyrosine, and V is valine.

DESCRIPTION OF PREFERRED EMBODIMENTS

The V antigen (SEQ ID NO.1) of Chlamydia trachomatis is encoded by open reading frame CT863, the complement of which is set forth in SEQ ID NO.2 which consists of nucleotides 6534 to 7982 of a 14,378 bp DNA fragment having GenBank Accession No. AE001359 which is set forth in SEQ ID NO.3. The Chlamydia trachomatis DNA sequence in GenBank was also reported by Stephens et al. in Science 282: 754-759 (1998). As used herein, the complement means that nucleotide sequence which is the complement of the nucleotide sequence set forth in SEQ ID NOs. 1 and 2. The complement nucleotide sequence is read in its 5' to 3' direction. A gene map based on the GenBank sequence showing open reading frame (ORF) CT863 is shown in Figure 1A. ORF CT863 has been designated by the inventors to be gene ccrV which encodes polypeptide CcrV, hereinafter, "V antigen" will be used to refer to CcrV. The gene ccrV, as shown below, is part of an operon that includes Chlamydia genes ccrH and ccrG. This operon is shown in Figure 1B. While the V antigen disclosed herein is from Chlamydia trachomatis, the
present invention further includes V antigen analogs in other species of Chlamydia, which include but is not limited to, Chlamydia pneumoniae and Chlamydia psittaci.

The Chlamydia trachomatis V antigen as shown below is an analog to polypeptide LcrV which is encoded by the lcrV gene of Yersinia pestis. In Y. pestis, the lcrV is within a 70-kb low-calcium response (LCR) plasmid and is part of the lcrGVH-yopBD operon wherein lcrV is flanked by the lcrG and lcrH genes. The V antigen is also an analog to polypeptide PcrV which is encoded by the pcrV gene of Pseudomonas aeruginosa. Vaccines consisting of either LcrV or PcrV were effective in protecting animals against disease caused by Y. pestis or P. aeruginosa, respectively.

That CT863 encodes an analog of LcrV or PcrV was determined by an analysis of the complete genome of Chlamydia trachomatis, serovar D (GenBank Accession No. AE001273; SEQ ID NO.3). The GenBank sequence showed that an lcrH-like gene (hereinafter “ccrH”) was located in the 1010775 to 1020775 bp region of the chromosome (see map in Figure 1A). The polypeptide encoded by the lcrH-like gene is the complement of nucleotides 6016 to 6612 set forth in SEQ ID NO.3. The amino acid identity between the polypeptide LcrH of Yersinia spp. and the Chlamydia LcrH-like polypeptide (herein after “CcrH”) was identified in the GenBank sequence annotation. However, the ORPs adjacent to the ccrH gene were not identified. Since in Yersinia lcrH is a part of the lcrGVH-yopBD operon, the Chlamydia sequences that are located upstream of ccrH were analyzed for the presence of DNA sequences that could encode LcrG- and LcrV-like polypeptides. Because ORF CT863 was immediately upstream of ccrH, the CT863 region was identified as likely to encode a V antigen analogous to LcrV or PcrV.
However, since *Chlamydiae* is not related to either *Y. pestis* or *P. aeruginosa* it would not have been expected that a *Chlamydia* spp. would encode an analog of the LcrV or PcrV. Furthermore, even though CcrH appeared to be an analog to LcrH, the GenBank DNA sequence did not identify an ORF that could encode an LcrG analog. Finally, as shown herein, the V antigen has limited amino acid identity to the amino acid sequences for LcrV or PcrV which in turn have an amino acid identity to each other of only about 40%. Furthermore, the V antigen consists of 482 amino acids with a predicted molecular mass of 53.7 kDa and a pI of 5.08. In contrast, LcrV is 326 amino acids with a molecular mass of 37.2 kDa and a pI of 5.44 and PcrV is 294 amino acids with a molecular mass of 32.3 kDa and a pI of 4.77. The primary similarity between all three antigens is that they globular, acidic, and do not contain a known procaryote signal sequence. Therefore, the identity of the *Chlamydia* V antigen analog was not readily apparent by amino acid sequence comparisons or by comparing polypeptide sizes or isoelectric points.

Furthermore, the identity of the V antigen encoded by CT863 as an analog of LcrV and PcrV was not apparent from the annotated *Chlamydia* sequence provided by GenBank. That is because the 859 bp region between the start of ORF CT863 and the end of ORF XerD (complement of nucleotides 8843 to 9745 of SEQ ID NO.3) was left blank in the *Chlamydia* genome map (Figure 1A), but a tRNA Leu sequence (nucleotides 8351 to 8432 of SEQ ID NO.3) was placed in this area in the GenBank sequence annotation. Therefore, while a *Chlamydia* LcrH had been identified, a *Chlamydia* analog to LcrG had not been identified. Without an LcrG analog identified, there would be little reason to support a contention that ORF CT863 encoded an LcrV analog because the absence of an
LcrG analog would suggest that CcrH was not an analog of LcrH and that an H-V-G operon did not exist in Chlamydia.

The inventors discovered an additional ORF was in this area (as shown in the map in Figure 1B) that was located downstream of the tRNA Leu gene; however, a BLAST search did not show any significant homology between the putative protein encoded by this ORF and any amino acid sequence in GenBank. This ORF is presented in SEQ ID NO.4. However, unexpectedly the inventors identified upstream of the tRNA Leu gene a DNA sequence that in one of the six possible open reading frames encoded polypeptide with an LcrG-like amino acid sequence (SEQ ID NO.5). The start and stop codons for this gene could not be identified because of presumed errors in the GenBank sequence which also made identifying an ORF in the region difficult. Nevertheless, significant similarity was detected between the amino acid sequence of the polypeptide encoded in this region and the amino acid sequence of LcrG of Yersinia. Therefore, this region was designated ccrG and its approximate coding region is shown in Figure 1B. The degree of identity between 44 amino acids (about half of LcrG) comprising the two proteins was 25% perfect matches, 23% high similarity, and 11% low similarity as determined by the CLUSTAL W (1.74) program (Thompson et al., Nuc. Acids Res. 22: 4673-4680 (1994)). The first 33 amino acids of this alignment showed 33% identity and 51% similarity as determined by the BLAST program (Altschul et al., Nuc. Acids Res. 25: 3389-3402 (1997)). Recently, heparin-binding motifs were described within LcrG of Y. enterocolitica, which lead to interference by heparin with the translocation of Yop cytotoxic proteins into host cells by the type III secretion machine (Boyd et al., Mol. Microbiol. 27:
425-436 (1998)). The inventors' analysis also showed the presence of putative heparin-binding motifs within the identified portion of CcrG (Figure 2). This may explain the well-known ability of heparin sulfate to inhibit Chlamydia infections (Zhang and Stephens, Cell 69: 861-869 (1992)). Based on the above, this region was determined to contain the ccrG gene which encodes the CcrG polypeptide which is an analog of the LcrG of Yersinia.

Therefore, the inventors have identified an LcrG-like coding sequence downstream of CT863. This information, coupled with the identity of an LcrH-like encoding sequence led the inventors to identify CT863 as encoding V antigen, an analog to LcrV or PcrV. LcrG/PcrG and LcrH/PcrH are known to be required for translocation of virulence factors in Yersinia and Pseudomonas and antibodies directed against the intervening product (LcrV or PcrV) provides solid immunity against their respective bacterial species. Since gene function is highly conserved within the same operon of disparate bacteria, it is expected that because CT863 is flanked by ccrH and ccrG, it is part of an operon similar to the lcrGVH or pcrGVH operons and; therefore, encodes a V antigen that can provide solid immunity against Chlamydia. Just like both LcrV and PcrV which provide solid immunity even though both polypeptides show little identity to each other.

The identification of a gene in Chlamydia trachomatis that encodes an antigen (V antigen) that is part of an operon that has been shown previously in Y. pestis or P. aerugatosa to produce an antigen that provides for efficacious vaccines indicates that vaccines of the present invention which comprise the V antigen or antibodies against the V antigen can provide
in a human or animal immunity against \textit{Chlamydia} infection. Thus, the present invention provides vaccines against \textit{Chlamydia} which includes polypeptide vaccines, recombinant vector vaccines, DNA vaccines, and antibody vaccines.

The route of administration for the vaccines of the present invention can include, but is not limited to, intramuscular, intraperitoneal, intradermal, subcutaneous, intravenous, intraarterial, intraocular, and oral as well as transdermal or by inhalation or suppository. The preferred routes of administration include intramuscular, intraperitoneal, intradermal, and subcutaneous injection. The vaccine can be administered by means including, but not limited to, syringes, needle-less injection devices, or microprojectile bombardment gene guns (biolistic bombardment).

The vaccines of the present invention are formulated in pharmaceutically accepted carriers according to the mode of administration to be used. One skilled in the art can readily formulate a vaccine that comprises the polypeptide or DNA of the present invention. In cases where intramuscular injection is preferred, an isotonic formulation is preferred. Generally, additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol, and lactose. In particular cases, isotonic solutions such as phosphate buffered saline are preferred. The formulations can further provide stabilizers such as gelatin and albumin. In some embodiments, a vasco-constriction agent is added to the formulation. The pharmaceutical preparations according to the present invention are provided sterile and pyrogen free. However, it is well known by those skilled in the art that the preferred formulations for the pharmaceutically accepted carrier which comprise the vaccines of the present invention are those
pharmaceutical carriers approved in the regulations promulgated by the United States Food and Drug Administration or equivalent government agency in a foreign country for polypeptide, recombinant vector, antibody, and DNA vaccines intended for human applications, and the United States Department of Agriculture or equivalent agency in a foreign country for vaccines intended for veterinary applications. Therefore, the pharmaceutically accepted carriers for the commercial production of the vaccines according to the present invention are those carriers that are already approved or will at some future date be approved by the appropriate governmental agency in the United States of America or foreign country.

Inoculation of an individual is preferably by a single vaccination which in the case of polypeptide, recombinant vector, and DNA vaccines produces a full, broad immunogenic response. In another embodiment of the present invention, the individual is subjected to a series of vaccinations to produce a full, broad immune response. When the vaccinations are provided in a series, the vaccinations can be provided between about 24 hours apart to two weeks or longer between vaccinations. In certain embodiments, the individual is vaccinated at different sites simultaneously.

The vaccines of the present invention are generally intended to be a prophylactic treatment which prevents a Chlamydia from establishing an effective infection in an individual. However, the vaccines are also intended for the therapeutic treatment of individuals already infected with Chlamydia. For example, antibody vaccines of the present invention are suitable for therapeutic purposes. However, vaccines that provide active immunity have also been shown to be effective when given as a therapeutic treatment against
various diseases. Thus, the immunity that is provided by the present invention can be either active immunity or passive immunity and the intended use of the vaccine can be either prophylactic or therapeutic.

The vaccine that elicits active immunity in a host can be a polypeptide vaccine or a DNA vaccine which produces the polypeptide in a vaccinated host. Alternatively, the vaccine can be a recombinant microorganism vaccine that expresses the V antigen or a recombinant virus vector that expresses the V antigen.

Thus, in one embodiment of the present invention, the active immunity is provided by a vaccine that consists of the isolated V antigen or the V antigen as a fusion polypeptide wherein the amino and/or carboxyl terminus is fused to another polypeptide, preferably a polypeptide that facilitates isolation of the fusion polypeptide.

The fusion polypeptide comprising the vaccine is preferably produced in vitro in an expression system from a DNA that encodes the V antigen which is in a microorganism such as bacteria, yeast, or fungi; in a eukaryote cells such as mammalian or insect cells; or, in a virus expression vector such as adenovirus, poxvirus, herpesvirus, Simliki forest virus, baculovirus, bacteriophage, or sendai virus. In particular, suitable bacterial strains for producing the V antigen or V antigen as a fusion polypeptide include Escherichia coli, Bacillus subtilis, or any other bacterium that is capable of expressing heterologous polypeptides. Suitable yeast for expressing the V antigen or the V antigen as a fusion polypeptide include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Candida, or any other yeast capable of expressing heterologous polypeptides. Methods for using the aforementioned and the like to produce recombinant
polypeptides for vaccines are well known in the art. For any of the above, transformed host cells are cultured under conditions which produce the V antigen or V antigen fusion polypeptide. The resulting expressed polypeptide can be purified from the culture, either the medium or cell extracts, using purification methods such as gel filtration, affinity chromatography, ion exchange chromatography, or centrifugation. For any of the above expression methods, the V antigen has the amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1 which is encoded by the DNA sequence with a nucleotide sequence substantially similar to the nucleotide sequence set forth in SEQ ID NO.2. It is understood that the present invention further includes V antigen analogs for other Chlamydia spp. and the polypeptide produced can comprise a polypeptide that includes only those V antigen epitopes which confer protective immunity against Chlamydia.

DNA encoding the V antigen can be obtained from a genome preparation using a polymerase chain reaction (PCR) method using DNA primers that correspond to the nucleotide sequences encoding the amino and carboxyl ends of the V antigen. Preferably the 5' ends of the primers contain a restriction enzyme site that facilitates the subsequent steps of constructing a V antigen expression system. Alternatively, the DNA primers can correspond to an internal region of the nucleotide sequence encoding the V antigen for producing a DNA encoding a particular epitope of the V antigen. Primer design and PCR methods are well known in the art.

In a preferred embodiment, the DNA is in a plasmid and is operably linked to a promoter which effects the expression of the V antigen in a microorganism, preferably E. coli. As used herein, the
term "operably linked" means that the polynucleotide of the present invention and an expression control sequence, i.e., transcription promoter and termination sequences, are situated in a vector or cell such that the expressed polypeptide is expressed by the host cell which has been transformed or transfected with the operably linked polynucleotide and expression control sequence. Methods for operably linking DNA to The V antigen so produced is isolated from the microorganism by methods well known in the art. Cloning DNA encoding the V antigen to make recombinant DNA plasmids and methods for expressing polypeptides from recombinant DNA plasmids are well known in the art. Expression of the V antigen in a microorganism enables the V antigen to be produced using fermentation technologies which are used commercially for producing large quantities of recombinant polypeptides.

To facilitate purification of the V antigen, a fusion polypeptide is made wherein the V antigen is linked to another polypeptide which enables purification by affinity chromatography. Preferably, fusion polypeptide is made using one of the aforementioned expression systems. Therefore, the DNA encoding the V antigen is linked to a DNA encoding a second polypeptide to produce a fusion polypeptide wherein the amino and/or carboxyl terminus of the V antigen is fused to a polypeptide which allows for the simplified recovery of the V antigen as a fusion polypeptide. The simplified recovery also prevents the V antigen from being degraded during purification. While a vaccine comprising the fusion polypeptide is efficacious, in some instances it can be desirable to remove the second polypeptide after the purification. Therefore, it is also contemplated that the fusion polypeptide comprise a cleavage site at the junction between the V antigen and the polypeptide.
The cleavage site consists of an amino acid sequence that is cleaved with an enzyme specific for the amino acid sequence of that site. Examples of such cleavage sites that are contemplated include the enterokinase cleavage site which is cleaved by enterokinase, the factor Xa cleavage site which is cleaved by factor Xa, and the GENENASE cleavage site which is cleaved by GENENASE (GENENASE is a trademark of New England Biolabs, Beverly, Massachusetts).

An example of a procaryote expression system for producing the V antigen is the Glutathione S-transferase (GST) Gene Fusion System available from Amersham Pharmacia Biotech, Piscataway, New Jersey which uses the pGEX-4T-1 expression vector plasmid. The DNA encoding the V antigen is fused in frame with the GST gene. The GST part of the fusion polypeptide allows the rapid purification of the fusion polypeptide using glutathione Sepharose 4B affinity chromatography. After purification, the GST portion of the fusion polypeptide can be removed by cleavage with a site-specific protease such as thrombin or factor Xa to produce a polypeptide free of the GST gene. The V antigen free of GST is produced by a second round of glutathione Sepharose 4B affinity chromatography.

Another example for producing the V antigen is a method which links in-frame with the gene encoding the V antigen, codons that encode polyhistidine. The polyhistidine preferably comprises six histidine residues which allows purification of the fusion polypeptide by metal affinity chromatography, preferably a nickel chromatography. To produce the native V antigen free of the polyhistidine, a cleavage site such as an enterokinase cleavage site is fused in frame between the codons encoding the polyhistidine and the codons encoding the V antigen. The native polypeptide
free of the polyhistidine is made by removing the polyhistidine by cleavage with enterokinase. The V antigen free of the polyhistidine is produced by a second round of metal affinity chromatography. This method was shown to be useful for preparing the LcrV antigen of *Y. pestis* which was disclosed in Motin et al. Infect. Immun. 64: 4313-4318 (1996) which is hereby incorporated herein by reference. The Xpress System available from Invitrogen, Carlsbad, California is an example of a commercial kit which is available for making and then isolating polyhistidine-polypeptide fusion proteins.

A method further still for producing the V antigen is disclosed in Motin et al., Infect. Immun. 64: 3021-3029 (1995) which discloses a DNA encoding a fusion polypeptide consisting of the DNA encoding LcrV of *Yersinia pestis* linked to DNA encoding a portion of protein A wherein DNA encoding an enterokinase cleavage site is interposed between the DNA encoding protein A and the LcrV. The above reference is hereby incorporated herein by reference. The protein A enables the fusion polypeptide to be isolated by IgG affinity chromatography, and the V antigen to be made free of the protein A by cleavage with an enterokinase. The protein A is then removed by a second round of IgG affinity chromatography.

Another method for producing polypeptide vaccines against *Chlamydia* is based on methods disclosed in U. S. Patent No. 5,725,863 to Daniels et al which is hereby incorporated herein by reference. The method in the above patent can be used to make the V antigen vaccine of the present invention which consists of an enterotoxin which has inserted therein upwards of 100 amino acid residues of the V antigen. Another method that can be used to make the polypeptide vaccines of the
present invention is disclosed in U.S. Patent No. 5,585,100 to Mond et al., which is hereby incorporated herein by reference, which provides methods for making various fusion polypeptide vaccines. Further methods are disclosed in U.S. Patent No. 5,589,384 to Liscombe which is hereby incorporated herein by reference. Finally, the pMAL Fusion and Purification System available from New England Biolabs is another example of a method for making a fusion polypeptide wherein a maltose binding protein is fused to the V antigen. The maltose binding protein facilitates isolation of the fusion polypeptide by amylose affinity chromatography. The maltose binding protein can subsequently be released by cleavage with any of the aforementioned cleavage enzymes.

While bacterial methods are used to produce the V antigen, it can be desirable to produce the V antigen in a eukaryote expression system. A particularly useful system is the baculovirus expression system which is disclosed in U.S. Patent No. 5,229,293 to Matsuura et al. which is hereby incorporated herein by reference. Baculovirus expression vectors suitable to produce the V antigen are the pBac and pMbac vectors from Stratagene; and the Bac-N-Blue vector, the pBlueBac4.5 vector, pBlueBacHis2-A,B,C, and the pMelBac available from Invitrogen, Carlsbad, California.

Another eukaryote system useful for expressing the V antigen is a yeast expression system such as the ESP Yeast Protein Expression and Purification System available from Stratagene. Another yeast expression system is any one of the Pichia-based Expression systems from Invitrogen. Mammalian expression systems are also embraced by the present invention. Examples of mammalian expression systems are the LacSwitch II system, the pBK Phagemid, pXT1 vector system, and the
pSG5 vector system from Stratagene; the pTarget mammalian expression vector system, the pSI mammalian expression vector, pCI mammalian expression vector, and pAdVantage vectors available from Promega Corporation, Madison, Wisconsin; and the Ecdysone-Inducible Mammalian Expression System, pCDM8, pcDNA1.1, and pcDNA1.1/Amp available from Invitrogen.

Another method for producing the V antigen in a eukaryote expression system is to insert the DNA encoding the V antigen into the genome of the eukaryote cell or in a eukaryote virus expression vector such as herpesvirus, poxvirus, or adenovirus to make a recombinant virus that expresses the V antigen. The recombinant virus vectors are used to infect mammalian cells wherein the V antigens are produced in the cell.

U.S. Patent No. 5,223,424 to Cochran et al. which is hereby incorporated herein by reference provides methods for inserting genes into herpesvirus expression vectors. U.S. Patent Nos. 5,338,683 and 5,494,807 to Paoletti et al. and U.S. Patent No. 5,935,777 to Moyer et al. which are hereby incorporated herein by reference provide methods for inserting genes into poxvirus expression vectors such as vaccinia virus, entomopoxvirus, and canary poxvirus. In another embodiment, the genes encoding the V antigen can be inserted into a defective virus such as the herpesvirus amplicon vector which is disclosed in U.S. Patent No. 5,928,913 to Efstathiou et al. which is hereby incorporated herein by reference.

In any of the aforementioned virus vectors, the genes encoding the V antigen are operably linked to a eukaryote promoter at the 5' end of the DNA encoding the protein and a eukaryote termination signal and poly(A) signal at the 3' end of the gene. Examples of such promoters are the cytomegalovirus immediate-early (CMV) promoter, the Rous sarcoma virus long terminal repeat
(RSV-LTR) promoter, the simian virus 40 (SV40) immediate-early promoter, and inducible promoters such as the metallothionein promoter. An example of a DNA having a termination and poly(A) signal is the SV40 late poly(A) region. Another example of a viral expression system suitable for producing the V antigen is the Sindbis Expression system available from Invitrogen. The use of these commercially available expression vectors and systems are well known in the art.

While subunit vaccines comprising the V antigen generally provide good humoral protection, it can be desirable to provide the V antigen as a component of a recombinant vector vaccine. Therefore, the present invention further embraces recombinant virus vector vaccines wherein DNA encoding the V antigen is inserted into a recombinant virus vector. In one embodiment of the recombinant virus vector vaccine, the DNA encoding the V antigen are inserted into a herpesvirus vector according to the method taught by Cochran et al. in U.S. Patent No. 5,233,424 which is hereby incorporated herein by reference. It is particularly desirable to have a recombinant virus vector vaccine against Chlamydia that is fetal safe. U.S. Patent Nos. 5,741,696 and 5,731,188 to Cochran et al., which are hereby incorporated herein by reference, teach methods for making and using live recombinant herpesvirus vaccine vectors which are fetal safe.

Other recombinant virus vector vaccines embraced by the present invention, include but are not limited to, adenovirus, adeno-associated virus, parvovirus, and various poxvirus vectors to express the V antigen. For example, U.S. Patent Nos. 5,338,683 and 5,494,807 to Paoletti et al. teach recombinant virus vaccines consisting of either vaccinia virus or canary poxvirus expressing foreign antigens; and U.S. Patent
No. 5,266,313 to Esposito et al. teaches recombinant raccoon poxvirus vectors expressing foreign antigens. Therefore, the present invention embraces recombinant poxvirus vaccines that express the V antigen made according to the methods taught in any one of U.S. Patent Nos. 5,338,683; 5,494,807; and 5,935,777 which are hereby incorporated herein by reference.

While the above refer to DNA sequences encoding the V antigen, the present invention also includes RNA sequences for encoding the V antigen. In particular, the present invention encompasses RNA expression systems, such as those based on RNA viruses, for expressing the V antigen or portion thereof from an RNA or PNA molecule having a sequence substantially similar to the DNA sequence set forth in SEQ ID NO.2. The RNA sequence would correspond to the complement of the DNA sequence in SEQ ID NO.2.

The present invention further embraces vaccines that comprise the V antigen or particular epitopes of the V antigen as components of a heat-stable spore delivery system made according to the method taught in U.S. Patent No. 5,800,821 to Acheson et al. which is hereby incorporated herein by reference. Therefore, the present invention provides a genetically engineered a bacterial cell with DNA encoding the V antigen. When the recombinant bacterial spore vaccine is orally administered to the individual, the spores germinate in the gastrointestinal tract of the animal and the bacteria expresses the V antigen which comes into contact with the animal's immune system and elicit an immune response. The vaccine has the advantage of being heat stable; therefore, it can be stored at room temperature for an indefinite period of time.

Another embodiment of the Chlamydia vaccine is a DNA vaccine which elicits an active immune response in
an individual. The DNA vaccine consists of DNA having a DNA sequence substantially similar to the DNA sequence in SEQ ID NO.2 which encodes the V antigen. The DNA encoding the V antigen is operably linked at or near its start codon to a promoter that enables transcription of the V antigen from the DNA when the DNA is in the cells of the individual. Preferably, the DNA is in a plasmid. Promoters for expression of DNAs in DNA vaccines are well known in the art and include among others such promoters as the RSV LTR promoter, the CMV immediate early promoter, the SV40 T antigen promoter. It is further preferred that the DNA is operably linked at the or near the termination codon of the sequence encoding V antigen to a DNA fragment comprising a transcription termination signal and poly(A) recognition signal. Preferably, the vaccine is in an accepted pharmaceutical carrier or in a lipid or liposome carrier similar to those disclosed in U.S. Patent No. 5,703,055 to Pelgner which is hereby incorporated herein by reference. The DNA can be provided to the individual by a variety of methods such as intramuscular injection, intrajet injection, or biolistic bombardment. Making DNA vaccines and methods for their use are provided in U.S. Patent Nos. 5,589,466 and 5,580,859, both to Pelgner, which are hereby incorporated herein by reference. Finally, a method for producing pharmaceutical grade plasmid DNA is taught in U.S. Patent No. 5,561,064 to Marquet et al. which is hereby incorporated herein by reference.

Therefore, using the abovementioned methods, DNA vaccines that express the V antigen are made and used to vaccinate equines against Chlamydia. The advantage of the DNA vaccine is that the DNA is conveniently propagated as a plasmid which is a simple and inexpensive means for producing a vaccine, and since
the vaccine is not live, the regulatory difficulties associated with getting recombinant virus vaccines approved are not present.

It should be understood while the polypeptide produced for the polypeptide vaccine or by the DNA vaccine can be the entire V antigen, the present invention also includes polypeptide and DNA vaccines wherein the vaccine consists of a subfragment of the V antigen which comprises one or more epitopes of the V antigen or a DNA encoding one or more epitopes of the V antigen. For example, the vaccine can consist of a subfragment of the region near or at the carboxyl terminus of the V antigen.

Furthermore, the polypeptide and DNA vaccines of the present invention can comprise synthetically produced polypeptides or DNA which are made by chemical synthesis methods well known in the art.

While the DNA and polypeptide provided herein is from Chlamydia trachomatis, the present invention further encompasses the V antigen or CT863 homologue in other species of Chlamydia such as Chlamydia pneumoniae and Chlamydia psittaci. Furthermore, it is anticipated that the vaccines and methods disclosed herein are useful for other bacterial species that are determined to encode a V antigen that is part of an operon that is flanked by genes that have sequences substantially similar to ccrG and ccrH.

In another embodiment of the present invention, the vaccine provides passive immunity to Chlamydia. A vaccine that elicits passive immunity against Chlamydia consists of polyclonal antibodies or monoclonal antibodies that are against the V antigen of Chlamydia.

To make a passive immunity vaccine comprising polyclonal antibodies, V antigen or an epitope therefrom
is injected into a suitable host for preparing the antibodies, preferably the host is a horse, swine, rabbit, or goat. Methods for producing polyclonal antibody vaccines from these hosts are well known in the art. By way of brief example, the V antigen is admixed with an adjuvant such as Freund’s complete or less toxic TiterMax available from CytRx Corp., Norcross, Georgia following the procedure disclosed by Nakajima and Brubaker in *Infect. Immun.* 61: 23-31 (1993) and Une and Brubaker in *J. Immunol.* 133: 226-2230 (1984) for the preparation of polyclonal antibodies against LcrV of *Y. pestis* isolated from the organism or as a recombinant protein as disclosed in Motin et al, *Infect. Immun.*, 62: 4192-4201 (1994). In a preferred embodiment, the vaccine comprises antibodies against one or more epitopes within the carboxyl one-third of the polypeptide.

The passive immunity vaccine can comprise one or more monoclonal antibodies against one or more epitopes of the V antigen. Methods and hybridomas for producing monoclonal antibodies are well known in the art. Particularly relevant methods for producing monoclonal antibodies against LcrV have been disclosed by Brubaker in *Contrib. Microbiol. Immunol.* 12: 127-133 (1991). These methods are suitable for producing monoclonal antibodies against particular epitopes of the V antigen of *Chlamydia*. In a preferred embodiment, the vaccine comprises one or more monoclonal antibodies, each against a particular epitope within the carboxyl one-third of the polypeptide. While monoclonal antibodies can be made using hybridoma technologies well known in the art, the monoclonal antibodies against the *Chlamydia* V antigen can also be made according to phage display methods as disclosed in U.S. Patent No. 5,977,322 to Marks et al which is hereby incorporated.
herein by reference and humanized antibodies against Chlamydia V antigen which can be made according to methods disclosed in U.S. Patent Nos. 5,693,762 and 5,693,761 both to Queen et al which are hereby incorporated herein by reference. A phage display kit that is useful for making a monoclonal antibodies is the Recombinant Phage Antibody System available from Amersham Pharmacia Biotech.

The V antigen can also be used as a treatment for the suppression of syngenic grafts and allograft rejection following the method disclosed by Motin et al. in Transplant. 63: 1040-1042 (1997) and Motin et al in Vaccines 97, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. pp. 185-190 (1997) which showed that the LcrV antigen of Y. pestis was able to postpone inflammation known to be associated with recognition and destruction of foreign tissues by T lymphocytes and elimination of initial wound trauma. The LcrV delayed the onset of graft rejection in allografts. This delay was significant, because it allowed the graft to undergo immediate vascularization without significant wound trauma. In syngenic grafts, the immediate vascularization of the graft accelerated the healing of the graft. Furthermore, the LcrV was speculated to facilitate the healing of other types of wounds by suppressing pro-inflammatory cytokines. As shown above, the V antigen is presumed to be an analog of LcrV; therefore, it is believed that the V antigen of the present invention is useful as a therapeutic agent that is capable of minimizing inflammation and the associated deleterious facets of cell-mediated immunity. Preferably, the V antigen is given by given intraperoneal injections. The amount of V antigen that is useful for exerting an anti-inflammatory effect and accelerate wound healing is between about 50 to 200 µg
of V antigen, preferably about 100 μg of V antigen.

Finally, the antibodies of the present invention can comprise a diagnostic assay which is used to determine whether an individual is infected with Chlamydia. Preferably, the diagnostic assay is an ELISA-based assay.

While the present invention is described herein with reference to illustrated embodiments, it should be understood that the invention is not limited hereto. Those having ordinary skill in the art and access to the teachings herein will recognize additional modifications and embodiments within the scope thereof. Therefore, the present invention is limited only by the Claims attached herein.
WE CLAIM:

A vaccine for providing passive immunity to Chlamydia infection comprising antibodies which are against at least one epitope of a V antigen that has an amino acid sequence that is substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis.

-2-

The vaccine of Claim 1 wherein the V antigen has an amino acid sequence which is substantially similar to the amino acid sequence set forth in SEQ ID NO.1.

-3-

The vaccine of Claim 1 wherein the antibodies are selected from the group consisting of polyclonal antibodies and monoclonal antibodies.

-4-

The vaccine of Claim 1 wherein the antibodies are against the epitope which is from a region at or near a carboxyl terminus of the V antigen.

-5-

The vaccine of claim 1 wherein the vaccine is provided in a pharmaceutically accepted carrier.
A vaccine for active immunization of an individual against a Chlamydia infection comprising at least one epitope of a V antigen that has an amino acid sequence that is substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis.

The vaccine of Claim 6 wherein the V antigen has an amino acid substantially similar to the amino acid set forth in SEQ ID NO.1.

The vaccine of Claim 6 wherein the V antigen is a recombinant polypeptide produced in a plasmid in a microorganism other than Chlamydia.

The vaccine of Claim 8 wherein the microorganism is an E. coli.

The vaccine of Claim 6 wherein the V antigen is encoded by a DNA sequence that is substantially similar to the DNA sequence set forth in SEQ ID NO.2.

The vaccine of Claim 6 wherein the V antigen is a fusion polypeptide wherein an amino end or a carboxyl end of the V antigen is fused to all or a portion of a polypeptide that facilitates isolation of the V antigen from the microorganism in which the V antigen is produced.
The vaccine of Claim 11 wherein the polypeptide is selected from the group consisting of glutathione S-transferase, protein A, maltose binding protein, and polyhistidine.

The vaccine of Claim wherein the vaccine is provided in a pharmaceutically accepted carrier.

A vaccine for protecting an individual from a Chlamydia infection comprising a DNA that encodes at least one epitope of a polypeptide that has an amino acid sequence that is substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis.

The vaccine of Claim 14 wherein the DNA is operably linked to a promoter to enable transcription of the DNA in a cell of an individual.

The vaccine of Claim 14 wherein the DNA encodes the polypeptide which has an amino acid sequence substantially similar to the amino acid set forth in SEQ ID NO.1.

The vaccine of Claim 14 wherein the DNA has a DNA sequence substantially similar to the DNA sequence set forth in SEQ ID NO.2.
The vaccine of Claim 14 wherein the vaccine is provided in a pharmaceutically accepted carrier.

A method for vaccinating an individual against a Chlamydia infection comprising:

(a) providing a recombinant V antigen of Chlamydia produced from a microorganism culture wherein the microorganism contains a DNA that encodes a V antigen that has an amino acid sequence that is substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis; and

(b) vaccinating the individual.

The method of Claim 19 wherein the recombinant V antigen is in a pharmaceutically accepted carrier.

The method of Claim 19 wherein the DNA has the DNA sequence substantially similar to the DNA sequence set forth in SEQ ID NO.2.

The method of Claim 19 wherein the recombinant V antigen has an amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1.
The method of Claim 19 wherein the recombinant V antigen is a fusion polypeptide which is fused at the amino terminus or carboxyl terminus to a polypeptide that facilitates the isolation of the recombinant V antigen.

The method of Claim 19 wherein the polypeptide includes all or a portion of the polypeptide selected from the group consisting of glutathione S-transferase, protein A, maltose binding protein, and polyhistidine.

The method of Claim 19 wherein the DNA is in a plasmid in a microorganism wherein the DNA is operably linked to a promoter which enables transcription of the DNA to produce the recombinant V antigen for the vaccine.

A method for vaccinating an individual against a Chlamydia infection comprising:

(a) providing in a carrier solution a DNA in a plasmid which encodes at least one epitope of a polypeptide that has an amino acid sequence that is substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis; and

(b) vaccinating the individual with the DNA in the carrier solution.
The method of Claim 26 wherein the DNA has a DNA sequence that is substantially similar to the DNA sequence set forth in SEQ ID NO.2.

The method of Claim 26 wherein the carrier solution is a saline solution.

The method of Claim 26 wherein the polypeptide has an amino acid sequence that is substantially similar to the amino acid sequence in SEQ ID NO.1.

The method of Claim 26 wherein the DNA is operably linked to a promoter to enable transcription of the DNA in a cell of the individual.

A method for providing passive immunity to a Chlamydia infection in an individual comprising:

(a) providing antibodies against at least one epitope of a V antigen that has an amino acid sequence that is substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis wherein the antibodies are selected from the group consisting of polyclonal antibodies and monoclonal antibodies; and

(b) inoculating the individual.

The method of Claim 31 wherein the antibodies are provided in a pharmaceutically accepted carrier.
The method of Claim 31 wherein the V antigen has an amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1.

The method of Claim 31 wherein the DNA has a DNA sequence substantially similar to the DNA sequence set forth in SEQ ID NO.2.

A method for producing a polypeptide comprising:

(a) providing a microorganism in a culture containing a DNA encoding a fusion polypeptide comprising at least one epitope of a V antigen that has an amino acid sequence that is substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis and a polypeptide that facilitates isolation of the fusion polypeptide;

(b) culturing the microorganism in a culture to produce the fusion polypeptide; and

(c) isolating the fusion polypeptide.

The method of Claim 35 wherein isolating the fusion polypeptide is by affinity chromatography.

The method of Claim 36 wherein the polypeptide is all or a portion of protein A and the affinity chromatography comprises an IgG-linked resin.
The method of Claim 36 wherein the polypeptide is polyhistidine and the affinity chromatography comprises a Ni²⁺ resin.

The method of Claim 36 wherein the polypeptide is glutathione S-transferase and the affinity chromatography comprises a glutathione Sepharose 4B resin.

The method of Claim 36 wherein the polypeptide is maltose binding protein and the affinity chromatography comprises an amylose resin.

The method of Claim 36 wherein the portion of the fusion polypeptide from Chlamydia has an amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1.
A method for producing an antibody comprising:

(a) providing a microorganism in a culture containing a DNA encoding a fusion polypeptide comprising at least one epitope of a V antigen that has an amino acid sequence substantially similar to the amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis and a polypeptide that facilitates isolation of the fusion polypeptide;

(b) culturing the microorganism in a culture to produce the fusion polypeptide;

(c) isolating the fusion polypeptide;

(d) producing the antibody from the polypeptide.

A method for producing a monoclonal antibody comprising:

(a) providing a microorganism in a culture containing a DNA encoding a fusion polypeptide comprising at least one epitope of a V antigen that corresponds to a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis and a polypeptide that facilitates isolation of the fusion polypeptide;

(b) culturing the microorganism in a culture to produce the fusion polypeptide;

(c) isolating the fusion polypeptide;

(d) producing the monoclonal antibody from the polypeptide.

The method of Claim 42 or 43 wherein isolating the fusion polypeptide is by affinity chromatography.
-41-

The method of Claim 42 or 43 wherein the polypeptide is all or a portion of protein A and the affinity chromatography comprises an IgG-linked resin.

-46-

The method of Claim 42 or 43 wherein the polypeptide is polyhistidine and the affinity chromatography comprises a Ni²⁺ resin.

-47-

The method of Claim 42 or 43 wherein the polypeptide is glutathione S-transferase and the affinity chromatography comprises a glutathione Sepharose 4B resin.

-48-

The method of Claim 42 or 43 wherein the polypeptide is maltose binding protein and the affinity chromatography comprises an amylose resin.

-49-

The method of Claim 42 or 43 wherein the portion of the fusion polypeptide from Chlamydia has an amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1.

-50-

A method for delaying an inflammatory reaction at an injury in an individual by administering to the injury a composition comprising a polypeptide comprising all or a portion of a V antigen that has an amino acid sequence that is substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis.
The method of Claim 50 wherein the injury is a skin graft.

The method of Claim 50 wherein the V antigen has an amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1.

The method of Claim 50 wherein the V antigen is a fusion protein which comprises all or a portion of a polypeptide selected from the group consisting of glutathione S-transferase, protein A, maltose binding protein, or polyhistidine.

The method of Claim 50 wherein the V antigen is in a pharmaceutically accepted carrier.

A method for delaying an inflammatory reaction at an injury in an individual by administering to the injury a composition comprising a DNA encoding all or a portion of a V antigen that has an amino acid sequence substantially similar to an amino acid sequence of a polypeptide encoded by open reading frame CT863 of Chlamydia trachomatis.

The method of Claim 55 wherein the injury is a skin graft.
The method of Claim 55 wherein the DNA encodes the V antigen which has an amino acid sequence substantially similar to the amino acid sequence set forth in SEQ ID NO.1.

The method of Claim 55 wherein the V antigen is in a pharmaceutically accepted carrier.

The method of Claim 55 wherein the DNA is operably linked to a promoter to enable the DNA to be transcribed in the cell of the individual.
**SEQUENCE LISTING**

110. Brubaker, Robert  
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130. RECOMBINANT CHLAMYDIA VACCINE  
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213. Chlamydia trachomatis  

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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(?) : Please See Extra Sheet.
US CL : Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
WEST, MEDLINE, BIOSIS, EMBASE, Sequence databases.
V antigen; SEQ ID NO: 1 and 2; Chlamydia trachomatis; CT365, inventor's names.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C. See patent family annex.

Date of the actual completion of the international search
25 JANUARY 2001

Date of mailing of the international search report
APR 05 2001

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-5280

Authorized officer
S. DEVJ Ph.D.
Telephone No. (703) 308-0196

Form PCT/ISA/910 (second sheet) (July 1998)*
**INTERNATIONAL SEARCH REPORT**

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(9)(a) for the following reasons:

1. □ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. X Claims Nos.: 13 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. X As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

□ The additional search fees were accompanied by the applicant's protest.

□ No protest accompanied the payment of additional search fees.
A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):
A61K 59/118, 59/08, 59/00, 45/00, 59/40, 51/70, 58/00; C07K 1/00, 16/00; C12P 21/08, 21/04, 1/04; C07H 21/04;
C18N 15/00; G01N 25/55.

A. CLASSIFICATION OF SUBJECT MATTER:
US CL:
426/261.1, 254.1, 190.1, 184.1, 388.1, 159.1, 150.1, 164.1; 514/44, 2; 556/23.7; 456/170, 71.1, 69.2; 550/350, 827,
387.9, 388.2, 388.4, 389.1, 389.5; 456/548.

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.
426/261.1, 254.1, 190.1, 184.1, 388.1, 159.1, 150.1, 164.1; 514/44, 2; 556/23.7; 455/170, 71.1, 69.2; 550/350, 827,
387.9, 388.2, 388.4, 389.1, 389.5; 456/548.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single
inventive concept under PCT Rule 15.1. In order for all inventions to be searched, the appropriate additional search
fees must be paid.

Group I, claims 1-5 and 31-34, drawn to a vaccine comprising antibodies against a V antigen of SEQ ID NO: 1 of
Chlamydia trachomatis and a method of using the same for providing passive immunity.

Group II, claims 6-19 and 20-25, drawn to a comprising a V antigen of SEQ ID NO: 1 of Chlamydia trachomatis and a
method of using the for vaccination.

Group III, claims 14-18 and 26-30, drawn to a vaccine comprising a DNA sequence of SEQ ID NO: 2 of Chlamydia
trachomatis and a method of using the for vaccination.

Group IV, claims 26-41, drawn to a method of using the DNA for producing the polypeptide.

Group V, claims 42-45, drawn to a method of using the polypeptide for producing an antibody.

Group VI, claims 46-48, drawn to a method for delaying an inflammatory reaction by administering a polypeptide
comprising the V antigen of Chlamydia trachomatis.

Group VII, claims 55-59, drawn to a method for delaying an inflammatory reaction by administering a DNA that
encodes a V antigen of Chlamydia trachomatis.

Claim 15 is not included in any of the groups above because it appears to be a dependent claim which is not
drafted in accordance with the second and third sentences of Rule 6.4(a).

The inventions listed as Groups I through VII do not relate to a single inventive concept under PCT Rule
15.1 because, under PCT Rule 15.2, they lack the same or corresponding special technical features for the following
reasons:

The special technical feature of the instant invention is a polynucleotide of SEQ ID NO: 2 and the
polypeptide encoded by the same. Inventions I, II and III are drawn respectively to three structurally, biologically and
immunogenically distinct products: an antibody, a polypeptide and a polynucleotide and the first method of using the
same. Inventions IV, V, VI and VII are drawn to a subsequent method of use of these products. Although the
polynucleotide or polypeptide product of the invention and method of using the product is a permitted combination
under PCT Rule 15.2, in the instant case, the special technical feature is already disclosed in the art, for instance, by
Stephens et al. Science 282: 754-759, 1998, and therefore is not a unifying feature. Clearly, the special technical feature
is not a unifying feature.