The Potential of *Lactobacillus* as a Carrier of *Chlamydia trachomatis* Cryptic Plasmid Antigens in Chlamydia Vaccine Development.

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ABSTRACT

Chlamydia trachomatis genital infection is a prevalent bacterial sexually transmitted disease that can have serious consequences such as infertility, ectopic pregnancy, and pelvic inflammatory disease if it goes untreated. Current vaccinology strategies are attempting to produce an effective vaccine that would confer immunity against genital chlamydial infection and eliminate chlamydial pathology. *Chlamydia trachomatis* possesses a cryptic 7.5-kb plasmid of unknown function. The Chlamydia cryptic plasmid (pCT) encodes eight putative open reading frames (ORFs), designated pORF1 to -8. pORF 5 encodes a 28-kDa protein, pgp3, with unknown function. The goal is to elucidate the role of CT cryptic plasmid in pathogenesis of Chlamydia by identifying immunopathogenic cryptic plasmid antigens that drive Chlamydia pathology. We hypothesize that cryptic plasmid antigens maybe used in the development of an effacious vaccine against Chlamydial genital infection. We used a genetic approach to assess the role of the functional genes in CT cryptic plasmid in the pathogenesis of infertility. *Lactobacillus,* a commensal bacterium of the human gastrointestinal flora, was used as a surrogate vehicle to express CT plasmid genes. In our laboratory, we have evaluated the effect of Chlamydia cryptic plasmid in fertility, and found that plasmid deficient Chlamydia does not cause pathology. Additionally, we have isolated the eight open reading frames, and developed a live recombinant vaccine scheme that utilizes *Lactobacillus fornicalis* as a live delivery vehicle of CT cryptic plasmid pgp3. A vaccine encompassing live recombinant *Lactobacillus* with CT cryptic plasmid antigen may possibly induce a strong cell-mediated and humoral immune response in a mouse model protecting against *Chlamydia trachomatis* challenge and pathology. Our vaccine scheme can be used for general application of vaccinology efforts towards other diseases such as prostate cancer. Supported by NIH grants GM08247 and A141231.

INTRODUCTION

Chlamydia genital infection is a caused by the bacterium, *Chlamydia trachomatis*. It is the most frequently reported bacterial sexually transmitted disease in the United States estimated at 4 million infections annually with prevalence rates of higher than 10% in sexually active adolescent females. In women, untreated infections may progress to serious reproductive sequelae including: ectopic pregnancy, pelvic inflammatory disease with tubal scarring, and infertility. Clinical manifestations result from destruction of cells and the host inflammatory response. Early and accurate diagnosis is necessary to prevent these complications and thereby controlling the spread of infection. *C. trachomatis* possesses a cryptic 7.5-kb plasmid of unknown function. The CT Cryptic Plasmid may contain pathogenic components that may induce CT pathologies. All plasmids from human *C. trachomatis* isolates are extremely similar, with less than 1% nucleotide sequence variation. The evolutionary conserved, 7.5-kb cryptical plasmid (pCT) is present in seven to ten copies per cell and is the target of most molecular diagnostic tests. The strong selection to maintain the plasmid by human chlamydial strains implies its importance in the pathogenesis of human infection or disease. The chlamydial cryptic plasmid encodes eight putative open reading frames (ORFs), designated pORF1 to -8. pORF5 encodes a 28-kDa protein, designated pgp3. Although the function of pgp3 is not clear, pgp3 has been shown to be recognized predominantly by antibodies from *Chlamydia*-infected animals and humans and it has been shown to induce protective immunity against chlamydial challenge in a mouse model. Additionally, pgp3 is the only protein detected mainly in the cytosol of *Chlamydia*-infected cells, while the other seven proteins were detected inside the chlamydial inclusions only. Purified pgp3 proteins have been shown to stimulate macrophages to release inflammatory cytokines, suggesting that pgp3 may contribute to the *Chlamydia* induced inflammatory pathologies. We aim to assess the contribution of the cryptic plasmid of *C. trachomatis* to the pathogenesis of Chlamydia disease that culminates in infertility; whereby, *Lactobacillus* will be used as a surrogate live delivery vehicle to express cryptic plasmid genes, particularly pgp3 in vivo. *Lactobacilli* are of the normal flora of the human genital and urinary tracts. The goal of this study is to elucidate the role CT cryptic plasmid in pathogenesis of Chlamydia by comparing the incidence of infertility in wild-type and plasmid –free Chlamydia.

Fig. 1 CT Cryptic Plasmid

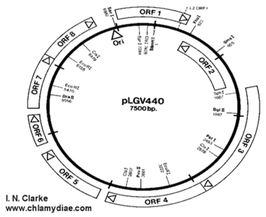

Figure 2: Restriction Site Map of Plasmid pGK12

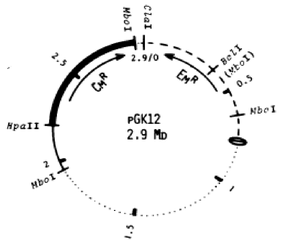

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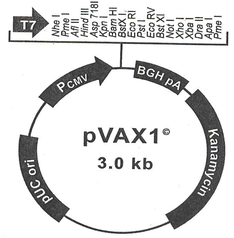
Figure 3: pVAX Vector System



Invitrogen

MATERIALS AND METHODS

Chlamydia Infection Fertility Study - Ten C57BL/6 female mice were divided into two experimental groups. Mice were respectively infected intravaginally with 10^5 IFU/ml of Chlamydia Wildtype or Plasmid Free Chlamydia LGV serovar L2. Five weeks post infection, infected female mice were mated with their male counterparts, one male to each cage of females. Female mice were weighed every 3 days to check the progression of their pregnancy. Following a gestation period of approximately 18 days, the female mice were scored for fertility. For the second round of mating, the male mice were randomly swapped between the different cages. On day 18, the male mice were removed from the cages and euthanized. All plasmid free and wild type Chlamydia infected female mice were sacrificed and their embryos enumerated.

Construction of Recombinant Plasmid pGKVAX with Cryptic Plasmid Genes - pgp plasmid construct was isolated from transformed XL1blue bacteria kindly provided by Dr. Guangming Zhong. The purified plasmids are digested to isolate the 8 genes, pgps, from the pGEX using a series of different combinations of restriction enzyme sites. Select enzyme combinations, EcoR I and Not I, BamH I and Xho I, BamH I and Not I, were used because of the primer combinations originally used in cloning. pGKVAX was previously constructed in our laboratory. It was constructed using pGK12 and the multiple cloning site of pVAX. pGKVAX was digested with the respective combinations of restriction enzymes and then dephosporylated using the New England Biolabs Antarctic Phosphatase system. Each pgp was ligated with the digested, dephosporylated pGKVAX using the by the Promega LigaFAST Rapid DNA Ligation System.

Bacterial Transformation

JM109 *E. coli* - The ligated constructs, pGKVAXpgp , were used in the transformation of JM109 *E. coli* by heat shock method. Positive JM109 *E. coli* transformants were cultured on LB chloramphenicol selective media plates. The plates were incubated at 37ºC for 48 hours. Samples of each colony were obtained and used to inoculate 8 milliliters of LB chloramphenicol broth. Qiagen Miniprep was performed to isolate the plasmids from each culture. Subsequent enzyme digestions were performed to remove each pgp from pGKVAX.

Lactobacillus fornicalis ATCC 700934 – The ligated constructs, pGKVAXpgp, was derived by Qiagen MiniPrep from transformant JM109 *E. coli* . The pGKVAXpgp was used in the electroporation transformation of *L. fornicalis.* Positive *L. fornicalis transformants* were cultured on MRS chloramphenicol plates.

RESULTS

Fertility Study

Mice infected with plasmid-deficient CT failed to induce pathology. Hydrosalpinx was only observed in mice infected with wildtype CT.

Fig. 4 Plasmid-free Infected Mouse

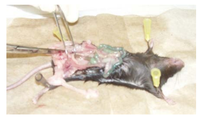


Fig. 5 Wildtype CT Infected

Construction of Recombinant Plasmid pGKVAX with Cryptic Plasmid pgp2 and pgp3a

Transformant JM109 colony formation on LB chloramphenicol selective media plates
EcoR I and Not I digested, dephosporylated pGKVAX was successfully ligated with EcoR I and Not I digested pgp2. Additionally, BamH I and Not I digested, dephosporylated pGKVAX was successfully ligated with BamH I and Not I digested pgp3.

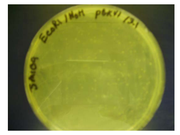


Fig. 6 Control – No colonies

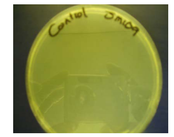


Fig. 7 Transformed JM109 pGKVAXpgp3

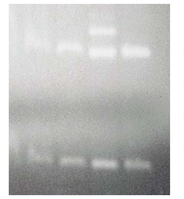
Recovery of plasmid pGKVAXpgp from *JM109* Transformants

Qiagen Miniprep Isolation

Lane 1 – 1kb Ladder
Lane 2 – Linearized pCT 02.2
Lane 3 – Linearized pCT 03.1
Lane 4 – pGKVAX-pgp2 miniprep
Lane 5 – Linearized pGKVAX

Fig. 8 Transformation was confirmed by the recovery of pGKVAXpgp DNA from transformant *JM109.*

Double Digestion of pGKVAXpgp Constructs



Lane 1 &2 - BamH I and Not I digested pGKVAX (upper band), pgp3 (lower band)

Lanes 3 & 4- EcoR I and Not I digested pGKVAX (upper band), pgp2 (lower band)

Fig. 9 Double digestion confirms the ligation of pGKVAX and pgp 2 and pgp3 respectively.

Transformation of *Lactobacillus fornicalis* with pGKVAX/pgp2 and pGKVAX/pgp3

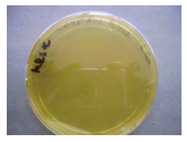


Fig. 10 Control - MRS + Chloramphenicol Nonpulsed *L. fornicalis* - No colonies

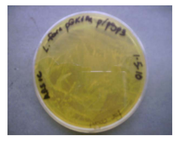
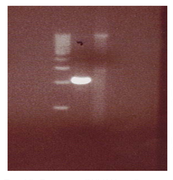


Fig. 11 MRS + Chloramphenicol *L. fornicalis* + pGKVAXpgp3 - Colonies

Fig. 12 MRS + Chloramphenicol *L. fornicalis* – pGKVAXpgp2 - Colonies

Qiagen Lyso-Miniprep Isolation



Lane 1- 1 kb Marker

Lane 2 - Isolated pGKVAX pgp3 from Transformed JM109

Lane 3 - Isolated pGKVAXpgp3 from Transformed *L. fornicalis*

Fig. 13 Plasmid pGKVAXpgp3 is isolated from *L. fornicalis* Transformants

CONCLUSIONS

Chlamydia cured of the cryptic plasmid retains infectivity in mice; however, the infected animals did not develop Chlamydia pathologies.

Host's responses to CT-cryptic plasmid encoded components mediate the development of pathologies following CT infection.

Cryptic plasmid antigens may be applicable to live recombinant *Lactobacillus* vaccine development.

Recombinant plasmid with chlamydial antigen may possibly induce a strong cell-mediated and humoral immune response in a mouse model protecting against *Chlamydia trachomatis* challenge.
L. vaginalis , as preferred strains of *Lactobacillus*, can be used as an effective vehicle to deliver chlamydia antigens to the genital mucosa.

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