Chlamydia Trachomatis Infection: Distribution Of The New Vaccine And Treatment Services

Introduction

The proposed project aims to identify the presence of the effecter protein secreted during the infection of the host cell by Chlamydia Trachomatis. The working hypothesis is to detect the quantity of the protein in the HeLa cells infected by the bacteria, compared to an identical culture that is not infected by using electrophoresis and western blot technique. Introduction of the type III secretion system inhibitor C1 compound, restrict the growth of the inferred protein. This will prove to be a solid foundation to guide the vaccine development and discovery intervention techniques.

Aims And Hypothesis:

- AIM 1 – To identify effector protein secreted during interaction with the host cell which is part of the type three secretion system, playing key role for the growth of chlamydia in host through detection of antigen-antibody complexes by electrophoresis in HeLa cells. Effector protein is secreted in the chlamydial development phase into the host cells and is the first protein found to be localized both into host cell cytoplasm and nucleus.
- HYPOTHESIS 1 – The mean weight of the effecter protein in the nucleus is more than other proteins responsible for the developmental phase.
- AIM 2 – To demonstrate the inhibition of secretion of effector protein, blocking chlamydial growth with the use of type three secretion system inhibitor C1 compound and proposing it as a potential adjuvant for the vaccine.
- HYPOTHESIS 2 – The addition of Type 3 secretion inhibitor C1 component in the infected HeLa cell inhibited the secretion of effector proteins leading to a decrease in the bacterial load per cell.

Significance:

Chlamydia trachomatis infection is one of the most common sexually transmitted diseases worldwide with most cases occurring in Asia, Sub-Saharan Africa, and South America (WHO, 1990, Behets, 2001)1. Worldwide, an estimated 90 million sexually transmitted Chlamydia trachomatis infections occur each year. Rates are about twofold higher (4.2%) among a random sample of young adults (18–26 years) in the United States, highlighting a universal epidemiological feature of C. trachomatis — that infection is mainly observed in adolescents and young adults2. When the infection remains untreated or with repeated infection episodes, fibrotic scarring and disease sequelae such as blinding trachoma, infertility, and cardiovascular disease ensue in a subset of individuals3. Chlamydia are Gram-negative, obligate intracellular pathogens that replicate within a membrane-bounded compartment termed an inclusion. Throughout their development, they actively modify the eukaryotic environment. The type III secretion (TTS) system is the main process by which the bacteria translocate effector proteins into the inclusion membrane and the host cell cytoplasm. Little is known about chlamydial effectors and how they manipulate host cellular processes4. Thus, searching for C. trachomatis-

secreted proteins has become a hot topic5. Identification of Chlamydia effectors is further complicated by the fact that the primary sequences of T3S substrates lack obvious consensus secretion signals6. Thus, the contribution of the current project is expected to identify the effector proteins playing key role in type III secretion (TTS) system and define the new horizons in the field of vaccine formation.

Chlamydial infections can often be asymptomatic, pointing toward preventative measures such as vaccination as the most effective option for control of chlamydial disease7. Intensive screening and antibiotic treatment are unable to completely prevent female reproductive dysfunction, thus, efforts have become focused on developing a vaccine8.To develop rational vaccines it is necessary to understand the complex life-cycle of the organism, the host immune response to infection and how these relate to disease9. A basic understanding of the Chlamydia T3SS is beginning to emerge, the full complement of components contributing to this mechanism, including those with effector functions, remains to be identified10. Hence, the evidence of the new effector proteins will add in further understanding of the mechanism of the infection of chlamydia. As the protein identified is part of type III secretion (TTS) system, inhibition of the same through the standard C1 compound used to block TTS will generate a basis as a potential vaccine candidate. This contribution will be significant because it will aid in knowledge of the pathogenic mechanism of chlamydial infection and propose the adjunct component in vaccine development.

Through the proposed project, we will broaden the insight of chlamydia infection by accomplishing the secretion of effector molecules into host cells for manipulating host signalling pathways. This is important for the future aspect as to successfully complete the intracellular replication/ developmental cycle, the C. trachomatis organisms must secrete the effector proteins. Also, all its biosynthetic processes within a cytoplasmic inclusion by importing nutrients and energy from host cells. However, searching for new effector molecules will continue to be a productive approach and as more effectors are identified, more tools will be availably dissecting the molecular pathways of interaction with host cells. The project will provide new directions in the vaccine research efforts. It will guide to the fresh thinking of using C1 compound inhibiting TTS as an adjuvant to the vaccine component and developing the effective vaccine which would be able to inhibit the bacterial multiplication, thereby preventing the occurrence of the disease.

Innovation:

There is no vaccine for Chlamydia at present. An important characteristic of a Chlamydia vaccine would be the ability to decrease bacterial shedding to reduce transmission and to produce an immune response to prevent Chlamydia-induced immunopathology11. The diagnosis of Chlamydia infection includes electrophoresis of the antibodies produced against the antigen. This takes up a long-time period as the disease doesn’t show up until it is potent enough. Identification of the protein, which is secreted through the lysis of cell membrane due to bacterial intrusion in the cell will be revolutionary. It will lead to highly specific, sensitive, rapid and economically efficient diagnosis even with the dormant and asymptomatic infection.

This research will move forward to the betterment of the cases coming up each year and potentially at-risk populations. The current treatment regimen for the management of Chlamydia infection includes various generations of non-specific antibiotics. It is a well-established fact that the improper or prolonged use of antibiotics develops into resistance. Resistance often leads to

mutated and complicated infections. It also provides a stance for other opportunistic infections surrounding the suppressed immune system. Development of the C1 compound inhibiting TTS will be a novel and target-specific drug delivery system and a potential candidate for vaccine development. It will not be a non-specific, resistance developing traditional molecule, but a guided drug for the specifically intended mechanism of action on a protein secretion pathway. It is important that a vaccine designed to protect against chlamydial genital infection induces a “protective” immune response with little or no “inflammatory” response12.

The identification of the signalling pathway protein will be helpful for vaccine development. It will be a perfect candidate for an accurate and timely diagnosis. Finally, it would lead to a novel drug delivery system (NDDS) for the unscreened cases developed over time without being screened.

Design

Chlamydia infection to the host cells can be identified and categorized by looking for the protein binding sites, type of expressed antigen and the available antibody load. The effecter protein that is secreted during the translocation of chlamydial bacteria into HeLa cells will be identified in the first phase. The study will start with the procurement of the HeLa cell cultures. Two identical cultures will be then selected for the comparison, one of which will be infused with the microbes. Due to the parasite-host activity, the infected cells will show a presence of the pathway protein. These levels will be compared with the uninfected cells for evaluation. Antigen-specific antibodies will be introduced into both cultures. The infected cells will show a rise in the content of the antigen-antibody binding complex. This will be visualized through the western blot technique to draw comparisons by electrophoresis. A similar pair of cultures with the HeLa cells will be developed for the analysis of Type 3 secretion inhibitor C1 component. Two types of cell masses, infected and non-infected will be utilized to detect the antagonist activities of the type three secretion system inhibitor C1 compound.

Both these findings will be quantified for the estimate of their loads. The numbers will be tested for the mean difference in the sample of infected and noninfected HeLa cell cultures. Student’s t-test and ANOVA will be performed to detect the difference in the quantities.

We assume that the Chlamydia infected cells will show a presence of the pathway protein and antigen-antibody complex in the nucleus as well as in the cytoplasm. The presence of the C1 compound will restrict the replication of Chlamydia by inhibiting the secretion of the reactive protein. Based on the evidence, we will come up with a potential candidate for vaccine development as a prevention measure as well as antagonists molecules discovery.

Limitations

- Specific protein might not be associated with all the infected cells and there can be a range of various proteins which do not share a similar trait. This evolving nature would prove to be a difficult to detect the difference. We need to collect and analyze enough sample for the detection if this is the case.
- The protein might be dormant which do not express the active nature which can be detected. It makes the quantity in the culture limited. The method has a practical limit of quantification (LOQ) below which the estimation is not possible.

Justification & Feasibility

Chlamydia trachomatis (Ct) is the most prevalent bacterial sexually transmitted infection13. There is a high number of patients out of the infected population, who show no symptoms. There is literature providing proof against protective immunity against the infection where the immunological response is generated pathologically in animal models. This, however, has not been evidently associated with human infection14.

Studies show that there are various sub-mechanisms in the way the host system mounted a response. Evidence on the single specific mechanism and a molecule will prove to drive the development on better preventive and/or treatment options for the eradication of the infectious morbidity15. The infection prosses a type III secretion system (T3SS) that allegedly possess and monitor the translocation in and out of the nucleus of the antigen proteins16. There was a study that detected an effecter protein in both intra-inclusion and host-cell cytoplasm and was distinct from Clamydia Protease like Activity Factor(CPAF). This protein was also inhibited by a C1 component, an inhibitor that is known to target type III secretion system of microbes17.

Such inhibition of the effector protein was detected by immunofluorescent microscopy as shown in the figure, suggesting that the protein fulfilled different functions during Chlamydial intracellular growth18.

Timeline

Phase 1

- Meeting
- Protocol outline and discussion
- Assigning the duties
- Procurement of the cells
- Identification of the collection of the strains of Chlamydia Trachomatis
- Development and maintenance of the cultures

Phase 2

- Collection of analyses
- Identification by western blot
- Maintaining the database

Phase 3

- Analysis of the database
- Final meeting
- Presentation and reporting

Future Direction

Upon completion of the study, we will have detailed insight into the pathogenesis of Chlamydia

into the host cells by signalling pathways. With the identification of the effector proteins, further studies will be directed towards finding site-specific vaccines and Novel Drug Delivery System (NDDS). With efficient allocation and distribution of the new vaccine and treatment services, we seek to reach to distant parts of the affected regions to decrease the number of cases infected by Chlamydia.

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