Reducing the risk of *Chlamydia trachomatis* transmission: male circumcision or a female vaccine?

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'...vaccine research is headed rapidly towards harnessing the Trojan horse that is *Chlamydia trachomatis*'.

Male circumcision – is it the answer to reducing the risk of acquiring and transmitting venereal diseases such as *Chlamydia* [1]? As a woman, the aforementioned strategy might appear to be one of the easiest solutions to deal with one of the oldest public health problems in the world. A relatively poor understanding of the regulation of the immune response of the female genital tract, the lack of adjuvants capable of targeting vaccines to the genital mucosa, our limited knowledge of which chlamydial antigens induce protective responses, the lack of a system to genetically manipulate chlamydiae and the observation that immune responses may be involved in pathogenesis, all suggest that the development of an efficacious chlamydial vaccine is a high-on-impossible task. As a female scientist, however, who has been working in the field of *Chlamydia* for the past 18 years, it is my opinion that vaccine research is headed rapidly towards harnessing the Trojan horse that is *Chlamydia trachomatis*.

Chlamydia trachomatis serovars D–K are important causes of sexually transmitted disease (STD) both in males and in females in developed and developing countries. Most recent international reports (as of early 2007) indicate that *Chlamydia* accounts for an estimated 92 million of the 500 million annual STDs worldwide affecting both women (50 million) and men (42 million) [2]. There is currently no vaccine available for either gender.

The human pathogen *C. trachomatis* has been described as a microbial Trojan horse as it is a highly evolved obligate intracellular bacterium that has developed unique mechanisms to evade the host immune system during infection. The surface of the infectious chlamydial elementary body (EB) is covered with antigenically diverse surface proteins that help avoid detection by antibodies [3]. The intracellular replication of

chlamydiae, within a membrane-bound inclusion, limits exposure of the organism to the immune system [4]. Once inside this intracellular niche, chlamydiae are thought to interact with the host via a type three secretion apparatus, which allows the chlamydiae to alter host-cell functions.

C. trachomatis serovars D–K are associated with acute urethral syndrome, urethritis and cervicitis in women and epididymitis, acute proctitis and acute proctocolitis in men. However, up to 75% of infections are often asymptomatic and subclinical in both males and females [5–8]. An estimated 40% of untreated genital chlamydial infections lead to sequelae, including pelvic inflammatory disease that can result in infertility, ectopic pregnancy and chronic pelvic pain in women [9]. *Chlamydia* infections in males can cause urethritis, prostatitis and epididymitis that can lead to infertility [10].

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Many infections are asymptomatic and hence effective treatments with antibiotics such as doxycycline and azithromycin cannot be commenced. These were until recently defined as effective therapy – but if high chlamydial loads are found *in vivo* then it is possible that women treated with these antibiotics are at increased risk of treatment failure [11]. Thus, the development of an efficacious vaccine would appear to be the most fiscally prudent approach to controlling chlamydial genital infections. Indeed, it has been shown by modeling studies that even a partially protective prophylactic vaccine that is capable of preventing severe disease sequelae would be a suitable short-term goal to control chlamydial infections [12]. The ideal vaccine would be one that protects against multiple serovars and prevents chronic infection sequelae, including blindness, pelvic inflammatory disease and infertility.

Antichlamydial protective immunity is essentially mediated by a T-helper type 1 (Th1), response that is dependent upon the presentation of antigen via major histocompatibility

complex (MHC) class II molecules. While antibody-secreting cells are not critical components of the primary effector response, they have been shown to be important for clearance of reinfection. Thus, an ideal vaccine would be one capable of inducing both a strong Th1 T-cell response and a strong mucosal antibody response. Currently, there are very few efficacious vaccine candidates that have been identified and characterized. More specifically, there is only a limited number of known T-cell antigens processed and presented by the human leukocyte antigen (HLA) class II molecules. This type of antigen is going to be essential to the development of an efficacious chlamydial vaccine.

Because it is currently not feasible to genetically modify chlamydiae in order to produce safe attenuated strains, the recombinant subunit approach is the one considered most likely to be successful. Among the chlamydial antigen candidates showing the greatest promise is a 40 kDa outer membrane antigen, known as major outer membrane protein MOMP, as well as the polymorphic outer membrane proteins (POMP), the PorB family of membrane antigens, an ADP/ATP translocase, a PGP3 plasmid protein and others [13–15].

The total number of characterized antigens is somewhat limited, possibly due to most research being focused on the development of MOMP as a single antigen vaccine. One of the main foci in chlamydial vaccine design has been on the use of the MOMP as a single antigen component. MOMP constitutes approximately 60% of the total protein mass of the bacterial outer membrane [16]. Interestingly, the last few years have seen an increase in the number of novel chlamydial antigens being reported, possibly reflecting a change in attitude towards chlamydial vaccine development and the fact that there are at least ten entire chlamydial genomes now available from The Institute for Genomic Research (TIGR).

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There is a great deal of diversity in the type of protein that has been reported as being immunogenic during chlamydial infection. There are a number of classical vaccine targets, such as the polymorphic outer membrane proteins and type three secretion system proteins (LcrD, LcrE, CopN, YscT and YscJ) [17–20]. However, there are also a number of unusual vaccine targets that

have been shown to be immunogenic and even able to confer protection, such as enolase, a ribosomal protein, a histone-like protein, RNA polymerase and chlamydial protease-like activity factor (CPAF) [21–23]. The discovery of immunogenic targets, such as NrdB, enolase, ribosomal proteins and RNA polymerase, suggest predicting immunogenicity based on a protein’s function is now becoming redundant.

Using expression library immunization we have screened the genome of *Chlamydia muridarum* and identified several promising gene fragments that protected genetically immunized mice by eliciting immune responses that contributed to increasing the clearance of live chlamydial infection from the genital tracts of these animals. The proteins we have identified as partially protective include a putative outer membrane protein (TC0512), two conserved hypothetical proteins (TC0757 and TC0767) an adherence factor protein (TC0439), a UvrD/REP helicase family protein (TC0490) and an ATP-dependent Clp protease, ATP-binding subunit ClpC protein (TC0559).

The fact that we have identified genes/proteins presumably not exposed on the surface of *Chlamydia* that are protective is not unexpected and concordant with findings of earlier studies reporting protection against *Mycobacterium avium* subsp. paratuberculosis infection provided by transport and chaperone proteins using expression library immunization [24]. Housekeeping genes have also been reported to mediate high levels of protection in the mouse pneumonia model of Chlamydial infection [25]. Resolution of primary Chlamydial infection is known to require MHC class II-restricted CD4⁺ Th1 and MHC class I-restricted CD8⁺ T cells mediated by the proinflammatory cytokine interferon (IFN)- γ [26–29]. Our results are therefore plausible since peptide fragments of the adherence factor protein or the REP helicase protein would have the opportunity to bind to the MHC class II complex and be recognized by the immune system in this complex.

In another of our recent studies [30], we have identified a number of unique vaccine candidates using a novel *in silico* approach. In an attempt to overcome HLA polymorphism, the whole chlamydial genome was screened for proteins containing epitopes predicted to bind multiple HLA class II molecules (i.e., predicted ‘promiscuous’ T-cell epitopes). A wide range of HLA class II molecules were used in this screen to identify vaccine antigens that could potentially offer broad and ethnically balanced

population coverage. This analysis identified a number of novel targets and was validated by the identification of a known chlamydial T-cell epitope.

Clinical persistence is a key concept in the pathogenesis of chlamydial infections. Sterility caused by genital infection with *C. trachomatis* and coronary artery disease associated with *Chlamydia pneumoniae* are both caused by inflammation-based pathology. Chlamydial antigens may persist for prolonged periods and there is evidence that these noncultivable forms may lead to continued inflammation.

During its intracellular developmental cycle, *C. trachomatis* maintains this intracellular niche since it possesses the tryptophan synthase virulence gene that has coevolved with its human host. This virulence factor acts as a primary mechanism for the invading pathogen to hide from the host's primary line of defense [31]. Chlamydiae also express a type III secretion system (T3SS) that secretes antihost (effector) proteins into the inclusion membrane known as inclusion membrane-localized proteins (Inc-proteins) and host cytosol that allow the organisms to establish and maintain the permissive intracellular niche in the host [32,33]. Persistence of the microorganisms by hiding from the immune system thus raises the question of optimal therapy as well as presenting a unique challenge to the development of effective vaccines.

Our own group has several potential chlamydial persistence antigens that can be tested in a vaccine that targets both the acute and the persistent stages of infection – both of which are the result of infection of the host cell cytoplasmic membrane and abnormal growth and persistence of the organism – two of the known stages of the chlamydial developmental cycle [34]. It would appear that a multisubunit vaccine would be

most effective, especially considering recent evidence reporting that a novel, multiple subunit vaccine induced a higher frequency of Th1 cells against *C. trachomatis* compared with a single subunit construct [35]. Thus, it would appear that an effective chlamydial vaccine will need to have multiple subunits, some of which are directed against acute antigens and others directed against persistent antigens.

'...candidate vaccine targets that induce partial immunity may well be able to induce sterilizing immunity...'

It will of course be imperative for any novel vaccine trials to evaluate what effect(s) immunizing against persistence antigens will have in situations where chlamydial infections of animals/people are either inapparent (subclinical) or persistently progressing (chronic).

There are now several novel vaccine candidates that can elicit partial protection against genital infection in various animal models of genital chlamydial infection. Novel antigens could be used either individually or as part of a multi-subunit construct with the appropriate delivery methods aimed to enhance uptake, expression and presentation of antigens. Used with improved effective delivery systems and potent adjuvants, candidate vaccine targets that induce partial immunity may well be able to induce sterilizing immunity if a higher frequency of Th1 cells can be induced after primary immunization with the novel chlamydial antigens.

So to answer my initial question, no, male circumcision is not the definitive answer. Vaccine research is indeed headed rapidly towards harnessing the Trojan horse of *C. trachomatis*. In fact, a female vaccine will only be one half of the story.

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