Persistent chlamydial infections: role in inflammation and challenges for vaccine development

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Persistent chlamydial infections and the challenges for vaccine development

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The development of chlamydial vaccines continues to be a major challenge for researchers. While acute infections are the main target of vaccine development groups, Chlamydia is well known for its ability to establish chronic or persistent infections in its host. To date, little effort has focussed specifically on the challenges of vaccines to successfully combat the chronic or persistent phase of the disease and yet this will be a necessary aspect of any fully successful chlamydial vaccine. This short review specifically examines the phenomenon of chlamydial persistence and the unique challenges that this brings to vaccine development.

The phenomenon of chlamydial persistence

Molecular evidence for chlamydial persistence was first reported by Beatty et al. back in 1994 and since then been supported by over 50 published reports on various aspects of the topic. The phenomenon of chlamydial persistence has best been described as “a viable but non-cultivable growth stage resulting in a long-term relationship with the infected host” (Hogan et al., 2004). Such relationships have been most thoroughly analysed in vitro, usually by perturbing the cell culture conditions in some way, such as; (a) amino acid deficiency (Coles et al., 1993), (b) specifically by limiting tryptophan, (c) iron depletion (Raulston et al., 1997), (d) exposure to interferon gamma (Pantoja et al., 2001), (e) antibiotic exposure (Kutlin et al., 1999), (f) phage infection (Hsia et al., 2000), (g) or a continuous infection model (Kutlin et al., 2001). In all cases, the morphological hallmarks of persistence are; (a) a block in the chlamydial developmental cycle such that reticulate bodies (RBs) do not convert back to elementary bodies (EBs) and hence the cycle is not completed, (b) a large reduction in the infectious titre, which results directly from the lack of production of EBs, (c) enlarged and abnormally shaped RBs
inside enlarged inclusions, (d) in some cases, increased resistance to antibiotics.

While persistence has been best characterised in vitro, there is also considerable in vivo evidence to support it. These include observations of altered morphological forms in vivo, detection of chlamydial DNA and antigen in diseased hosts but without the ability to recover culturable chlamydiae, as well as recurrences of infections in a single host that do not appear to be due to reinfection.

While it continues to be difficult to visualise persistent forms in the diseased host, there have been several reports of such altered forms in both humans and animals. Atypical, pleomorphic RBs with poorly defined outer membranes were observed by Nanagara et al. (1995) in infected fibroblasts and macrophages in synovial membrane samples from patients with C.trachomatis-associated reactive arthritis. More recently, abnormal C.pneumoniae forms have been observed within aortic valve samples from patients with degenerative aortic valve stenosis (Skowasch et al., 2003).

Detection of chlamydial DNA and/or antigens in the absence of recoverable infectivity is not uncommon and has been reported in in a range of human disease states. Chlamydial DNA and antigen for instance can often be detected in tubal biopsy specimens, particularly following inadequate antibiotic treatment (Patton et al., 1994). Similarly, chlamydial RNA, which indicates viable Chlamydiae, has been detected in the absence of cultivability in experimental trachoma infections of primates (Holland et al., 1992) as well as in synovial biopsy samples from patients with reactive arthritis or Reiter’s syndrome (Gerard et al., 1998).

The third area of proof of persistent forms in vivo comes from observations such as Dean et al. (2000) in a study of women with genital C.trachomatis infections who demonstrated recurrences of the same genotype of C.trachomatis in patients over a 2 to 4.5 year period. This strongly argues
against new infections, but rather suggests a long term chronic or persistent infection in these women.

Probably the most significant evidence for persistence however has been the molecular analyses that have occurred in the past 4 – 5 years. A series of transcriptomic and more recently proteomic studies are clearly demonstrating that Chlamydia possess a persistence regulon that responds to harsh external conditions by altering the chlamydial transcriptional pathways used. By using various in vitro models, such as interferon gamma induction, iron depletion or penicillin treatment, several groups have shown altered RNA expression profiles (Hogan et al., 2003; Belland et al., 2003; Nicholson and Stephens 2002; Mukhophaday et al., 2005; Timms et al., 2005). Hogan et al. (2003) first used the long term continuous model of persistence and more recently (Hogan et al., unpublished data) the interferon gamma induction model combined with quantitative RT-PCR analyses on C.pneumoniae to show significant up- and down-regulation of several key genes under persistence conditions. Nicholson and Stephens (2002) reported the first whole genome microarray data with C.trachomatis persistence using penicillin as the persistence inducing factor. Belland et al. (2003) subsequently performed a whole genome microarray experiment on C.trachomatis using the most widely accepted interferon gamma induction method. They also demonstrated that the persistence response was reversed when tryptophan was added back to the system. One aspect of chlamydial persistence has been the fact that while some gene responses seem to be consistently observed between models, others appear to vary. Timms et al. (unpublished data) recently studied this aspect by comparing the interferon gamma induction and iron limitation models of persistence with C.pneumoniae. They drew some conclusions about the most consistent features of chlamydial persistence, at least at the transcriptomic level. They noted that of the genes that have been analysed in several previous studies, only four show consistent changes in all Chlamydia and across all persistence models; hctB, omcB, ompA and crpA are all down-regulated while htrA is usually up-regulated. The down-regulation of hctB is probably predictable, being a late gene involved in DNA condensation, and it it appears to be one of the better late gene markers of persistence. The
consistent down-regulation of the cell wall/envelope genes, omcB, pmpA and crpA is interesting and could possibly suggest that Chlamydia has evolved a mechanism to dampen its immune induction by limiting the expression of its antigenic wall components. The interferon-gamma induction model has been the best studied persistence model by far, and in addition to the four model-wide hallmarks mentioned above, five other genes appear to give consistent changes in both chlamydial species specifically in the interferon-gamma induction model; groES, dnaK, grpE and ndk are all down-regulated while tyrP is up-regulated. Timms et al. (2005) also noted that the down-regulation of several stress response genes is less obvious and either suggests that this component of the stress response is deleterious to parasite survival, or else that transcriptional control of these genes is not as important as translational control mechanisms. Comparison of persistence markers between the chlamydial species is less straightforward, as many studies have not compared the same genes. It seems obvious however that there are numerous examples of species differences in the transcriptional response to persistence and this might suggest important differences in the evolution of the two species in their response to host-induced persistence.

Taken together however, the transcriptomic and more recently the proteomic studies on several persistence models demonstrate that this is not just a stress response by the Chlamydia, but is clearly a directed response by the parasite, presumably to ensure its longer term survival.

Implications for vaccine development

The development of effective chlamydial vaccines has been a major challenge for the past 20 years or more (reviewed by Igetsieme). This difficulty is partly due to the need to identify the most protective chlamydial antigens but also to the need to induce strong mucosal immunity. The currently held view is that a strong antibody response is able to neutralise the EBs when they are first encountered at the site of infection while a strong T cell response is required to detect and destroy infected cells once the infection has become established.
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 *C. pneumoniae* are both caused by inflammation-based pathology. Chlamydial antigens may persist for prolonged periods and there is evidence that these non-cultivable forms may lead to continued inflammation. Persistence of the microorganisms thus raises the question of optimal therapy as well as presenting a unique challenge to the development of effective vaccines.

Immune responses that are associated with persistent infection with *C. trachomatis* seem to induce pathology as a result of chronic inflammation and tissue damage. The induction of a delayed-type hypersensitivity (DTH) reaction to *C. trachomatis* antigens leads to repeated and persistent infection of the upper reproductive tract. Indeed there is a strong correlation between *Chlamydia*-specific immune responses, such as antibodies and T cells specific for heat-shock protein 60 (HSP60) from chlamydia spp., and PID and fallopian tube pathology (Brunham and Peeling 1994) It is known that T cells that are reactive to *C. trachomatis* HSP60 and produce IL-10 have been found in infertile women (Kinnunen et al., 2003) and thus may be involved in the suppression of *C. trachomatis* specific responses, which could contribute to the ability of *C. trachomatis* to persist. Heat shock protein 60 (HSP60) from *Chlamydia pneumoniae* also triggers cytokine responses including TNF and IL-12p40 inflammatory responses via Toll-like receptor 2 and 4 *in vivo* and it can be also found in atherosclerotic plaques of patients (DaCosta et al. 2004).

Some clinical and animal studies have also shown that heat shock protein 10 (HSP10) is another chlamydial antigen implicated in the immunopathogenesis of chlamydial infection. Chlamydial heat shock protein 10 was identified as a correlate to the immunopathogenic process in women with tubal factor infertility (LaVerda et al. 2000). Serum IgA antibodies to HSP60 and HSP10 were significantly higher in the female partners of subfertile (time to pregnancy > or = 12 months) couples than in their fertile controls (Karinen, et al., 2004). Chlamydial HSP60 and HSP10 antigen-specific antibodies were
also associated with uterine and salpingeal fibrosis in Chlamydia-infected koalas. (Higgins et al., 2005)

A recent study in the macaque model however reports that recombinant chlamydial heat shock protein 60 (rcHSP60) was the only antigen shown to induce significant delayed-type hypersensitivity from a panel of many other chlamydial antigens tested that included UV-inactivated organisms recombinant major outer membrane protein (MOMP), purified outer membrane proteins (OMP) and heat shock protein 10 (HSP10) (Lichtenwalner et al., 2004). However it was noted in this study that mild reactions to MOMP, OMP and HSP10 were also observed but the differences from controls were not statistically significant. It is possible that weak DTH reactions induced by testing with antigens other than rcHSP60 may not have been detected.

In terms of designing an effective C.trachomatis vaccine that does not induce a delayed type hypersensitivity (DTH) reaction it would appear that it is imperative to omit antigens to chlamydial HSP60 from a vaccine for use against genital chlamydial infections in the naïve vaccinated host. If the population of women to be vaccinated have previously been exposed to Chlamydiae and already have tubal factor infertility (TFI) it would be prudent to also exclude antigens to C-reactive protein (CRP) and HSP10 from the vaccine (since among women with similar exposure to chlamydiae the serologic responses were recorded against chlamydial CRP (denHartog 2005 and the responses to HSP10 showed a stronger correlation with TFI than did the responses to HSP60 or MOMP (LaVerda et al., 2000). In vaccines designed to protect against C.pneumoniae infection that is associated with atherosclerosis (an emerging risk factor in coronary artery disease) chlamydial HSP60 and lipopolysaccharide should likely be omitted from the formulation since these chlamydial antigens have been shown to dysregulate lipid metabolism, induce inflammatory cytokine cascades and trigger production of cross-reactive antibodies that initiate and promote atherogenesis (Kalayoglu, 2002).
If persistent infections are established in susceptible hosts then those infections may serve as reservoirs for new infections, contribute to the immunopathological consequences of infection or require alternative therapeutic approaches. An effective chlamydial vaccine must therefore target the persistent phase and not just initial phase of infection in these hosts.

Sera of subfertile women with tubal pathology test positive for the presence of IgG and IgA antibodies to *C. trachomatis*, IgG antibodies to cHSP60 and C-reactive protein (CRP) (denHartog et al., 2005) all serological markers of persistent *C. trachomatis* infections. Therefore antibodies are not likely to be effective against the persistent stage of chlamydial infection. This salient observation from the results of many clinical and animals studies on chlamydial genital tract infections is an important one to consider for vaccine development. Effective vaccine design needs to consider not only the fact that (1) Th1 cells are essential for resolution of chlamydial genital tract infection but also that (2) the microorganism is capable of abnormal growth and persistence as part of its developmental cycle and therefore (3) two distinct populations of humans/animals will be involved in vaccine studies. The first population are naïve hosts in whom it will be imperative that the vaccine used stimulates a rapid and strong MHC classII-restricted, CD4+ T-helper type 1 (Th1) response mediated by IFN-γ and TNF-a production which is crucial for resolving primary infection (reviewed by Igietseme et al., 2004) and which should not result in the establishment of chronic infection. An inadequate or sub-optimal Th1 response may lead to the establishment of persistent infection resulting in the second population that will need to be vaccinated. In this second population it will be crucial to design a vaccine that omits chlamydial antigens capable of stimulating DTH responses and that also targets the abnormal growth and persistence phase of the organisms life cycle.

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 (1) infection of the host cell
cytoplasmic membrane and (2) abnormal growth and persistence of the organism—two of the known stages of the chlamydial developmental cycle (AbdelRahman, and Belland, 2005). It would appear that the most effective vaccine would be a multi-subunit one especially considering recent evidence reporting that a novel, multiple subunit vaccine induced a higher frequency of Th1 cells against *Chlamydia trachomatis* compared with a single subunit construct (Eko et al., 2004). Thus it would appear that an effective chlamydial vaccine will need to have multiple sub-units some of which are directed against acute antigens and others directed against persistent antigens.

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

**Literature Cited**


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