Chlamydial Genomics and Vaccine Antigen Discovery

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Vaccine development for Chlamydia species has progressed on several research fronts, including knowledge of the components of the immune response required for immunity and resolution of infection as well as effective modes of delivery. The antigens required to elicit protective immune responses have not been identified. The full biologic potential for Chlamydia trachomatis and Chlamydia pneumoniae was exposed by the completion of the genome sequence for each organism. This knowledge will aid vaccine antigen discovery by facilitating the identification, testing, and evaluation of antigens by way of vaccine delivery approaches that elicit protective immunity.

The completed genome sequences for Chlamydia trachomatis and Chlamydia pneumoniae provide a comprehensive inventory of all proteins potentially produced by these organisms [1, 2]. A significant question is how this information guides vaccine development. It does not address delivery issues, type of humoral or cellular response elicited or, a priori, which proteins will provide the best protection; however, it does provide the knowledge for rational vaccine development and the means for evaluation of the completeness of processive or random vaccine approaches. This effort is facilitated by having two complete chlamydial genomes to compare with each other and with genome sequences for other organisms.

Comparison of the genome-wide protein-coding sequences for C. trachomatis and C. pneumoniae permits organization of the encoded proteins into four categories (table 1): (1) proteins that are shared by the two species and that are also related to proteins identified in other organisms, (2) proteins that are shared between the species but do not have identifiable relatives in other organisms (i.e., unique to chlamydiae), (3) proteins that are unique to either C. trachomatis or C. pneumoniae yet have relatives shared by other organisms, and (4) proteins that are unique to either C. trachomatis or C. pneumoniae but lack identifiable relatives in other organisms. These categories provide a convenient framework in which to discuss vaccine antigen discovery.

Genomics and Vaccine Discovery

Proteins present in C. trachomatis and C. pneumoniae with relatives in other organisms (category 1) typically have metabolic functions that provide the biologic foundation common to most organisms. These are not likely candidates for a chlamydia-specific vaccine. Category 1 proteins represent the majority of chlamydia-coding sequences and include proteins involved with translation, transcription, replication, recombination, and metabolic pathways. Although these proteins are typically not high priority vaccine candidates, exceptions to consider include proteins such as the type III secretion system structural proteins, hsp60, and proteins with analogous function in other organisms that are antigenically distinct. Given the primary sequence similarity of several of the type III secretion system structural proteins that are expected to be surface exposed and required for infection of host cells, these are attractive candidates to investigate.

Proteins that are shared by the two chlamydial species but lack relatives in other organisms (category 2) are of interest for vaccine design because their unique presence in chlamydiae suggests that most function in common chlamydial structure or biochemistry. Examples in this category are the chlamydial outer membrane proteins, type III secretion system effector proteins [3], and inclusion membrane proteins [4, 5]. Each is an exceptionally important vaccine candidate either because of its presence on the surface of the organism or because of its presence in cytosolic compartments of the host cell and consequently may be expected to be presented by major histocompatibility complex (MHC) class I.

Proteins that are unique to one chlamydial species yet have identified relatives in other organisms (category 3) are very few and typically add to the metabolic capacity of the organism. Examples for C. trachomatis are the tryptophan operon not found in C. pneumoniae. For C. pneumoniae, examples are genes encoding additional nucleotide metabolism capacity. This set does not seem to hold much promise for vaccine antigen discovery.

Proteins that are unique to one of the two species and that lack relatives in other organisms (category 4) represent the landscape of capacity that likely characterizes C. pneumoniae as C. pneumoniae and not as C. trachomatis, and vice versa. These capacities should reflect mutually exclusive biology and viru-
inflammation is not a response that chlamydiae directly elicit. By engaging the host cell’s signal transduction regulatory networks, chlamydiae cause the cell to up-regulate and secrete proinflammatory chemokines. This is not a generalized host response to antigenic stimulation by chlamydial infection but rather cellular responses engineered and orchestrated by chlamydiae. In this case, consideration of vaccination or therapeutics with the aim of reducing virulence and pathogenesis rather than direct microbial eradication provides a rational alternative. 

Table 1. Numbers of protein coding sequences for Chlamydia trachomatis (CT), Chlamydia pneumoniae (CPn), or both, and numbers of protein sequences lacking or with relatives identified from other organisms (protein coding sequence).

<table>
<thead>
<tr>
<th></th>
<th>CT and CPn</th>
<th>CT only</th>
<th>CPn only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthologs to other organisms</td>
<td>578 (1)</td>
<td>10 (3)</td>
<td>28 (3)</td>
</tr>
<tr>
<td>No orthologs to other organisms</td>
<td>271 (2)</td>
<td>50 (4)</td>
<td>187 (4)</td>
</tr>
<tr>
<td>Total</td>
<td>849⁴</td>
<td>60</td>
<td>215</td>
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⁴ Includes 34 additional C. pneumoniae paralogs for C. trachomatis.

Technical Approaches to Vaccine Antigen Discovery

There are two broad technical approaches to vaccine antigen discovery for C. pneumoniae. The first is a targeted approach in which rational deductive identification of genes encoding proteins that, because of their predicted function or specificity for chlamydiae, are likely candidates for vaccine testing. The chlamydial MOMP encoded by the ompA gene has long been a primary vaccine candidate for C. trachomatis. Although peptides and denatured C. trachomatis MOMP protein have failed to provide microbiologic protection [10], the key to understanding these failures may rest in the requirement for conformation-dependent antibody responses that may be solely responsible for effective in vitro neutralization [11]. For C. pneumoniae, this is emphasized by the general paucity of antibodies produced from infection to denatured OmpA as evidenced by the weak reaction of OmpA detection in immunoblots [12]. The species-specific antibodies detected in the microimmunofluorescence test are most likely specific to OmpA but may be conformation dependent.

Other outer membrane surface components predicted from the genome sequence are also vaccine candidates, including the polymorphic membrane protein (Pmp) family [13]. Nine genes are present in the C. trachomatis genome and 21 in the C. pneumoniae genome. Some other proteins encoded in the genome can be identified by their postulated requirement in chlamydial pathogenesis such as type III secretion system proteins that represent additional new targets for vaccine testing [14]. Of particular interest are the effector proteins delivered by the type III secretion system. These proteins are likely delivered to the host cell cytoplasm and hence should be presented by MHC class I on the surface of infected cells and stimulate CD8 T cells to secrete inhibitory cytokines (e.g., interferon-γ) and directly kill infected cells [15]. These targets are of special interest for C. pneumoniae as this species may infect long-lived cells and without elimination would promote microbial persistence.

The second approach to vaccine antigen discovery is empiric. Genomic information provides a new opportunity for empiric evaluation as high-throughput cloning and expression of chlamydial genes permits a comprehensive evaluation of all chlamydial gene products. These proteins can then be directly tested in animals for protection against challenge or time to resolution

lence traits, but because they have no relatives to assist in the identification of their function, nearly all (50 for C. trachomatis and 187 for C. pneumoniae) have no predicted function. Given the lack of any idea of function, one cannot discount these coding sequences.

An important question related to antigen discovery is whether vaccination is to protect against acute infection or is for therapeutic resolution of chronic, persistent infection. For C. trachomatis, the primary aim may be to prevent acute infection, but for C. pneumoniae the primary aim may be elimination of persistent infection. These goals may not be mutually exclusive. However, because of the apparent ability of C. pneumoniae to persist in long-lived cells coupled with the lack of major outer membrane protein (MOMP) antigenic variation that characterizes C. trachomatis, one may conclude that the immune mechanisms for preventing acute infection and resolution of chronic infection differ significantly for each species. The C. trachomatis MOMP varies antigenically among many strains, whereas the C. pneumoniae MOMP is not antigenically variable. As previously suggested by the lack of antigenic variation among Chlamydia psittaci MOMP of related strains [6], the selective pressure responsible for antigenic variation seen for C. trachomatis [7] is not a significant force or advantage for C. pneumoniae or C. psittaci. This suggests that C. pneumoniae spend much more time persistent within cells of the host and less time exposed to the selective pressure of anti-MOMP neutralizing antibodies than do C. trachomatis. The alternative explanation is that antibodies to C. pneumoniae MOMP do not neutralize, although this possibility is unknown.

Given the tens of millions of years that chlamydia-like organisms have parasitized eukaryotic organisms and the tens of thousands of years they have infected birds and mammals, infection per se appears to be relatively benign except for the inflammatory response that is the primary process that accounts for disease. The inflammatory process may be antigen driven, resulting in immune sensitization and autoimmune reactivities. This is the popular view and may have a basis in some chlamydial elicited diseases such as reactive arthritis [8], but few direct data support this notion for scarring disease processes typical of trachoma and the upper genital tract. Rasmussen et al. [9] recently proposed an alternative hypothesis of chlamydial pathogenesis. Inflammation is not a response that chlamydiae
of infection. The advantage of this approach is that it does not rely on knowledge of protein function or other attributes such as in vivo expression. However, it does require an animal model that is faithful to human infection immunobiology.

A possible drawback of both the rational and empirical approaches is that they only test proteins directly encoded by chlamydial genes and not compounds synthesized by chlamydial proteins. The most obvious example is chlamydial lipopolysaccharide that requires several genes to synthesize the authentic chlamydial compound. Other products likely produced or modified by chlamydial enzymes are the heparin sulfate-like ligand [16], the exoglycolipid [17], and the high-mannose oligosaccharide [18]. Because these may have important roles in chlamydial invasion of eukaryotic host cells, they could be relevant for immune targeting of chlamydial infection. Such uncertainties notwithstanding, chlamydial genomic information provides not only a wealth of information about these unique organisms and insights into new vaccine candidates but also expedites the deployment of new methodologies for vaccine antigen discovery.

References