Chlamydia trachomatis Strains and Virulence: Rethinking Links to Infection Prevalence and Disease Severity

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An unanswered question concerning prevalence and disease severity of Chlamydia trachomatis genital infection is whether more prevalent strains or strains more likely to cause serious disease complications are causally associated with specific virulence attributes. The major method for distinguishing chlamydial strains is based on differences in the major outer membrane protein (MOMP). A subset of MOMP serovars (D and E serovars) are easily the most prevalent strains identified worldwide, but MOMP serovar and genovar analyses have not yielded consistent strain-dependent virulence distinctions. Expansion of the definitions of chlamydial strains beyond the MOMP paradigm are needed to better understand virulence properties for this pathogen and how these properties reflect disease severity. Substantive genetic and phenotypic differences have emerged for the 2 major C. trachomatis pathobiotypes associated with either trachoma or sexually transmitted diseases, but differences within the sexually transmitted disease group have not yielded reliable disease severity attributes.

A number of candidate virulence factors have been identified, including the polymorphic outer membrane autotransporter family of proteins, the putative large cytotoxin, type III secretion effectors, stress response proteins, and proteins or other regulatory factors produced by the cryptic plasmid. Continued work on development of a chlamydial gene transfer system and application of genomic approaches to large collections of clinical isolates will be required to associate key chlamydial virulence factors with prevalence and disease severity in a definitive way.

Identification and sorting of different Chlamydia trachomatis genital tract isolates have been central to epidemiologic studies of chlamydial genital tract infections, in defining sexual networks, in establishing tests of cure parameters, and in comparing the role of persistent infection versus reinfection in the development of infection complications. There has also been a great deal of interest in establishing strain-dependent virulence associations, especially for upper genital tract disease in women or other serious complications of chlamydial genital tract infection [1]. Thus far, no clear strain-dependent patterns have emerged, but continued studies focused on causally linking specific strains to disease severity are warranted. In fact, it is not entirely clear how best to define chlamydial strains. It is clear, however, that it is time to think about a new definition for chlamydial strains that is based on functional variability of proteins that may be targets of evolutionary selection [2].

One purpose of this review is to help provide a basis for defining chlamydial strains in a meaningful way. It is important to continue evaluating clinical isolates with the goal of identifying unique attributes that help define chlamydial strains functionally and provide clues for this pathogen that may be associated with disease severity and infection prevalence. This information can then be used to inform studies in basic research laboratories directed toward providing mechanistic in-
sights that can then be translated into improved management strategies for *C. trachomatis* genital tract infection.

The most frequently cited strain-specific *C. trachomatis* variants reflect differences in the chlamydial major outer membrane protein (MOMP). Despite occasional credible reports to the contrary [3], MOMP differences have yet to be consistently associated with disease severity or even disease phenotype. MOMP serovars fail to show relationships to virulence, because MOMP variation is not linked to *C. trachomatis* biologic variation in ways that reflect the pathogenic potential of this organism (see below). Because of the increase in reported *C. trachomatis* genital tract infection case rates, especially in populations in which improved screening and treatment programs are in place [4], it is now essential to consider the possibility of strain variants that may predict disease severity. It is also critical to extend studies beyond the MOMP paradigm of strain definition to better assess the impact of *C. trachomatis* genotype on disease phenotype. The purpose of this review is to describe likely chlamydial components that may be useful in comparative studies focused on defining disease-specific phenotypes. Chlamydiae are inherently problematic in that, although genetic manipulation of the pathogen has been reported [5], the capacity to create and compare isogenic strain pairs has not yet been developed. This deficiency severely limits definitive work on laboratory-based studies of chlamydial virulence factors.

**METHODS**

Information on chlamydial strain distinctions and virulence was obtained by search of the PubMed journal database with use of the term “Chlamydia trachomatis.” This yielded ∼10,000 citations that were categorized according to topic. Topics included (1) attributes of specific strains that foster the development of acute disease, increase the reinfection rate, or promote better transmission or the development of chronic disease; and (2) virulence factors and how they contribute to disease pathogenesis. Originally, mechanisms for variant strain production and a role for polymicrobial environments in disease severity were included in the topic list. The former was excluded mainly because definitive information is just now emerging, and the latter was excluded because it was deemed a topic large enough for separate review. Eventually, ∼125 articles were chosen for more detailed study, and 75 of those have been incorporated in this overview. Categories of virulence attributes were selected on the basis of the frequency of their appearance in the literature and their relevance to serious sequelae in chlamydial sexually transmitted diseases. Relevance is by its nature a subjective criterion, but it was applied in culling the larger list to those contributions selected. Therefore, relevant omissions are wholly the responsibility of the author.

**CHLAMYDIAL STRAIN DEFINITIONS AND MOMP**

The conventional definition of *C. trachomatis* strains is based on the historical serovar distinctions as measured by microimmunofluorescence test and reviewed by Wang and Grayston [6] >40 years ago. It is now known that this test is based on serologic differences elicited by variable segments of the chlamydial MOMP, and genovar sequence differences for the MOMP gene (*ompA*) accurately reflect MOMP serovars [7, 8]. The original Wang and Grayston [6] classification defined 15 *C. trachomatis* MOMP serovars. Subsequent isolation of serovariant strains coupled with additional *ompA* sequence data has expanded the *ompA*-based classification of chlamydial strains to >20 genovars, serovars, and serovariants [1]. Because variable MOMP regions are exposed on the surface of chlamydial elementary bodies, it is hypothesized that immune pressure fuels MOMP variation [9, 2]. Arguments also have been made that suggest that MOMP variability reflects differences in strain virulence, and the idea that MOMP mosaics [10] provide a mechanism for differences in the pathogenic potential among strains suggests that natural recombination plays a role in generating variability among *C. trachomatis* genital tract strains. Chromosomal genetic recombination for chlamydiae has recently been credibly validated in the laboratory [5], but the role of genetic exchange in supporting chlamydial variability in nature remains highly speculative.

In addition, extensive inspection of *ompA* sequence data has failed to reveal pathobiologic distinctions among the various genovars [11]. In fact, just the opposite has been found. Genovar analysis confirms long-held serovar-related arrangements of *C. trachomatis* in pathobiology complexes that are unrelated to each other. For example, chlamydial MOMP similarities group a subset of genital phenotypes (D and E serovars) with endemic trachoma phenotypes (B and Ba serovars) and with lymphogranuloma venereum (LGV) serovars (L1 and L2). Likewise, the genital strains H, I, Ia, J, and K are grouped with endemic trachoma serovars A and C and the LGV L3 serovar. There are occasional reports [12] implicating disease severity with particular genital serovars, but often these types of studies are limited in scope because of a small numbers of samples and do not hold up to more rigorous scrutiny. The vast majority of published data clearly reveal that, although D and E serovars are isolated from genital tract infections most frequently, *ompA* variability is not critical in identifying chlamydial strains with higher pathogenic potential.

The chlamydial *ompA* gene, however, has undergone genetic changes at a highly accelerated rate when compared with the rest of the *C. trachomatis* genome [13], although this variability is independent of functional differences among chlamydial isolates. Recombination hot spots are argued to exist at the *ompA*
locus [14], but this interesting observation has thus far not provided clues as to why there are no obvious pathobiologic phenotypic differences between MOMP serovars [15]. Variant MOMP strains circumvent cell culture–based antibody neutralization assays [16], but these data do not help explain why serovars E and D are most prevalent as genital tract isolates worldwide. If natural immunity exerts selective pressure, serovars D and E should be no more prevalent than other serovars. If immune selection is responsible for ompA variability, it is curious that serovar E demonstrates the least genetic and serospecific variability [9]. It is possible that serovars E and D are less immunogenic than other serovars and, therefore, remain the most prevalent strain in all populations examined. Alternatively, it is possible that still-undefined virulence attributes (eg, factors related to transmission) are linked to serovars D and E and provide a biologic advantage for these serovars.

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**DISCRIMINATION AMONG DISEASE PHENOTYPES BY CHLAMYDIAL POLYMORPHIC OUTER MEMBRANE PROTEINS**

Chlamydial polymorphic outer membrane protein (pmp) genes were immediately recognized as potentially important on the basis of the original C. trachomatis sequencing report by Stephens et al [17], who identified a family of 9 genes that appeared to be related to large surface proteins in Chlamydia psittaci identified by Longbottom et al [18]. All members of the pmp gene family (pmp A–I) in C. trachomatis appear to be transcribed, and some may be developmentally regulated and encode for proteins ranging in size from 95.5 kDa (Pmp A) to 187 kDa (Pmp C). The entire 9-member gene family represents nearly 14% of the entire coding capacity for C. trachomatis—a considerable genetic investment [19, 20].

The precise functions of Pmp proteins in chlamydiae are not entirely clear. Pmp A and D are highly conserved among C. trachomatis isolates, but other pmp genes may be quite variable from strain to strain. Pmp D and H are documented to be surface expressed. The chlamydial pmp genes code for proteins that share key motifs with a group of autotransporter proteins [21]. This autotransporter-mediated process defines the type V secretion system in various gram-negative pathogens [22]. Bacteria use a number of protein secretion mechanisms. Some of these comprise elaborate multicomponent apparatuses (eg, type III) that deliver effector proteins directly to specific host cell membranes, organelles, or cytosol. The type V autotransporter mechanism is, by contrast, relatively simple and straightforward. Type V autotransporters have common structural and functional motifs that include an amino terminal leader peptide for secretion in the periplasm using the general secretion pathway [20]; the passenger domain, which may or may not be cleaved; and a C-terminal outer membrane pore domain, through which the passenger domain passes [22]. The chlamydial pmp family shares these autotransporter attributes. In general, the N-terminal leader peptide and the C-terminal pore are conserved in autotransporters, but the passenger domains provide unique functions for this group of proteins. In some instances, they are cleaved and released, whereas in others, they remain uncleaved and, thus, physically associated with the bacterial surface [23]. Common examples of passenger domain functions among gram-negative bacteria include the immunoglobulin A proteases of Neisseria and Haemophilus and other serine proteases, including Shigella and Escherichia coli toxins. Adhesin functions have been described, as have actin recruiting and serum resistance [22].

Several chlamydial Pmp proteins have been detected on the elementary body surface (eg, Pmps D, E, G, and H). Although chlamydial Pmp function has not been extensively studied, it is clear that chlamydial Pmps B, D, and H are strongly immunogenic and can elicit proinflammatory cytokine responses [20]. Thus, both by analogy to autotransporters from other pathogens [22] and on the basis of the relatively small amount of functional data for chlamydiae (reviewed in [20]), Pmps are likely to be important in chlamydial virulence in ways that include adherence to host cells and modulation of inflammation. Some pmp genes (eg, pmp A and pmp D) are nearly invariant between strains, but others (eg, pmp E, pmp F, pmp H, and pmp I) show a great deal of variability. Of significance, Stothard et al [24] and, subsequently, Gomes et al [25] reported that ≥2 Pmp proteins clustered C. trachomatis isolates according to pathobiotypic and tissue tropism. These data may not yet be used constructively in assigning disease severity phenotypes in a C. trachomatis phenotype cluster, but the pmp family of genes are excellent candidates for more in-depth study of strain-dependent variability in virulence.

**CHLAMYDIAL TYPE III SECRETION SYSTEMS**

Type III secretion systems (TTSSs) are complex arrangements of structures that are designed to promote delivery of pathogen effector proteins after contact with the host cell [26]. The early work of Hsia et al [27] on the chlamydial TTSS was accelerated greatly by publication of the complete genome sequence for C. trachomatis [17]. This secretion mechanism became the candidate system for the family of chlamydial proteins that localize to the inclusion membrane during intracellular chlamydial growth, as originally described by Rockey et al [28]. Investigation of TTSSs in a variety of gram-negative pathogens has led to a wealth of information regarding virulence factors that
are secreted in the host cell cytoplasm or integrated in host cytoplasmic or organelle membranes in ways that promote invasion, intracellular survival, and modulation of host cell function [29].

The structural and effector genes comprising the TTSSs of most pathogens are clustered on the chromosome in pathogenicity islands [30]. This is convenient for investigators, because it allows rapid identification of putative effector proteins and, thus, facilitates advantageous approaches for identification of virulence factors and comparative virulence differences among strains. Unfortunately, this does not occur for genes involved in the chlamydial TTSS [31], which has thus far been found to be scattered in ≥10 operons that use the sigma factor for constitutive gene expression, suggesting that additional activators or repressors are required if components of the chlamydial TTSS are developmentally regulated [32].

Despite these limitations, several putative chlamydial TTSS proteins have been identified, including structural components [33, 34], chaperones [35], and effectors. One of these effector proteins (IncA) is proposed to mediate inclusion fusion in C. trachomatis [36, 37], and other Inc proteins have been studied with respect to host cell cytoplasmic binding partners, to understand their function in the context of pathogenesis [38]. The epidemiology of natural inca mutants has been studied [39], and these strains are associated with reduced virulence, indicating that systematic evaluation of this subset of putative TTSS effectors may provide relevant insight into strain-dependent disease severity phenotypes.

A second putative TTSS effector that may distinguish biotypes and differential disease severity strains is a translocated actin recruiting phosphoprotein, which is thought to be delivered into the host cell cytoplasm during the initial attachment stage of uptake. After phosphorylation, translocated actin recruiting phosphoprotein is thought to promote internalization via an actin recruiting mechanism [40–42]. This protein shows differences in the number of tandem repeats between pathobiotypes [40] and, therefore, may be useful in helping to define disease severity genotypes among classic genital serovars and genovars.

CHLAMYDIAL TOXIN

A putative chlamydial cytotoxin has been described [43], although original cytotoxic reports [44] involved LGV isolates that apparently do not possess the actual putative cytotoxin gene. On the other hand, the putative chlamydial cytotoxin does share amino acid sequence similarities to the clostridial toxin B protein [45], and a study [46] demonstrated that the ectopically expressed chlamydial protein glycosylates the small guanosine triphosphatase Rac1 in HeLa cells and causes actin reorganization in a manner similar to ectopically expressed authentic clostridial toxin B. Significantly, cytotoxin gene arrangements have been useful in defining C. trachomatis disease phenotypes [43], in that genital biovars contain a single gene with a large central deletion, LGV strains lack the toxin gene, and the closely related C. muridarum strain has 3 copies of the full-length putative toxin gene. In addition, cytotoxin gene polymorphisms have been useful in distinguishing ocular and genital C. trachomatis isolates [47]. Thus far, extensive comparisons among clinical genital isolates have not been made, nor has the actual function of the protein or cellular localization been established with certainty. The putative chlamydial cytotoxin is a protein that warrants further study to establish whether causal links to disease severity may emerge among strains with differing toxic potential, although current evidence suggests that little selective advantage is provided by cytotoxin-bearing strains in human populations, because the trend in toxin-coding region of the chromosome (the so-called plasticity zone) is for loss of function [45, 47].

CHLAMYDIAL STRESS RESPONSE PROTEINS

Immunologic studies have implicated host responses to a subset of chlamydial stress response proteins (chlamydial GroEL and GroES homologues) and disease severity [48–51]. Chlamydial GroEL has been reported to signal through the Toll-like receptor (TLR) system [52] and, thus, is a potential mediator of inflammation during chlamydial disease. However, the chlamydial groE operon (groES and groEL) is highly conserved in [17] and among chlamydial species, making disease-associated variability of these genes unlikely. One thought is that, because these genes are conserved, their immunologic association with complications of genital tract disease reflects long-term chronic infection or re-infection rather than any sort of autoimmune process. This thought, coupled with the inflammation-inducing potential of these proteins, certainly implicates the groE operon as a mediator of disease but does not provide strain-specific distinctions. It is curious, however, that chlamydiae have 3 versions of the groEL gene [17]. There is considerable sequence diversity among the 3 versions, and these proteins exhibit very different properties when expressed in heterologous bacterial strains (V. Onguri and G.I.B., unpublished observation). Differences in either expression or sequence variability for GroEL 2 and 3 among strains may be exploited to investigate differences in disease severity among genital isolates.

CHLAMYDIAL LIPOPOLYSACCHARIDE (LPS) AND OTHER GLYCOLIPIDS

Chlamydial glycolipid exoantigens have been reported for chlamydiae [53], but these reports have not been independently confirmed. The C. trachomatis LPS has been examined structurally to only a limited extent and is known to be a “rough”-type molecule [54] with a lipid A portion that is pentaacylated with longer than usual fatty acids [55]. These features (penta,
rather than hexaacylated; >C14 fatty acids, rather than <C14 fatty acids) are thought to confer the rather weak levels of macrophage activation observed for the chlamydial molecule [56]. There is some debate concerning whether chlamydial LPS signals via TLR-2 or TLR-4 pathogen pattern recognition receptors [57], although it is clear that chlamydial LPS, although unusual in its composition, is not an LPS antagonist. The chlamydial LPS has long been recognized as a genus-specific antigen [58], and although structural features of this molecule cannot be ruled out in helping to define disease severity patterns, these types of analyses would be facilitated by in silico identification of novel biosynthetic enzymes, followed by actual chemical analysis of unique versions of chlamydial LPS in conferring different disease phenotypes.

THE CRYPTIC PLASMID

Interest in the chlamydial plasmid has peaked in recent years. Several investigators have identified plasmidless strains [59, 60]. The finding by O’Connell et al [61] that a plasmidless C. muridarum strain failed to induce upper genital tract pathology in a mouse model was later expanded to include loss of virulence for an LGV plasmidless strain in mice [62]. There is some suggestion that plasmid transcriptional activity contributes to the regulation of chlamydial chromosomal gene expression [62], but direct impact of ≥1 plasmid gene product on virulence is also possible [61, 63]. Certainly, interest in the mutant originally identified in Sweden with a deletion of a portion of the chlamydial plasmid that prevented C. trachomatis detection with use of a commercially available nucleic acid amplification test has resulted in concern about fail-safe detection methods, but this strain variant has not resulted in widespread problems, nor has it been associated with an increase in disease severity [64, 65]. Because a plasmidless strain studied in a tractable model system conferred protection in the absence of disease pathology [61], more attention to the presence and absence of the plasmid in clinical isolates is warranted, especially in the context of inapparent infections and the development of natural immunity.

METABOLIC PROCESSES AND VIRULENCE

Similar to other bacteria, C. trachomatis has the capacity to recognize and respond to environmental changes by a variety of mechanisms, including the classic 2-component system comprising an environmental sensor coupled to a transcriptional regulator designed to modulate gene expression in response to environmental changes [66], which is probably responsible for transcriptional regulation during late-stage chlamydial development. Other putative chlamydial regulators also have been identified [67], including ≥1 species of small, noncoding RNA [68]. It is unclear how broad or narrow the repertoire of transcriptional regulators is across the genus and whether clues to disease severity phenotypes may be revealed by characterizing the repertoire of regulators of gene transcription; however, clearly, similar molecules have been associated with virulence phenotypes for many other important human pathogens, and there is no reason to suspect that C. trachomatis is any different in this regard.

Hypoferremia and transferrin receptor modulation are known consequences of the inflammatory response [69], and chlamydiae respond to iron limitation in a regulated way, typical of many pathogenic prokaryotes [70, 71]. Iron in usable form is often limiting in any niche, and this may present more acutely in the female genital tract. Thus, the capacity of chlamydiae to sequester iron effectively may be an important virulence consideration in genital tract infections.

Other immune-related environmental changes that are significant to the chlamydiae are a reflection of the immune-regulated modulation of tryptophan availability resulting from the induction by interferon-γ of the tryptophan-decylizing enzyme idoleamine-2,3-dioxigenase [72]. Genital strains of C. trachomatis have evolved a unique method of surviving under these nutrient-limiting conditions by activating tryptophan biosynthetic genes [73]. The unique feature of this method of acquiring tryptophan is that indole is required as a substrate, and neither the host nor the pathogen is capable of producing this compound. The current thought is that genital C. trachomatis must acquire indole from other bacterial flora (eg, vaginal flora) and, thus, be metabolically poised to complete the developmental cycle and be transmitted. Of interest, it has been found that all genital isolates examined at the molecular level possess functional partial tryptophan operons, whereas none of the tested ocular strains do [74]. This is significant because it provides a metabolic distinction between ocular and genital strains that is nearly axiomatic with tissue-specific virulence. This important finding also provides a clear rationale to suggest that other metabolic pathways may be equally important in defining disease severity phenotypes among genital isolates.

OTHER FACTORS

The aforementioned ways in which genital C. trachomatis strains may affect disease severity, especially in the upper genital tract of women, is by no means complete. It simply reflects systems that have been studied extensively enough to warrant comment. We are still learning about how chlamydiae grow and survive in an environment that is at the same time competing for essential nutrients and responding to chlamydiae in ways that are designed for pathogen destruction. Certainly, it will be important to learn more about how chlamydiae elicit inflammation and what role this plays in disease severity. Pro-
teins, such as the macrophage infectivity potentiator [75], are candidates for examination of variants that may be associated with modulating disease severity. Much work has been done on C. trachomatis adhesions or invasins, but consensus has yet to be reached. It would be interesting to learn whether C. trachomatis has >1 way to move from cell to cell and whether efficient methods of ascending the genital tract are correlated with disease severity in a strain-dependent way.

We have a lot to learn about chlamydial virulence factors, their expression, and how they affect disease severity. The bad news is that chlamydial genital tract infections continue to be an enormous public health problem; the good news is that methods are available to definitively study these organisms in ways that are likely to translate into improved understanding of their pathogenic potential and the development of better infection management strategies, with the ultimate goal of eliminating this pathogen from the list of important human infectious agents.

CONCLUSIONS AND PROSPECTUS

In many ways, we are just scratching the surface in defining chlamydial gene products that may be associated with virulence and disease severity. It must be recognized from the outset that it is difficult to assign the relative importance of any specific chlamydial gene product, set of gene products, or disease severity phenotype in the absence of host genetics, coupled with any number of other considerations, including the individualized polymicrobial microenvironment at the time of infection, the likelihood of complications resulting from primary versus repeat infections, and even the age of the infected individual at the time of infection. These and other variables may be important in determining disease severity. Clearly, there are differences that modulate outcome. Only a portion of women with uncomplicated infection eventually develop untoward sequelae. There must be reasons to account for this critically important observation. The answer to this question will no doubt involve sorting through complex data sets that include both host and pathogen factors; however, comparative bioinformatic approaches are beginning to expand our ability to identify true causal factors in all sorts of complicated data sets, and tractable genetic systems in chlamydiae finally may be a reality [5]. These approaches will be helpful for analysis of the natural history of chlamydial genital tract infection. Continued identification and study of pathogen factors will be central to our understanding of the causes for fluctuations in the prevalence of chlamydial genital tract infection, how best to manage this public health concern, and most importantly, how best to reduce the burden of upper genital tract complications among infected women.

References