Chlamydia
Intracellular Biology, Pathogenesis, and Immunity
Chlamydia

Intracellular Biology, Pathogenesis, and Immunity

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Cover photo: Immunofluorescence staining of Chlamydia trachomatis within its intracellular vacuole. Heparan sulfate-deficient CHO745 cells were infected with chlamydiae and stained with a monoclonal antibody specific for heparan sulfate. Courtesy of Stephanie Rasmussen-Lathrop, University of California.
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PREFACE

The last book that comprehensively covered the biology of *Chlamydia* was published over 10 years ago (A. L. Barron [ed.], *Microbiology of Chlamydia*, CRC Press, Boca Raton, Fla., 1988). Since that time, the field has seen an increase in the rate and scope of research that has significantly advanced our knowledge of *Chlamydia* and the diseases it causes. Five fundamental changes in the field have been the following: (i) the identification and classification of a new chlamydial species that is an important pathogen of humans, *Chlamydia pneumoniae*; (ii) new noninvasive diagnostic methods for *C. trachomatis* infections; (iii) a new, effective single-dose antibiotic; (iv) a nexus of understanding of the intersection of chlamydial and host cell biology; and (v) the determination of the genome sequences of *C. trachomatis* and *C. pneumoniae*.

The aim of this book is to provide the reader with an integrated view of our current understanding of chlamydiae based on critical examination of the research literature. The book is organized by disciplines, and we endeavor to use the knowledge of each chlamydial species to compare and contrast within each disciplinary area. This provides a platform to understand the pathobiology that forms the common basis of these unique organisms and the differences that define each species and the relationships with its hosts and diseases. Every effort has been made to provide information that is as current as possible. This has been facilitated by the timing of this book, which follows the Ninth International Symposium on Human Chlamydial Infection (held in June 1998), at which the most current, original, and unpublished research in this field was presented and discussed (R. S. Stephens et al. [ed.], *Chlamydial Infections: Proceedings of the Ninth International Symposium on Human Chlamydial Infection*, San Francisco, Calif., 1998).

This is an exciting time for chlamydial research, as horizons of understanding are expanding and researchers not previously working on chlamydiae are drawn into the field. If it was not enough that *C. trachomatis* remains a major public health problem as a cause of ocular disease, blindness, and sexually transmitted diseases, especially among teenagers, new impetus is derived by the potential association of *C. pneumoniae* with coronary artery disease, the leading cause of death in many countries. In addition, with the genome sequences now revealed for these organisms, an explosion of research activity on chlamydiae is anticipated, and it is hoped that this book will facilitate the introduction of new researchers into the field.

I acknowledge the exceptional contributions provided by the authors of each chapter, the many scientific contributions by investigators dedicated to our field, and Claudia Lammel for editing this book.

Richard S. Stephens
INTRODUCTION

Richard S. Stephens

ADVANCES, CHALLENGES, AND CHANGING PARADIGMS

Scientific advances have been made in every chlamydial research arena, including understanding chlamydial developmental biology, physiology, cell biology, disease spectrum, diagnostics, treatment, epidemiology, and immunology. That this research in some cases consolidates the previous knowledge of these organisms and in other cases requires a change in paradigm indicates that this has been a very productive decade of chlamydial research. An overview of highlights of the research advances follows.

WHAT IS CHLAMYDIA?

Terminology

Following the suggestions made by Wyrick (1998), it is useful to reiterate some of the specific terminology used in the chlamydial field. The genus is Chlamydia (capitalized and italicized), but the trivial names “chlamydiae” (plural) and “chlamydia” (singular) are often used in addition to the adjective “chlamydial.” The two major developmental forms, the elementary body and the reticulate body, are referred to as the EB and the RB. Chlamydiae have a “developmental cycle,” not a “life cycle” whose end would result in death or terminal differentiation. The intracellular vacuole in which chlamydiae grow is called an “inclusion.”

Nomenclature and Taxonomy

Although chlamydiae were originally thought to be protozoa and later viruses, it became clear that chlamydiae had all the requisite properties of bacteria. The relationship of chlamydiae to other bacteria is analyzed and presented in chapter 1. Chlamydiae have been placed in their own order, Chlamydiales, with one family, Chlamydiaceae, and a single genus, Chlamydia (Moulder et al., 1984). Molecular evaluation of rRNA sequences confirms that chlamydiae are eubacteria, but with only very distant relationships to other eubacterial orders (Weisburg et al., 1986). The genus Chlamydia consists of three major species, Chlamydia trachomatis, C. psittaci (Moulder et al., 1984), and a new species, C. pneumoniae (Grayston et al., 1989). There is increasing acceptance of a species designation for a subset of C. psittaci called C. pecorum (Fukushi and Hirai, 1992; Pudjiatmoko et al., 1997). C. trachomatis has been divided into three biovariants (biovars): trachoma, lympho-
granuloma venereum (LGV), and murine (mouse pneumonitis [MoPn] agent). DNA homology studies of genomic DNA and comparison of DNA sequences of specific genes have shown that the trachoma and LGV biovars appear to be essentially identical and the murine biovar is more distantly related. The trachoma and LGV biovars are distinguished by significantly different clinical features. LGV biovar strains readily cause systemic infections and proliferate in lymph nodes, whereas growth of the trachoma biovar is believed to be limited to columnar epithelial cells at mucosal surfaces. Differences in virulence are also reflected in tissue culture and animal models. The trachoma biovar consists of prototypical serovariants (serovars) designated by the letters A through K, including serovars Ba, Da, and Ia. The LGV biovar consists of four serovars, L1, L2, L2a, and L3. Serovars are determined by serological assays, not by DNA sequencing.

It has long been understood that C. psittaci-classified organisms represent a diverse group of quite different organisms including pathogens of birds and non-human mammals. These have remained grouped because of the lack of biological markers to distinguish between these groups of organisms—a standard that is both utilitarian and intrinsically meaningful. Recent efforts by Everett and her colleagues at the U.S. Department of Agriculture to devise a new set of genus and species designations based upon the singular sequence of a small intergenic rRNA region (Everett and Andersen, 1997) may operationally define evolutionary relationships among chlamydial organisms (see chapter 1 for a discussion of this method). However, the presumption that use of long-term evolutionary measures alone to describe biological taxonomy will lead the field toward a vital understanding of chlamydial species remains to be tested. Because horizontal gene transfer punctuates and redirects evolutionary trajectories, while the evolutionary pace of rRNA changes remains relatively constant, rRNA may not be expected to reflect significant biological differences among organisms. This is particularly notable among pathogens and their unique abilities that define, for example, host tropism. Indeed, genomic-level analyses of biological relationships among organisms have not been strongly supported by rRNA-based phylogenetic relationships among organisms (Pennisi, 1998). Proposing a new taxonomy and names for the chlamydiae also raises a concern from a public health perspective. The name Chlamydia has not served well to promote the public education necessary for effective control of chlamydial infections because it is difficult to say. However, changing the name to something else might have a significant impact on public recognition by losing the currency finally established for public awareness of the name “Chlamydia.”

Genomics

It has been chronically problematic that the chlamydial research field has not had a method for stable transformation of chlamydiae with DNA, thereby effectively eliminating genetic experimental designs. The recent completion of the C. trachomatis genome sequence will help fill that void (Stephens et al., 1998). The origin of the chlamydial genome project stems from the Eighth International Chlamydial Symposium held in Chantilly, France, where the decision was made to support the commitment of effort and resources to determine the complete genome
sequence. Thus, the result of this effort represents a success of our entire community of scientists. No other single data set has provided a more comprehensive understanding of chlamydiae and challenged many of our strongly held beliefs and scientific paradigms (see chapter 2). This information has expanded our understanding of the structural composition of chlamydiae, with implications for defining new virulence determinants (see chapter 3), and is particularly revealing in terms of understanding chlamydial metabolism (see chapter 4). This also provides a basis for reconstructing chlamydial evolutionary history and provides indicators of the environmental composition of their unique intravacuolar niche. The genome sequence sets the stage for an era of research that will alter the basis of conducting research. More profoundly, genomics provides a fundamentally different process for establishing hypotheses and will provide new experimental designs in testing them.

**INTRACELLULAR BIOLOGY**

**Developmental Biology**

Research in the fundamental biology of chlamydiae has also advanced, but, as a reflection of good science, these advances raise often provocative new questions. Although 10 years ago it was known that *Chlamydia* differentially transcribes some of its genes late in the developmental cycle (Stephens, 1988), the mechanism for gene transcription regulation was undefined. It was clear that transcriptional promoters for chlamydiae differed from those in most other bacteria. The finding of an RNA polymerase sigma factor led to an expectation for understanding the molecular mechanisms of chlamydial gene transcription (Engel and Ganem, 1990; Koehler et al., 1990). In vitro transcription assays demonstrated that this chlamydia sigma subunit recognized promoter sequences with less specificity than expected (Mathews and Sriprakash, 1994). This apparently inherent promoter recognition flexibility seems antithetical to requirements for control of gene transcription and the evident precise control of its developmental differentiation.

How, then, chlamydiae regulate gene transcription, especially of gene families associated with developmental stages, remains an enigma (see chapter 3). One paradigm is that this major sigma factor does not play a major role in differentiation and that alternative sigma factors regulate gene transcription related to developmental stage transitions. Early efforts to identify such alternative sigma subunits failed; however, the genome sequence for *C. trachomatis* revealed two alternative sigma subunits (Stephens et al., 1998). An unaddressed consideration is the effect of the nucleoid condensation on specific gene transcription that marks the differentiation of a vegetative RB to an infectious EB. The current challenge is defining the roles of each sigma factor and the coupled effect and mechanism of nucleoid condensation on sigma factor transcription initiation (Mathews and Stephens, in press).

The remarkably condensed nucleoid observed in EBs is unlike any other nucleoid in other bacteria (Costerton et al., 1976). Early efforts to characterize the mechanism identified two highly basic proteins associated with EB but not RB
chromosomes (Wagar and Stephens, 1988). During searches for late developmental gene products, the genes for each of these were identified (Hackstadt, 1991; Hackstadt et al., 1991; Perara et al., 1992). Both proteins have homology to eukaryotic histone H1, principally due to the basic amino acid composition; nevertheless, a role in DNA condensation and nucleoid development was directly demonstrated following expression in Escherichia coli (Barry et al., 1992) and by the repression of transcription and translation in vitro (Barry et al., 1993; Pedersen et al., 1994). The mechanism of interaction of these proteins with DNA and especially the process of decondensation required for vegetative growth are unknown. Whether the reported proteolytic degradation of these histones (Kaul et al., 1997) is an antecedent of a primary modification mechanism that is responsible for apparently rapid decondensation process will be important for understanding this unique process. The genome sequence revealed at least three additional proteins (CT460, CT643, and CT737), whose orthologs are associated with eukaryotic chromatin structure, that are likely involved in chlamydial nucleoid condensation (Stephens et al., 1998). An inclusive model of higher-ordered nucleoid structure and its regulation will be an important future research challenge. The practical relevance of understanding the mechanisms of this unique developmental biology relates to the paradigm of clinical persistence of chlamydial infections. The ability of chlamydiae to persist for long periods in their hosts has implications for understanding the cell biology, immunity, pathogenesis, diagnosis, and treatment. The concept of persistence is supported by experimental evidence. Although largely anecdotal for human infection, evidence has been provided for persistence in natural animal infection, animal models, and tissue culture (see chapters 7 and 8). The selective forces responsible for the acquisition and maintenance of the chlamydial chromatin structure found in EBs must be strong, and one role could be promoting a developmental pathway that leads to a microbiologically persistent state.

**Cellular Microbiology**

Whether one views the intracellular site for chlamydial growth as a “hostile environment,” as proposed by Moulder (1991), or a “comfort zone” providing rich resources ripe for exploitation, it has become clear that Chlamydia plays a very active role in manipulating its host cell and its vacuolar environment (see chapter 5). One of the most fundamental and essential steps for chlamydial infection is adhesion to and entry into the target host cell (i.e., invasion). The early data on cellular invasion are interpreted as a process of receptor-mediated endocytosis (Moulder, 1991). No eukaryotic host cell receptor has been identified, and this remains a high priority for chlamydial research. In contrast, a number of potential candidates have been implicated as chlamydial ligands which bind the putative host cell receptor that mediates uptake of chlamydiae (Wyrick, 1998), although compelling, if not controversial, evidence has been presented for a heparan sulfate-like ligand (Stephens, 1994). This hypothesis includes the ability of chlamydiae to bind exogenous heparan sulfate (Kihlstrom et al., 1992; Zhang and Stephens, 1992; Chen et al., 1996) and then use it to mediate invasion by engaging a host cell receptor (Zhang and Stephens, 1992; Chen and Stephens, 1994). The ability of chlamydiae
to bind heparan sulfate has led to some confusion in terms of a potential role in binding to host cell heparan sulfate-containing glycosaminoglycans. While it is clear that chlamydial organisms can bind such host cell-associated molecules (Kihlstrom et al., 1992; Zhang and Stephens, 1992; Chen et al., 1996), this is neither required nor sufficient to mediate chlamydial entry, as chlamydiae infect, in a heparan sulfate-inhibitable process, mutant cells that do not produce glycosaminoglycans (Zhang and Stephens, 1992; Su et al., 1996). Indeed, polystyrene microspheres coated with heparan sulfate “invade” fibroblast cell monolayers, although, like antibiotic-treated chlamydiae, polystyrene microspheres fuse with lysosomes (van Ooij et al., 1997). The uptake of coated microspheres is inhibitable by native chlamydial organisms (Stephens, 1998). These data suggest that heparan sulfate presented on a solid support alone is sufficient to mimic the entry mechanism of chlamydiae. Independent of attachment mechanism differences among chlamydiae (Chen and Stephens, 1994), uptake is always readily inhibited by heparan sulfate (or related analogs), suggesting that this mechanism is not only sufficient but likely necessary for productive interactions with eukaryotic host cells.

As many highly sulfated polysaccharides inhibit chlamydial infectivity, whether such compounds would inhibit infectivity in vivo was tested (Burillo et al., 1998; Su and Caldwell, 1998). In both of these studies, compounds such as heparin and dextran sulfate did not inhibit chlamydial infection in mice. As the compounds tested can not only inhibit but also promote the interaction of chlamydiae (Zhang and Stephens, 1992), a test of this hypothesis is insufficient until compounds that have been shown to inhibit chlamydial infectivity but that are not capable of promoting chlamydial interaction with the host cell are tested. Such a compound has been identified by in vitro testing (Chen et al., 1996), although it has not yet been tested in vivo experiments. Identification and characterization of the host cell receptor can be expected to provide definitive understanding of the mechanism(s) and ligand(s) principally required for this process.

In addition to the development of our understanding of the initial stages of chlamydial infection, significant and exciting progress has been revealed for interactions of chlamydiae with the host cell following entry. The early paradigm was one in which chlamydial EBs had, preformed, all that was required not only for invasion but also for subsequent steps such as inhibition of lysosomal fusion (Moulder, 1991). It is now clear that chlamydiae play a more active role in lysosomal fusion inhibition than previously thought (Scidmore et al., 1996). That early paradigm has been replaced by compelling data that implicate sophisticated exploitation and manipulation of the host cell by chlamydiae. The birth of this new era of “cellular microbiology” was the finding of a selective redistribution of a family of annexins (Majeed et al., 1994) that helped to mechanistically explain the phenotypic observation of the connection between F-actin and calcium ions in the translocation of early chlamydial inclusions (Majeed et al., 1993).

The identification and characterization of chlamydial proteins that are found exclusively localized to the inclusion membrane substantially expand the new paradigm (Rockey and Rosquist, 1994; Rockey et al., 1995). These proteins are involved in remodeling the inclusion membrane and are likely involved in unique properties of the inclusion membrane, such as inhibition of fusion with lysosomes
and regulating inclusion-inclusion fusion, and in transport of components necessary for chlamydial growth and survival. This is supported by the finding of their exposure to the cytoplasmic face of the inclusion membrane and the phosphorylation of at least one member of this family (Rockey et al., 1997). It can be anticipated that many more chlamydial proteins localized to the inclusion will be identified.

By its origin and nature, the chlamydial inclusion appears to represent a unique cellular compartment that becomes dissociated from the endocytic pathway and intercepts vesicles from the exocytic pathway, perhaps accounting for the rapid and substantial increase in inclusion size during chlamydial growth (Hackstadt et al., 1997). The inclusion does not acidify (Schramm et al., 1996) and is characterized by its paucity of cellular organelle markers and by its acquisition of trans-Golgi-derived sphingomyelin (Heinzen et al., 1996; van Ooj et al., 1997). A related finding was the modification of mitochondrial glycerophospholipids observed in chlamydia-infected cells that is apparently part of chlamydial lipid metabolism (Hatch and McClarty, 1998). These findings were linked to increased mitochondrial metabolism, which has been shown to be a specific result of chlamydial infection (Ojcius et al., 1998).

The level of interaction of chlamydiae with their eukaryotic hosts has recently been extended to induction of proinflammatory chemokines (Rasmussen et al., 1997) and the lack of induction and, indeed, the inhibition of programmed cell death (apoptosis) following invasion by chlamydiae (Fan et al., 1998). Both of these processes require chlamydial protein synthesis and likely depend upon an ability of chlamydiae to specifically modify the host cell's signal transduction processes. A potential common denominator is that cellular infection by chlamydiae triggers the mobilization of transcription factor NF-κB (Rasmussen et al., 1998), which activates transcription of cellular proinflammatory chemokines and suppresses apoptosis. Thus, it has become clear that investigation of the intersection of chlamydial biology with host cell biology promises to continue to provide significant new insights into what has clearly become an expanded appreciation of the complexity of this host-parasite relationship.

### PATHOGENESIS

#### Epidemiology

Defining the distribution of natural chlamydial infections of humans and its relationship to pathogenesis provides the fundamental basis for management and control of chlamydial infections and diseases (see chapter 6). This also provides the public health and medical imperatives for advancing our knowledge of chlamydiae.

The discovery of *C. pneumoniae* as a respiratory pathogen of humans and its apparent associations with a variety of respiratory and systemic diseases (Grayston et al., 1989) have significant implications for broadening the agenda and resources for chlamydial research. While research with *C. pneumoniae* had a slow start following the organism’s discovery, research activity has increased in recent years following the isolation and availability of numerous strains worldwide. The growing
evidence for the association of *C. pneumoniae* infection with atherosclerosis, while still uncertain, represents a radical new paradigm as an infectious cause for coronary artery disease with significant implications for treatment. An important challenge for *C. pneumoniae* research is the lack of noninvasive diagnostic tests for patients with active infection. Without such an objective and specific test of infection, understanding *C. pneumoniae* epidemiology and the diseases it causes will remain problematic.

New tools that facilitate *C. trachomatis* epidemiological research have been developed. The ability to rapidly and cost-effectively determine the DNA sequence of the polymorphic *ompA* gene (Dean et al., 1992) provides an unprecedented level of resolution for determining the pathways of spread and distribution of *C. trachomatis* infections in both trachoma and sexually transmitted disease epidemiological settings.

New diagnostic tests for *C. trachomatis*, especially their remarkably effective application using urine specimens (Schachter et al., 1995), and new, single-dose drugs for treatment (i.e., azithromycin) have been introduced and together offer new perspectives and opportunities for intervention and control.

**Immunology**

During the past 10 years there have been hundreds of publications primarily concerned with immune responses to *Chlamydia* or to chlamydial proteins. Immunity and pathogenesis of chlamydial infections appear to be inexorably linked. While there is as yet little understanding of chlamydial immunity or the immune basis of pathogenesis, this area has not been without advances. Chapter 7 provides perspectives on how chlamydiae cause disease. Chapter 8 addresses the question, Is there immunity following natural chlamydial infection? Finally, chapter 9 reviews information obtained from animal models concerning mechanisms of immune responses and how these can be manipulated by vaccination.

Based upon the original and early observations by Brunham et al. (1987) that sera from patients with worse disease had a different pattern on immunoblots of chlamydial lysates, distinctly around the 60,000-molecular-weight region, and upon observations by Bavoil et al. (1990) that a soluble 60,000-molecular-weight protein could be eluted from EB surfaces, Wagar et al. (1990) conducted immunoblot analyses using lysates that were differentially extracted and probed with a panel of patient sera reactive to *C. trachomatis*. A remarkable bias in serological responses only to the soluble 60,000-molecular-weight protein was discovered among some patients. Unlike patients with acute infection, approximately 80% of women diagnosed with ectopic pregnancy had antibodies to this soluble protein (Wagar et al., 1990). It was additionally shown that the protein was related to the then so-called “common antigen” or heat shock protein 60 (Hsp60) of bacteria. The *C. trachomatis* Hsp60 gene (*groEL_1*) was cloned, and the same sera were retested by using recombinant Hsp60, with the same results, definitively demonstrating that the chlamydial GroEL_1 homolog is the target antigen associated with the biased immune reactivity (Cerrone et al., 1991). Other researchers have confirmed the original results in a variety of populations by using this recombinant protein (see
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While the data for this relationship remain unchallenged, data supporting a causal relationship have not been forthcoming. The question remains whether the higher frequency of anti-Hsp60 antibody is confounded merely by longer-term exposure to chlamydial antigens, and hence more severe disease sequelae, or whether a specific immune reactivity to Hsp60 contributes to disease. The discoveries that chlamydiae elicit a family of proinflammatory chemokines in cells they infect and, more significantly, that chlamydiae elicit a persistent proinflammatory chemokine host cell response (Rasmussen et al., 1997) offer a fresh perspective and hypothesis on the immune mechanisms of chlamydial pathogenesis. One might ask why chlamydiae would elicit in their host, especially with apparent purpose, such potent and persistent inflammatory responses that are normally associated with resolution of infections? It is difficult to conclude that such a response would facilitate chlamydial intracellular survival and growth. Thus, the advantage of inflammation for chlamydiae is likely to facilitate transmission—for intrahost spread as well as promoting host-to-host transmission. Inflammation also likely contributes to host susceptibility to chlamydial infection, thereby sustaining endemicity at the population level. These represent very strong selective pressures that could overcome the negative effects of inflammation on local growth and survival. One of the implications of this paradigm is that reduction in inflammation will not only reduce disease sequelae but also reduce host susceptibility and transmission. Notwithstanding alternative explanations, evidence to support this conclusion may include the inability of trachoma to sustain itself at high endemic levels following relatively small increases in community standards of living, or behavioral activities such as face washing, and seasonal increases in trachoma coincident with bacterial and viral conjunctivitis (see chapter 3).

The basis of immunity and the development of a vaccine remain enigmatic (Rasmussen, 1998). Having correlates of immunity that can be evaluated in vitro has been important in the development of vaccines for other infectious disease systems. The development of a C. trachomatis neutralization assay should facilitate neutralization research that was previously confounded by antibody receptors on the host cells used for neutralization studies (Su et al., 1991). This assay (Byrne et al., 1993) provides the opportunity to evaluate the ability of antibodies with different specificities to neutralize infection. This is particularly important for strains of C. trachomatis that infect humans, in which antigenic variation plays an important role in immune evasion (Brunham et al., 1993; Stephens, 1989). The major outer membrane protein (MOMP) was established as a target of neutralizing antibodies (Su and Caldwell, 1991). Although antibodies that recognize linear MOMP epitopes can inhibit C. trachomatis infectivity, such epitopes, while strongly immunogenic, do not elicit potent neutralizing antibodies. The complexity of the antigenic determinants for MOMP (Stephens et al., 1982; Baehr et al., 1988; Stephens et al., 1988) raised the question of the role of conformation-dependent epitopes (Jones et al., 1992; Zhong et al., 1994). The question was answered when it was shown that polyvalent immune sera obtained following infection were dissected by adsorption using MOMP peptides or conformationally intact recombinant MOMP. Despite the presence of antibodies to linear MOMP peptides, the antibodies
responsible for mediating neutralization were only those that recognized conformational antigenic determinants of MOMP (Fan and Stephens, 1997).

As *Chlamydia* is an intracellular pathogen, one might conclude that its antigens would be processed and presented through the endogenous pathway and recognized by CD8+ cytotoxic T cells. Although the early literature provided conflicting evidence, the presence of CD8+ T cells that are capable of recognizing chlamydia-infected cells in vitro (Beatty and Stephens, 1994) and of chlamydia-specific CD8+ T cell lines that are protective in murine studies (Starnbach et al., 1994) suggests that CD8+ T cells are elicited and active in chlamydial infections. There appears to be a role of CD8+ T cells in immunity, at least in murine models (Ramsey and Rank, 1991; Igietseme et al., 1994; Magee et al., 1995), although a role in mediating disease remains a possibility (Van Voorhis et al., 1997). The roles of biased immune responses of individuals to $T_{H1}$ versus $T_{H2}$ CD4+ T-cell responses (Mosmann and Coffman, 1989) in the context of chlamydial infections may provide a model for understanding some of the differences between immune mechanisms of chlamydial immunity and disease. In murine models, $T_{H1}$ responses are associated with immunity to chlamydiae (Cain and Rank, 1995; Holland et al., 1996; Yang et al., 1996; Perry et al., 1997). For chlamydial infections of humans, analogous distinctions have not provided consistent findings (Holland et al., 1996; Van Voorhis et al., 1997).

One of the primary goals of chlamydial immunology research is the development of a vaccine that would resolve infection and/or protect against disease. Recent technological advances have provided significant strides toward understanding requirements for vaccine development. The uses of intact nonviable organisms carried by dendritic cells (Su et al., 1998), outer membrane complex fractions (Pal et al., 1997), and DNA vaccine constructs using the MOMP gene (Zhang et al., 1997), have each been shown to provide significant protection of mice against challenge with the chlamydial mouse pneumonitis strain. Common denominators of these experiments include eliciting strong $T_{H1}$-type T-cell responses and including MOMP in a native, nondenatured conformation.

**CONCLUSIONS**

Conducting research with chlamydiae is not an easy endeavor (Stephens, 1992). Investigating the clinical spectrum, epidemiology, biochemistry, genetics, and immunology of chlamydiae depends upon new and creative applications of technology to rapidly move the field forward. The following chapters set the stage for the next decade of research by presenting critical appraisals of research to date and identification of major research needs. Among the paramount research challenges to be met are:

1. Effective control strategies for *C. trachomatis* infection
2. A diagnostic test for *C. pneumoniae* infection and definition of the role of *C. pneumoniae* in coronary artery and other diseases
3. Identification of the host cell receptor(s)
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4. Identification and function of proteins delivered by the type III secretion apparatus
5. Characterization of inclusion membrane modification and remodeling
6. Mechanisms and effects of chlamydial manipulation of host cell signal transduction pathways
7. A method of genetic transformation
8. A mechanistic understanding of pathogenesis
9. Understanding the clinical implications and molecular mechanisms of chlamydial persistence
10. How to induce immunity through vaccination

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