Chapter 5.

Characteristics of Chlamydia Developmental Cycle.

As the previous chapters outlined, with effective laboratory testing we could isolate a wide variety of microbes from the seminal fluid of patients who were complaining either of infertility or symptomatic prostatitis. Similarly, a variety of bacteria were isolated from our female patients who came for either treatment for infertility, recurring miscarriages or for symptomatic genital tract infections.

For detecting the presence of Chlamydia trachomatis elementary bodies our laboratory has used the same DFA test procedure ever since its introduction in the early 1980s. We followed identical testing procedures through the years, and performed flawlessly under strict quality control measures. We thus believe that the 30% chlamydia positive reading in the early 1980s and the jump to 65% positive chlamydia reading by the end of the first decade of the 21st century represents a true increase in the spread of chlamydia infection in our infertility patient population rather than an improvement in laboratory testing. This finding is in synchrony with CDC statistics (1). The 70+ % infection rate in a recent subgroup of our patients who have visited us for the treatment of chronic prostatitis is sobering and needs further investigation.

While there were numerous other bacteria isolated in both infertility and prostatitis groups, no other bacterium was ever recovered with such predictable frequency as chlamydia.

Still, to treat Chlamydia caused infections in both male and female genital organs, either to relieve a symptomatic condition or to solve a state of infertility, one must have a basic understanding of the behavior of this bacterium.

The members of the Chlamydia family are so versatile, and mutate so readily that curing chlamydia infection with one dose, single drug antibiotic therapy or stemming the epidemic with effective vaccination have become fading dreams. When planning antibiotic therapies, one must know the best antibiotics to choose, the best way to deliver them and the best possible result to hope for.

During screening for chlamydia, one should keep in mind that though the infection is most commonly transferred through sexual contact, it can also cause inflammation in the oral cavity, ear, nasal passages, and intestinal colonization is also known.

Due to its significance in causing multiple diseases in humans and its negative effect on the reproductive process, it behooves the writer of this book to devote a full chapter to discuss the special features of the life cycle of these organisms.

Research of the last couple of decades has proven that these organisms are widely spread both in animals and in humans alike. Only the detailed understanding of the life cycle of chlamydia, its ability to survive in living cells and its resourceful adaptation to a new environment will enable us to comprehend the spectrum of diseases chlamydia causes and how to choose the proper therapy.
Halberstaedter and Von Prowazek working in Java were the first to discover the presence of chlamydia in humans and could inoculate orangutans with scrapings taken from the diseased eye. They reported it as a specific, transmissible disease in 1907. Ever since their publication the organism has been known as the causative agent of a chronic, scarring disease of the conjunctiva leading to the blinding tropical disease, called trachoma.

Our knowledge of its significance in the reproductive process and in causing genital tract infections is merely decades old. Reports from around the world, including USA, Canada, Sweden, Norway and Finland have testified for the dramatic increases in the number of newly diagnosed chlamydia infections during the last 30 years and the associated financial burden. It is estimated that screening adolescents for Chlamydia trachomatis in the United States alone will cost $2.54 billion between 2016 and 2021 (2). The World Health Organization also reported high level of transmission in Sub-Saharan Africa and in Southeast Asia (3, 4). The emergence of multidrug resistance of some of these infections is becoming reality (5). To gain control over the epidemic, health care officials face compounded hurdles.

- A significant number of infected patients are asymptomatic, vectors for new infections.
- The vertical transmission to the intra uterine baby extends the problem to subsequent generations.
- The delayed symptom development in effected organs postpone initiation of prompt therapy.
- Organs, distant from the acquisition site can become involved secondary to the high antigenicity of chlamydia, confusing the clinical picture.
- The choice for effective antibiotic therapy, and patient follow-up lack strict guidelines.
- The ability of chlamydia to rapidly mutate frustrates production of effective vaccination.
- Development of multi-drug resistant chlamydia strains became a reality with increasing therapeutic challenge.

The name Chlamydia trachomatis derives from the Greek word ‘chlamys’ – coat, describing the intracellular inclusions wrapped around the cell’s nucleus and ‘Trachoma’ a tropical eye disease known since antiquity as “rough eye”.

A large family of a variety of organisms belongs to the order of Chlamydiales. For humans, four species of this huge order are of significance: Chlamydia trachomatis, Chlamydia pneumoniae, Chlamydia psitaci and Chlamydia pecorum. Chlamydia trachomatis is the most significant genital tract pathogen, Chlamydia pneumoniae is a widespread respiratory tract pathogen and suspected to cause, cardio- and cerebrovascular disease. Chlamydia psitaci and Chlamydia pecorum are primarily animal pathogens and they are significant for veterinary personnel.
While the primary transmission route for Chlamydia trachomatis is mucosal transfer, the other three infect humans primarily through inhalation.

A common antigen group characterizes all four groups, but within these groups there are different, so-called phenotypes with different pathogenicity. In humans, by far the genital serovars (small differences in the surface antigens) of Chlamydia trachomatis are the most significant and they are responsible for a worldwide known sexually transmitted disease.

All members of the family Chlamydiaceae need living cells of a wide variety of hosts to survive. In histological samples, they are Gram-negative particles and easily discernible in a peri-nuclear location.

The genus Chlamydia trachomatis currently contains nine family members and they are denoted by their serological characteristics and labeled with the upper-case letters of the alphabet.

A, B, Ba, and C serovars or biovars (similar antigenic characteristics) belong to the Trachoma biovars, the leading cause of blindness worldwide – Trachoma.

The second group, biovars D to K causes noninvasive genital tract infections including urethritis, epididymitis, and prostatitis, in the men, and infertility, ectopic pregnancies and chronic pelvic inflammatory disease, in women.

The third group is the LGV, lymphogranuloma group. Members of this group preferentially invade lymph glands and cause suppurative lymphadenitis.

The reader should refer to Table 1 summarizing the basic characteristics of the Chlamydiaceae family.

<table>
<thead>
<tr>
<th>Species</th>
<th>C. trachomatis</th>
<th>C. pneumoniae</th>
<th>C. psittaci</th>
<th>C. pecorum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Trachoma</td>
<td>TWAR</td>
<td>1, 2, 3-9</td>
<td>No data</td>
</tr>
<tr>
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<td>Human</td>
<td>Human</td>
<td>Animals</td>
<td>Animals</td>
</tr>
<tr>
<td>Infection route</td>
<td>Contact</td>
<td>Inhalation</td>
<td>Inhalation</td>
<td>Inhalation</td>
</tr>
<tr>
<td>Place of Infection</td>
<td>Conjunctival and genitourinary epithelial cells</td>
<td>Respiratory epithelium</td>
<td>Respiratory epithelium</td>
<td>Respiratory epithelium</td>
</tr>
</tbody>
</table>
Chlamydia trachomatis and Chlamydia pneumoniae are human pathogens chlamydia psitaci and Chlamydia pecorum cause primarily animal diseases.

Table 1. Basic characteristics of the Chlamydiaceae family.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Trachoma, venereal granuloma, infection of the urogenital system, inclusion conjunctivitis, reactive arthritis, conjunctivitis and pneumonia in children</th>
<th>Pneumonia, Arteriosclerosis</th>
<th>Zoonosis</th>
<th>Zoonosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Serotypes</td>
<td>18</td>
<td>1</td>
<td>Numerous</td>
<td>3</td>
</tr>
</tbody>
</table>

Serological types of Chlamydia Trachomatis and Diseases Caused by Them

Species

Genotype

Serotype

Chlamydia Trachomatis

Trachoma

LGV

Trachoma

Inguinal Lymphogranuloma venereum

Genitourinary infections, conjunctivitis in adults and children, and pneumonia in children
This Figure 1. Visual representation of the members belonging to the genotype Chlamydia trachomatis and the diseases they cause.


It is an ongoing multifaceted research project that seeks to understand exactly how chlamydia enters the genital canal and leads to tissue damage, scarring of vital structures and ultimately leads to infertility or chronic infection. During the last two decades, significant advances have been made in the understanding how immunity to chlamydia infection develops.

Figure 2. Chlamydial developmental cycle. This illustration is modified from:

All members of the Chlamydiaceae group share a characteristic dual developmental cycle. They can exist in a contagious extracellular form: called EB (elementary body), measuring 0.2 µm. The elementary body is metabolically inert, and has been likened to a spore like form of the organism. The bacterial nucleoid is highly compacted and occupies eccentric location. An elaborate mechanism (secretory system) allows the elementary body to attach to cell surfaces, the first step before the elementary body invades the host cell, including some of the immune cells (6).

In Figure 2 attachment of the elementary body is at 12 o’clock. The third step of this attachment process injects the genetic material through specialized receptors (TTSS). Between 2 and 3 o’clock the primary differentiation is illustrated. Once this differentiation is completed cell division (at 4 o’clock) and rapid multiplication of the elementary body follows. The elementary body is now rapidly transforms into a larger, multiplicating reticulate body (RB), measuring 0.8 µm. Inside the cell the preferential location for the development of the reticulate body is next to the nucleus.

The reticulate body is not infectious but becomes metabolically active, undergoes multiple divisions (see illustration at 9 o’clock) and eventually uses up the host cell’s metabolites. Once the host cell dies and the cell membrane breaks open, the reticulate bodies convert back into elementary bodies and become infectious, ready to invade new cells. This entire process will take approximately 48 to 72 hours.

The details of this classic primary infection become much more complicated when the infection moved into the chronic phase or repeated infections with Chlamydia takes place. The influence of various factors can delay the maturation of the elementary body and its transformation into reticulate body. Interaction with the immune system and off the immune cells of the host, varying antibiotic courses taken for the initial infection are the two most important factors slowing down the chlamydia turnover rate. Some of these factors include penicillin that inhibits the RB transformation into EB, INF-gamma (Interferon-gamma) inhibits the intracellular multiplication of RBs and dietary factors that reduce the level of exogenous tryptophan (6, 7 and 8).

When and if PCR test or DFA tests are not available, in histological specimens, chlamydia is easily visualized as Gram negative, peri-nuclear inclusions. They are located after counter staining the slide with hematoxylin and eosin for cell structure identification.
Figure 2. Note peri-nuclear inclusion bodies of Chlamydia trachomatis in a case of chronic prostatitis. Biopsy slide stained with Gram stain and counterstained with hematoxylin and eosin. Original magnification X 1000, oil immersion.

Figure 3. This photomicrograph captures the moment of cell rupture as the intracellular reticulate bodies (right pointing horizontal arrow) start leaving the cell as elementary bodies (arrow pointing down). The left pointing horizontal arrow marks the picnotic nucleus of the dying cell. Gram stained slide with hematoxylin counter staining. Original magnification X 1000, oil immersion.
There are two paradigms of primary chlamydia infection:

1. The cellular paradigm

Many of those chlamydia species that infect humans or animals preferentially invade mucosal epithelial cells.

A chlamydia-free individual’s first exposure to chlamydia occurs most commonly during sexual intercourse. At that stage, the human body has no immune memory of the organism. The most common entry sites for elementary bodies of chlamydia are the surface epithelium of the male’s urethra and the mucosa of the female’s cervical canal.

There is a so-called *innate* or *inborn* defense reaction composed of white blood cells that will line up against the elementary body (EB) of the chlamydia bacterium as it enters the body the very first time. As the bacterium attaches to the cell wall of the mucosal epithelium with a special, so-called secretory system (TTSS) it damages the cell and numerous active compounds are liberated from broken down epithelial cells causing tissue damage. Within hours, the broken down, antigenically highly active chlamydia particles are picked up by macrophages and so-called dendritic cells and like messengers they will carry the chlamydia fragments to the lymphatic system: spleen, lymph nodes and thymus, where antibodies against chlamydia will form and the initiation of a secondary cell group, specialized, so-called T4 lymphocytes are activated with the memory of the chlamydia antigens. They are trained to fight chlamydia. These altered cells, T-cells, then migrate to the entry site of the original infection, most commonly the mucus membrane of the urethra and cervix (9).

When a healthy immune system is operating, it is a very quick learning process, the result of which is the formation of a line of defense by the deployment of T-cells defending against subsequent infections. Chronic infections will generate a steady supply of modified (T4) lymphocytes and contribute to local tissue damage with progressing scar formation. This tissue damage will initiate the second phase of the primary chlamydia infection, the response by the immune system that is triggered by the antigens located on the surface of the chlamydia bacterium.

Chlamydia has 4 groups of antigens: group-specific, species-specific, type-specific and sub species-specific.

*Group (genus)-specific antigens*: these antigens are similar to those found on Gram negative bacteria and mostly composed of lipopolysaccharides.

*Species-specific antigens are proteins* of the main outer membrane (MOMP) and the heat shock protein. The structural protein, MOMP is responsible for the high immunogenicity of Chlamydia. The heat shock proteins are less immunogenic. This group of antigens is the most significant in regulating the immune response associated with chlamydia infection.

*Type-specific antigens* are polypeptides and they are used to differentiate between serotypes of Chlamydia.
Sub species specific antigens were used to classify Chlamydia trachomatis into the three main sub species: those affecting the eyes causing trachoma, the group that causes mainly genital and urinary tract infections and the lymphogranuloma group.

2. The immunological paradigm

Simultaneously with the activation of the cellular defenses the second phase of Chlamydia infection will proceed with the formation of blood born anti-chlamydia antibodies and among many other active compounds, the appearance of interferon-gamma at the infection site. The result is the development of a complex cellular and blood-born immune response targeting the invading chlamydia. The magnitude of this response and the associated tissue damage can differ from individual to individual and can greatly vary against different strains of chlamydia. The subsequently developing scar tissue can determine the extent of functional limitations in the effected organs (10,11,12,13).

In the male, it is the prostate that is the primary filtering station for chlamydia infection and the site of primary and secondary infections and associated defenses. In the female similar role is played by the cervix that protects the upper genital tract. Nuance changes in the function of these two organs can reveal degrees and progression of chlamydia infection.

In the prostate, the location of the scarred areas can affect functions both in the urinary and in the ejaculatory spheres. There is good correlation between sonographic imagery of the prostate and functional changes reported by the patient on the completed Function–Symptom Questionnaire. In the cervix, sonography and evaluation of the cervical mucous can be used to gauge disease progression.

In the pathogenesis of the developing immune defense against chlamydia infections the following immune compounds are the most significant:

1. Interferon–γ, in high concentration completely inhibits the developmental cycle of chlamydia. In law concentration, it causes the development of noninfectious, resting forms by depleting the local tryptophan level.

2. The infected cells produce a series of cytokines (interleukins, IL 1-17), mostly produced by CD4 lymphocytes and white blood cells. These compounds are responsible for the inflammatory response to infection by controlling the differentiation and function of granulocytes and macrophages. Some members of these active compounds can cause protracted inflammation, extensive scarring and functional complications.

Persistent form of Chlamydia (Aberrant form, AB).

The above described, simplified presentation of the biphasic developmental cycle of chlamydia does not address all clinical problems and therapeutic challenges this organism creates. Over 50 years we have evidence that under certain conditions chlamydia can become dormant and this state could mimic a state of cure (14, 15).
It has been known since 1961 that Chlamydia can be maintained in a noninfectious form (so-called aberrant form, AB) up to nine months in the presence of antibiotic and antibody. In these particles, the metabolism is slowed and divisions suspended. With the removal of antibiotic and antibody the aberrant forms (AB) revert to infectious elementary bodies (EB). The chlamydia research field refers to this aberrant form of chlamydia existence as ‘persistence’. We prefer to call the persistence of chlamydia as dormant chlamydia infection and refer to the aberrant forms as the spore forms. Interferon, a soluble component of the humoral response to chlamydia infection is one of the most important component that can cause the transformation of the actively multiplying reticulate body into spore form. Chlamydia also can change into spore form inside monocytes. Exposure to cigarette smoke components can alter intra-cellular development of chlamydia by interfering with interferon, direct toxic effects of the cigarette smoke components including nicotine can directly reach the genital tract in quantities sufficient to cause host DNA damage and smoking can add to the constant environmental pollutants that can have impact on chlamydia development causing persistence of infection. Intracellular viruses also compete with chlamydia development and can lead to chronic dormant infections. The best studied among these viruses is herpes simplex (HSV).

During the last 30 years numerous studies were performed in vitro, in tissue cultures examining many aspects of these altered chlamydia forms. Animal studies support the existence of these altered chlamydia particles; however, no studies demonstrate the conversion of these particles into infectious agents in humans.

If proven that the periodically reactivated spore forms are responsible for symptom recurrences, the clinical course of chronic prostatitis could be the ideal in vivo model.

In our clinic practice, when a patient presents with prior adverse pregnancy outcome and her test reveals chlamydia infection we treat her with a combination of intravenous and locally given antibiotic therapy. We add steroids to the locally given antibiotics hoping to activate the dormant forms. We advise for at least two subsequent chlamydia tests, performed in one-month intervals with negative results, before starting to try to become pregnancy. An indirect proof for the existence of dormant forms is a pregnancy that is conceived after antibiotic treated chlamydia infection with repeatedly documented negative post therapy chlamydia tests that turns positive few weeks after the pregnancy is confirmed. We postulate that this finding is due to the sudden increase in blood steroid levels that can activate previously dormant chlamydia forms.

Can chlamydia infection lead to prostate cancer?

A few definitions will help the reader become familiar with basic terminology for future reading of scientific papers in this field.

**What is a genome?**

A genome is an organism’s complete set of DNAs (deoxyribonucleic acid), including all its genes. Each genome contains all the information needed to build and
maintain the organism. In humans, a copy of the entire genome – more than 3,000,000,000 DNA base pairs – is contained in all cells within the mucus.

What is a nucleotide?

A group of molecules, usually a set of amino acids, that, when linked together, form the building blocks of DNA or RNA (nucleic acids found outside of the nucleus of the cell, usually in the cytoplasmic “R”ibosomes)

What is aneuploidy?

Any variation in chromosome number that involves individual chromosomes rather than entire sets of chromosomes. There may be fewer chromosomes, as in Turner’s syndrome (one X chromosomes in females), or more chromosomes as in Down syndrome (three copies of chromosome 21).

In general, it is now recognized that infections are major contributors to the development of cancer (16, 17). Pathogens through toxic compounds can cause damage in the DNA structure of a cell. Recent research has zeroed in on some of the critical steps this damage could take place. Especially viruses are known to manipulate intracellular environment and interfere with DNA damage response (DDR, performed by a complex protein group surrounding DNA molecules). During DNA division, this repair mechanism oversees correcting random abnormalities, breaks in the single-strand of the DNA molecule or where a nucleotide (usually a group of amino acids) is missing.

Putting it in a simplified form, if a virus interferes with the DDR process means, that after the double helix becomes single helix both the normal DNA strand and the other, with the ever so slight damage on it, keep on multiplying; one follows the normal pathway, forming a healthy new cell and the other replicating into an abnormal cell with chromosomal abnormality or this unrepaired side of the multiplying DNA strand becomes altogether irregularly proliferating cancer cell.

Number of viruses are associated with human cancer: Epstein-Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), human T – lymphotropic virus 1 (HTML – 1), human papilloma virus (HPV), Kaposi sarcoma – associated herpesvirus (KSHV).

Bacterial infections are also known to be associated with cancer development. The most studied bacterium is Helicobacter pylori (18). It is further known that chronic inflammation can promote cancer progression as with chronic gallbladder and colon infections.

Research on associating chlamydia with prostate cancer is relatively recent. The ongoing studies however suggest that this organism having a unique intracellular phase in its development may have all the potentials that viruses have in tumorigenesis (19, 20, 21).
References to Chapter 5:

1. CDC 2015 sexually-transmitted disease surveillance – chlamydia (October 19, 2016)

