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Department of Clinical Microbiology, University School of Medicine, Lublin
Katedra i Zakład Mikrobiologii Lekarskiej Akademii Medycznej w Lublinie

MARIA KOZIOŁ-MONTEWKA

Chlamydiae — new taxonomy, biology and pathogenic factors

Chlamydie — nowa systematyka, biologia i czynniki chorobotwórczości

Chlamydiae are nonmotile, gram-negative, obligatory intracellular bacteria with unique developmental cycle of replication, belonging to the bacterial order *Chlamydiales*. They are best known for the diseases they cause in humans and animals but also many *chlamydiae* coexist in an asymptomatic state within specific vertebrates or amoebae, and these hosts provide a natural reservoir for these species [21]. The order *Chlamydiales* possesses family *Chlamydiaceae*. Analyses of the 16S and the 23 S rRNA showed that *Chlamydiaceae* contains two monophilogenetic lineages and nine species-level groups [4]. There are three species pathogenic for humans: *Chlamydia pneumoniae*, *Chlamydia trachomatis* and *Chlamydia psittaci* [15].

Chlamydia trachomatis

Chlamydia trachomatis has been isolated only from people and is comprised of two human biovars that are transmitted by sexual or other contact: trachoma (14 serovars) and lymphogranuloma venereum (LGV, 4 serovars which can invade lymphatic tissue). Serovars in both *Chlamydia trachomatis* biovars cause trachoma, sexually transmitted disease, some forms of arthritis, and neonatal inclusion conjunctivitis and pneumonia, and are found in the temporomandibular joint of patients with TMJ disease [8]. Chlamydial genital tract infections have been associated with increase rates of transmission of human immunodeficiency virus (HIV). Many, but not all *Chlamydia trachomatis* strains have an extrachromosomal plasmid and are generally sensitive to sulfadiazine and tetracyclines.

Chlamydia psittaci

Human psittaciosis is a zoonosis usually contracted from exposure to an infected avian species. *Chlamydia psittaci* in birds is often systemic and can be inapparent, severe, acute or chronic with intermittent shedding. Most organs become infected, as

well as the conjunctiva, respiratory system, and gastrointestinal tract. The organism is shed in the feces, contaminates the environment, and is readily spread by aerosols. Human chlamydial infections resulting from exposure to infected domestic animals do occur but seem to be relatively uncommon. They appears to be a greater risk to pregnant women who are exposed to infected farm animals [18].

Chlamydia pneumoniae

Chlamydia pneumoniae has three biovars, TWAR which infects humans without an animal reservoir, Koala and Equine. Three TWAR strains genome sequences have been independently determined, and two of them that are available in GenBank, differ in only a few bases [16]. TWAR is primarily a respiratory pathogen, and has also been associated with atherosclerosis, asthma and other acute and chronic respiratory disease [3, 6]. Manifestation of infection include pharingitis, bronchitis and mild pneumoniae. Within households, transmission occurs via respiratory secretions in schools and workplace environments and among military personnel in close living quarters. Age-specific prevalence and incidence rates suggest that infections are commonly acquired in later childhood, adolescence and early adulthood. Seroepidemiological studies indicate that infections are very common world-wide.

Pathogenic factors of *chlamydiae*, especially important in chronic infection include: unique intracellular biphasic life cycle, outer membrane proteins, lipopolisaccharide antigens and chlamydial heat shock protein 60.

DEVELOPMENTAL CYCLE

The infection process is initiated by the attachment of elementary bodies (EBs) to epithelial cells. EBs are quite resistant to environmental conditions outside the host cell, making them adapted to cell-cell and host-host transits. Entry of elementary bodies proceeds by host-driven endocytosis. Within a vacuole, elementary bodies undergo a significant morphological change into the larger, metabolitically active reticulate bodies (RBs), which are not capable to survive outside the host. The cell wall of RBs is much less rigid probably due to reductive opening of the covalent disulphide cross-linkings allowing for enhanced metabolic activities and transport of nutrients. By unknown mechanisms, phagosome-lysosome fusion is inhibited. Reticulate bodies multiply forming microcolonies as so-called inclusion bodies. Finally, reticulate bodies condense, enhance the rate of biosynthesis of outer membrane proteins and reorganise into infectious elementary bodies, which are released by exocytosis or lysis of the host cells.

OUTER MEMBRANE PROTEINS

The major outer membrane protein (omp-1), which is similar in molecular mass (~ 40 kDa) and structure among the chlamydial species constitutes an important antigenic site on the surface of chlamydial cell walls. The cell walls of EBs are rigidi-

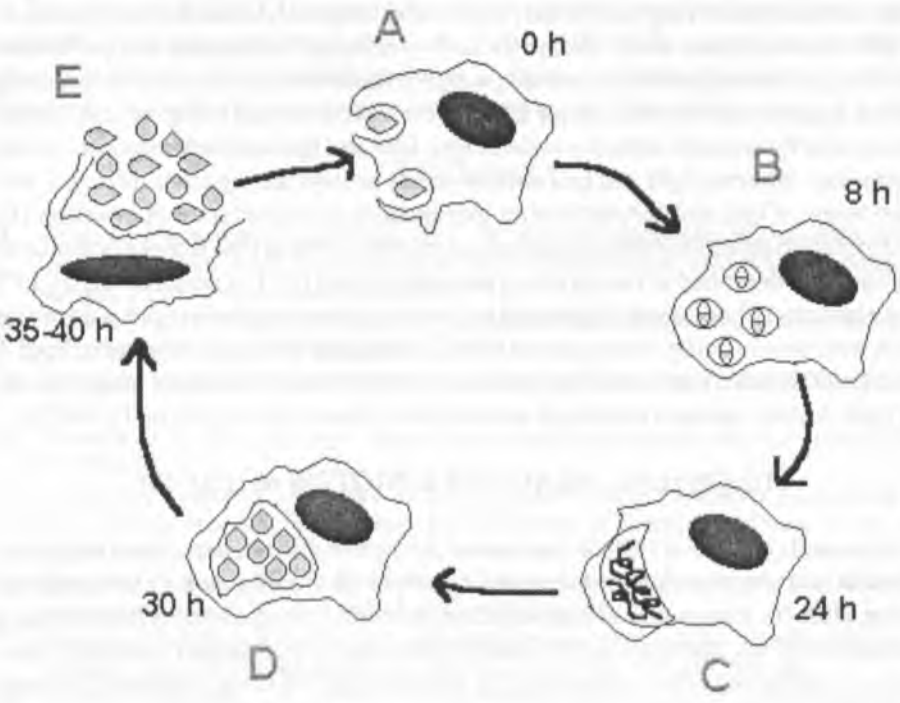

Fig. 1. J.G. Thomas, K.S. Long; Chapter 21: "Chlamydia, Mycoplasma and Ureoplasma" (611 pg). *Textbook of Diagnostic Microbiology*. Connie R. Mahon, G. Manuselis. 1995. Authors' permission has been obtained.

fied by major outer membrane protein units being highly cross-linked via disulphide bridges. In addition, the chlamydial major outer membrane protein omp-1 is glycoprotein of the high-mannose type, and manno-oligosaccharides have been found to mediate attachment and infectivity of *chlamydiae*. Meanwhile it has been demonstrated that human mannose-binding protein is capable to bind high-mannose structures on the surface of *chlamydiae* and thus may be involved in host defence mechanisms against chlamydial infections. Heparan sulphate-like glycans being present on the surface of the host cell have been proposed to be involved in the adhesion process, but did not show protective properties when used as inhibitors [22].

THE GENUS-SPECIFIC LIPOPOLISACCHARIDE ANTIGEN

The second major antigenic site located in the outer membrane is composed of a glycolipid that is shared by all chlamydial species, thus constituting a genus-specific epitope. The occurrence of a 2-keto-3-deoxy carbohydrate was described as the immunodominant group [12]. The chemical structure, antigenic and immunogenic prop-

erties were studied in molecular detail employing bacterial antigens as well as synthetic compounds serving as artificial haptens and antigens. Antibodies were generated after immunization with *chlamydiae* and selection of antibodies was performed according to binding to the immunising antigen. Based on the results of the antibody studies, a genus-specific solid phase ELISA test was developed being used in clinical laboratories for sensitive determination of IgG, IgM and IgA antibody response against *chlamydiae*. Whereas IgM and IgG antibodies are present during acute infection, elevated levels of IgG and IgA antibodies may indicate a chronic state of infection [1]. Interpretation of antibody results measured by MIF method that is more common in use, has been described in details in our previous paper [13]. For lipid A analysis, LPS from elementary bodies was hydrolysed to give the monophosphoryl lipid A derivates which were separated by reverse phase HPLC. Biological effects of chlamydial lipid A result from reduced number of fatty acids as compared to, for example, enterobacterial lipid A show reduced endotoxic activities and lowered induction of TNF-α [9].

CHLAMYDIAL HEAT SHOCK PROTEIN 60 (HSP 60)

Increased chlamydial HSP 60 expression characters chronic, persistent chlamydial infection and can stimulate autoimmune reactions by cross-homology to analogical human HSP 60. Expression of human HSPs increases during a variety of conditions such as heat shock, nutrient deprivation, infections and inflammatory reactions, functioning to stabilise cellular proteins [23]. Human HSP 60 when expressed by heat-shocked endothelial cells, can provoke an autoimmune reaction mediating endothelial cytotoxicity. Microbial HSP 60, abundantly produced during a chronic chlamydial infection of vessel wall might augment atherosclerosis, or stimulate humoral and cellular immunity in atheroma. Serological evidence of *Chlamydia pneumoniae* infection association with atherosclerosis may be explain by contribution of mechanism of HSP 60 antibody-dependent inflammation, cytotoxicity and cell injury [11].

Summarising the biological characteristics which are important for pathology of chlamydial infection we can include the following features.

Chlamydiae may exist inside cells in aberrant forms such as giant reticulate bodies or as persistent culture-negative but potentially infectious agents leading to chronic inflammatory responses. Diminished metabolic reactions make them resistant to antibiotic therapy.

Protective antibodies against outer membrane proteins make omp-1 as candidate target for vaccine development.

Chlamydial lipid A shows reduced endotoxic activities and lowered induction of TNF-α.

Presence of persistent *chlamydiae* is accompanied by increased expression of chlamydial heat-shock protein hsp-60, which displays several sequence homologies with the human heat-shock protein hsp-60 and in this way is implicated in a delayed-type hypersensitivity immune response.

Chlamydia pneumoniae as intracellular bacterium with numerous unique and atypical features remains a plausible candidate as an etiologic factor for some diseases

like atherosclerosis, coronary artery diseases, abdominal aortic aneurysm and asthma in various ways [10, 17]. Infection with these organisms may precede asthma onset, exacerbate asthma, or make asthma control more difficult. Numerous studies investigate contribution of chlamydial infection in asthma in immunity aspect [7], proper diagnostic tests [19, 20], treatment and prevention in children and adults [2, 5, 14].

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STRESZCZENIE

Postęp w dziedzinie biologii molekularnej i zastosowanie analizy 16S i 23S rRNA pozwoliło na zrewidowanie dotychczasowej systematyki rodziny *Chlamydiaceae*. Potwierdzono obecność dwóch monofilogenetycznych rodzajów, w tym dziewięć gatunków, z czego tylko trzy są chorobotwórcze dla człowieka. Jako czynniki determinujące chorobotwórczość należy wymienić zdolność do wewnątrzkomórkowego namnażania w formie dwóch postaci, obniżony metabolizm i wenątrzkomórkowe namnażanie decydujące o oporności na większość immunologicznych mechanizmów obronnych i niektóre antybiotyki. Lipid A charakteryzuje niewielka toksyczność oraz mniejsza zdolność do aktywacji TNF-α. Sekwencyjna homologia chlamydiowych białek szoku termicznego HSP 60 z analogicznymi ludzkimi HSP-60 odgrywa ważną rolę w przewlekłych infekcjach, uczestnicząc w procesach immunopatologicznych.