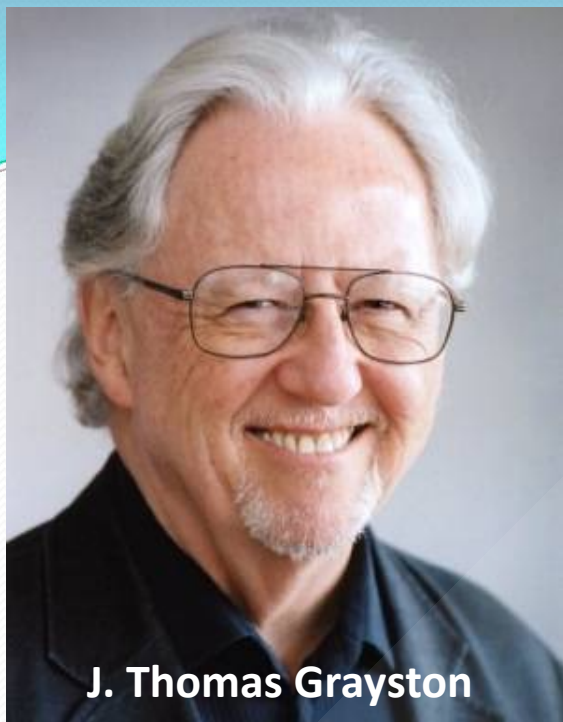




Imunoblot v sérologickej diagnostike *Chlamydophila pneumoniae*

**MUDr. Rudolf Botek, RNDr. Silvia Vašková,
Mgr. Kristína Hájková**



J. Thomas Grayston

Bull. Org. mond. Santé } 1962 26, 783-787
Bull. Wld Hlth Org.

1962 TWAR

Trachoma Virus Isolation Studies on Taiwan*

R. L. WOOLRIDGE, D.Sc., S. P. WANG, M.D. & J. T. GRAYSTON, M.D.

Studies on trachoma have been carried out on Taiwan since 1958 by the US Naval Medical Research Unit No. 2. Routine methods for the collection of virus specimens, storage and processing of successful virus isolation are presented in this paper.

A special virus isolation study on clinically active cases has shown that 49% positive diagnoses were made at the first examination, 58% at the second and 63% at the third. Detection of virus positive specimens by inclusion bodies alone would have diagnosed only 19%, 30% and 34% of the cases. Combining results of both tests increased the positive diagnosis to 57%, 64% and 68% respectively. Administration of oxytetracycline ophthalmic ointment for two series of intermittent treatments reduced virus isolations by 48% in a single test. The clinical findings in the treated cases showed only minor changes.

1986 *C. psittaci* TWAR

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1986, p. 1034-1037
0095-1137/86/121034-04\$02.00/0
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Vol. 24, No. 6

Identification of a New Group of *Chlamydia psittaci* Strains Called TWAR

CHO-CHOU KUO,^{1*} HSIN-HSING CHEN,¹ SAN-PIN WANG,¹ AND J. THOMAS GRAYSTON^{1,2}

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Received 6 June 1986/Accepted 25 August 1986

A new group of *Chlamydia psittaci* strains has been identified. They are called TWAR after the laboratory designation of the first two isolates. Twelve strains were isolated from pharyngeal swabs of different persons with acute respiratory disease in Seattle, Wash., during 1983 to 1986. One strain was obtained from the eye of a child during the trachoma vaccine study in Taiwan in 1965. Nine strains were characterized in this study. TWAR organisms formed intracytoplasmic inclusions in HeLa cells which were morphologically typical of *C. psittaci* and iodine stain negative (contained no glycogen). Immunological analysis with various chlamydia-specific monoclonal antibodies revealed that TWAR strains belong to the genus *Chlamydia*, are distinct from *C. trachomatis*, and are serologically unique among *C. psittaci*. All TWAR strains so far isolated appear identical serologically. TWAR organisms grew poorly in egg and cell cultures and demonstrated low virulence to mice by intracerebral, intranasal, and intravenous inoculation. Available data suggest that the TWAR strain is a primary human pathogen.



San-Ping Wang

Chlamydia pneumoniae sp. nov. for *Chlamydia* sp. Strain TWAR

J. THOMAS GRAYSTON,^{1,2*} CHO-CHOU KUO,¹ LEE ANN CAMPBELL,¹ AND SAN-PIN WANG¹

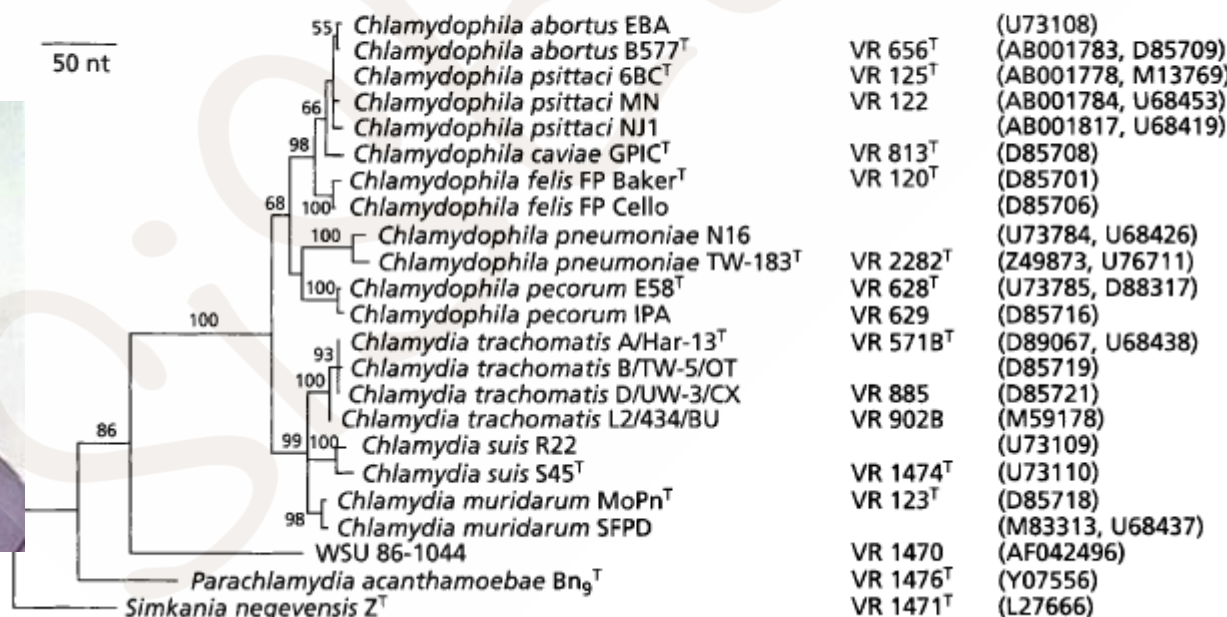
Departments of Pathobiology¹ and Epidemiology,² University of Washington, Seattle, Washington 98195

A third species, *Chlamydia pneumoniae*, is proposed for the genus *Chlamydia*. This bacterium is a human respiratory pathogen, which has been referred to as the TWAR strain of *Chlamydia*. Species identification is based on ultrastructural differences in the elementary bodies, deoxyribonucleic acid analysis, and serology.

K. D. E. Everett, R. M. Bush and A. A. Andersen



Karin D. E. Everett



- Chlamydiae (class)
 - Chlamydiales
 - Chlamydiaceae
 - Criblamydiaceae
 - Parachlamydiaceae
 - Rhabdochlamydiaceae
 - Simkaniaceae
 - Waddliaceae
 - Unclassified Chlamydiales
 - environmental samples
 - environmental samples
- uncultured Chlamydiae bacterium

- Chlamydiaceae
 - Candidatus Clavochlamydia
 - Chlamydia
 - *Chlamydia muridarum*
 - *Chlamydia suis*
 - *Chlamydia trachomatis*
 - Chlamydophila
 - *Chlamydophila abortus*
 - *Chlamydophila caviae*
 - *Chlamydophila felis*
 - *Chlamydophila pecorum*

Chlamydophila pneumoniae

- *Chlamydophila psittaci*
- >16 nešpecifikovaných druhov

upravené

<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi>

PERZISTENCIA/LATENCIA

Persistent Chlamydiae: from Cell Culture to a Paradigm for Chlamydial Pathogenesis

WANDY L. BEATTY,¹ RICHARD P. MORRISON,² AND GERALD I. BYRNE^{1*} MICROBIOLOGICAL REVIEWS, Vol. 58, No. 4, Dec. 1994

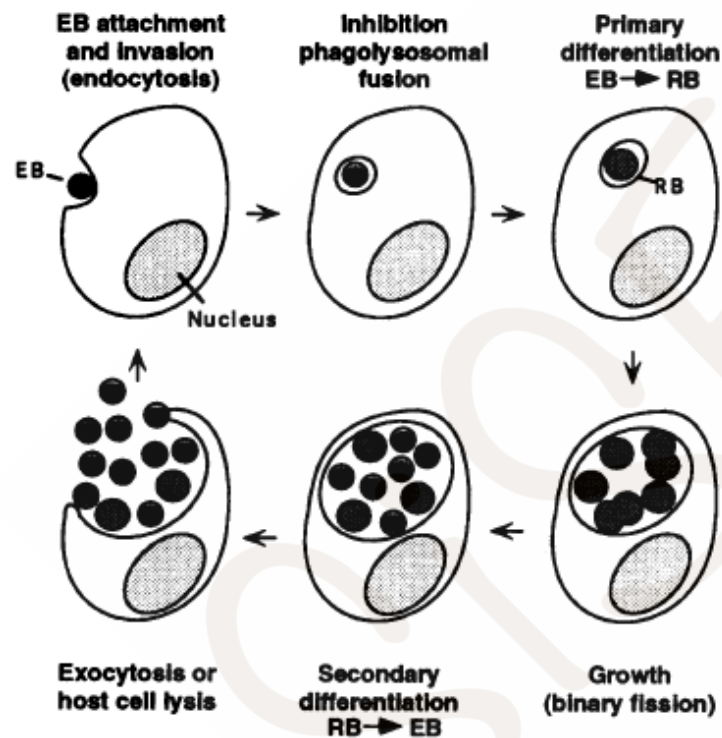


FIG. 1. Schematic diagram of the *Chlamydia* developmental cycle.

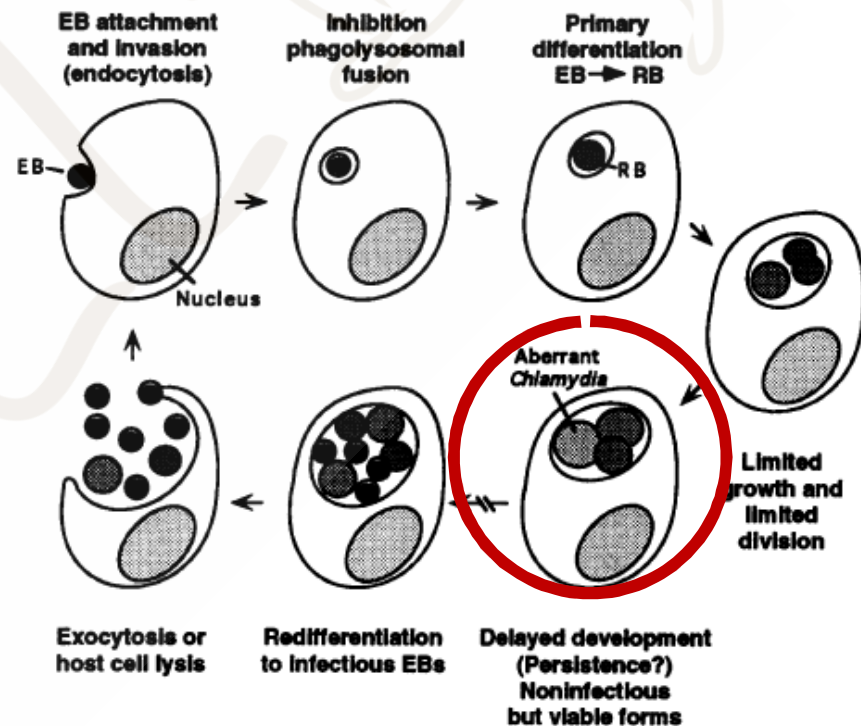


FIG. 2. Schematic diagram of altered intracellular *Chlamydia* development.

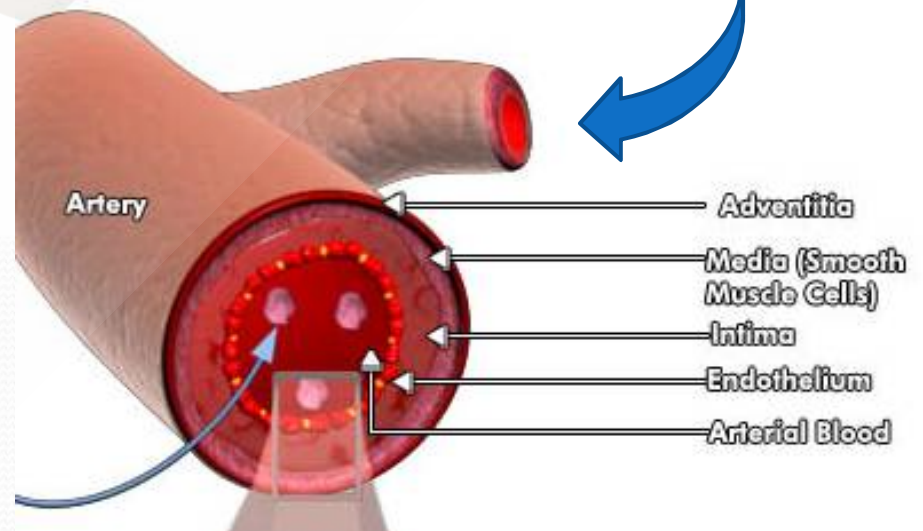
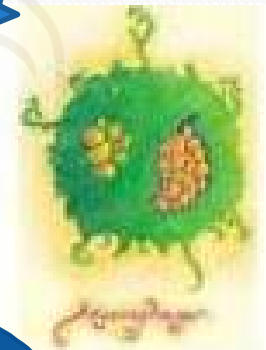
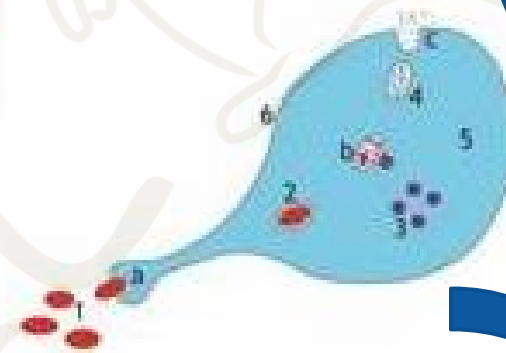
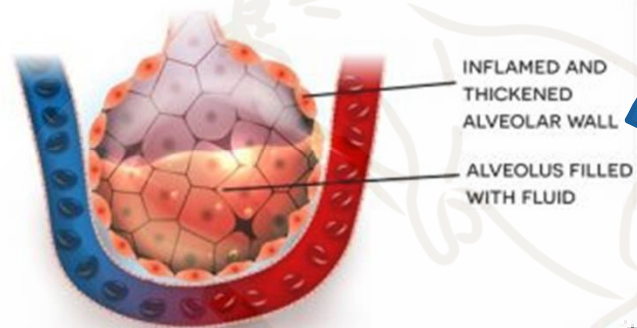
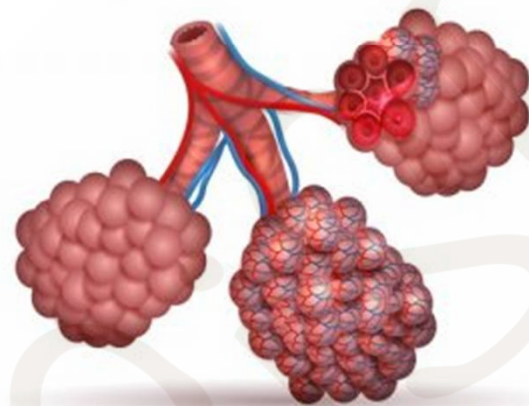
Patogenita chlamýdií

Chlamýdie majú afinitu k :

- Epitelu
- Endotelu
- Bunkám hladkého svalu
- Fibroblastom
- Bunkám monocyto/makrofágového systému

Chlamydophila pneumoniae

- Kardiovaskulárne ochorenia
- Ochorenia respiračného traktu
- Pneumónia
- Alveitída a bronchiolitída
 - Chronická bronchitída
 - Tracheitída, larygitída, faryngitída
 - Sinusitída, otitída
- Imunopatologické ochorenia
 - Astma
 - Reaktívna artritída
- Ochorenia CNS



Chlamýdiae interferujú s
mitochondriami sprostredkovanou
apoptózou

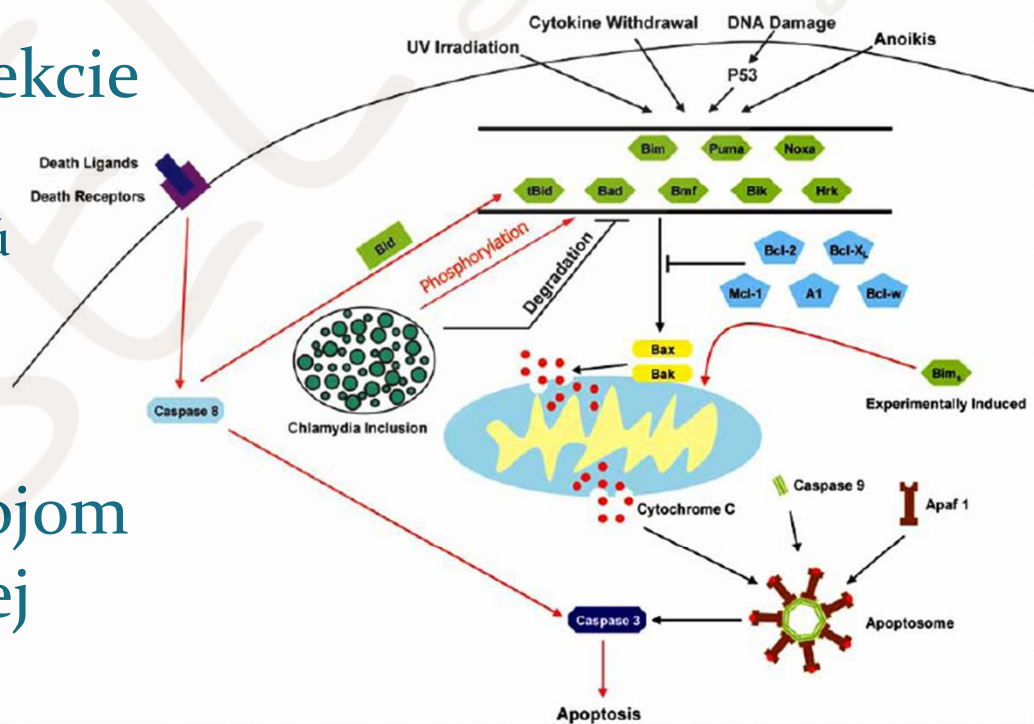
Prežívajúce bunky sú
nositeľom cryptickej infekcie

Neihibujú receptorom indukovanú
nekrózu cestou kaspázy 8

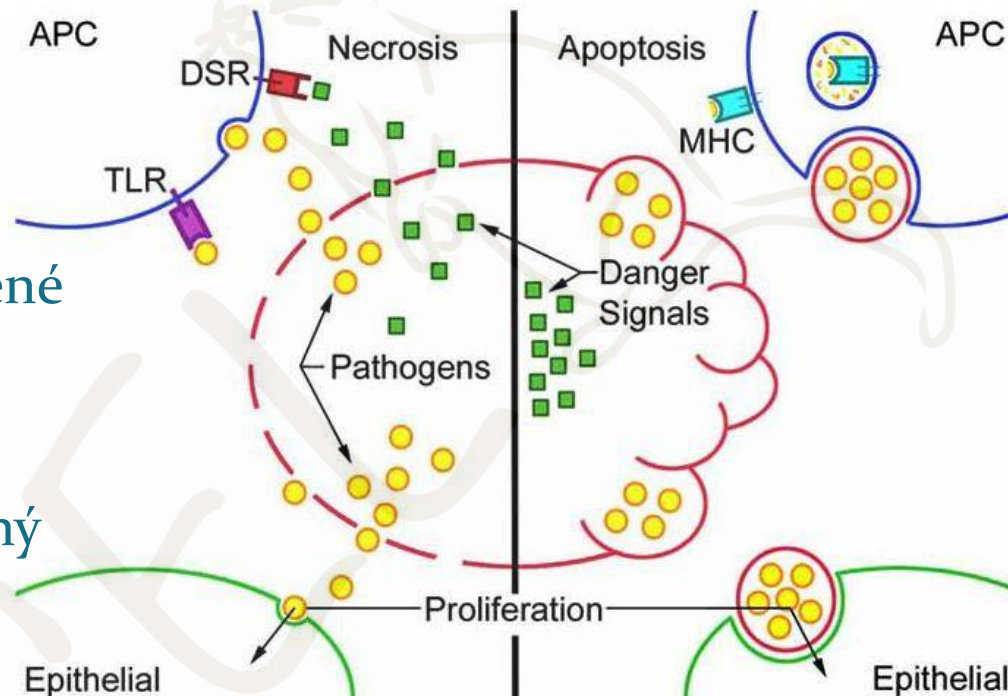
Rozpadnuté bunky sú zdrojom
antigénnej a prozápalovej
stimulácie

Host-Cell Survival and Death During Chlamydia Infection

Current Immunology Reviews, 2007, Vol. 3, No. 1

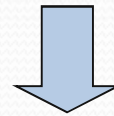


Epiteliálne bunky usmrtené „via apoptosis“ prezentujú Na svojich povrchoch aj chlamýdiami derivovaný antigén cestou MHC a touto cestou môže byť prenesený aj na susedné bunky a cestou APB - okrem iného - stimuluje prozápalové mediátory

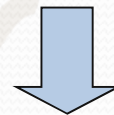


Songmin Ying et al.: Host_Cell Survival and Death During *Chlamydia* Infection. Current Immunology Reviews, 2007

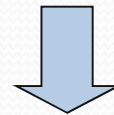
Lymfocytárna aktivácia, produkcia cytokínov, expresia adhezívnych molekúl, etc.)



***Chronický oligosymptomatologický
fibroproduktívny zápal***



***(Subinhibičná produkcia TNF, IL 1;6, INFgama, TGFbeta,
etc.)***



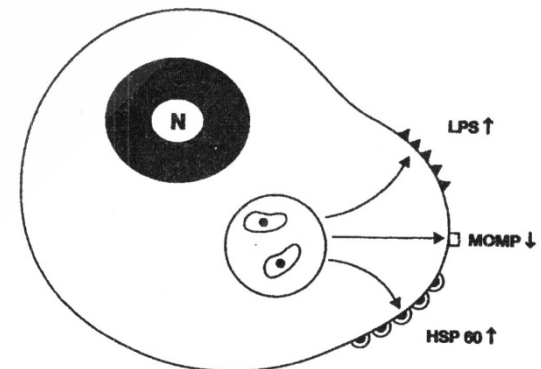
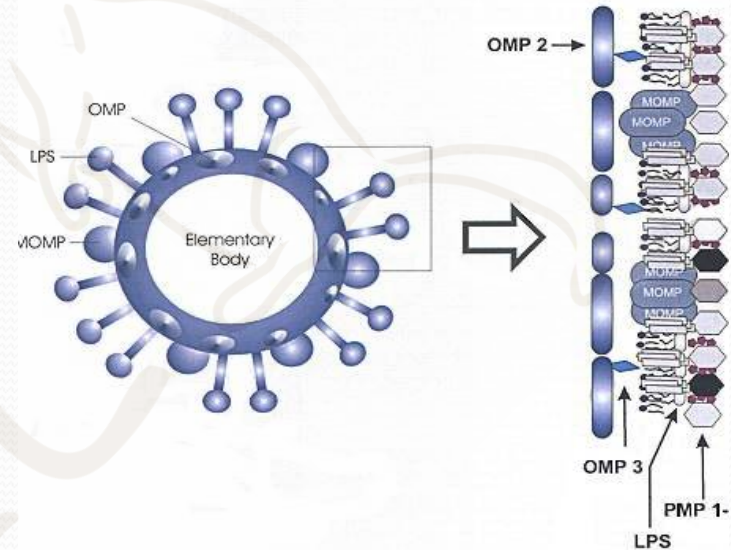
Autoimunopatologické prejavy

Antigenic Structure

- Chlamydiae possess **shared group (genus)-specific antigens**.
 - These are heat-stable lipopolysaccharides with 2-keto-3-deoxyoctanoic acid as an immunodominant component.
 - Antibody to these genus-specific antigens can be detected by CF and immunofluorescence.
- **Species-specific** or **serovar-specific** antigens are mainly outer membrane proteins.
 - Specific antigens can best be detected by **immunofluorescence**, particularly using monoclonal antibodies.
 - Specific antigens are shared by only a limited number of chlamydiae, but a given organism may contain several specific antigens.
 - There are at least 16 **serovars** of *C trachomatis*; these include A, B, Ba, C–K, and L1, L2, L2a, L3.
 - Several serovars of *C psittaci* can be demonstrated by **complement fixation (CF)** and **microimmunofluorescence** tests.
 - Only one serovar of *C pneumoniae* has been described.

Patogenita chlamýdií

- Lipopolysacharidový komplex polyklonový aktivátor makrofágov a B buniek
špecifická väzba na CD14 receptor
- Heat shock proteins
 - autoimunita mechanizmom skríženej imunity
- Outer membrane proteins
 - autoimunita mechanizmom skríženej imunity
- Glykolipidový exoantigén (GLXA)
 - stimuluje cytotoxickú aktivitu



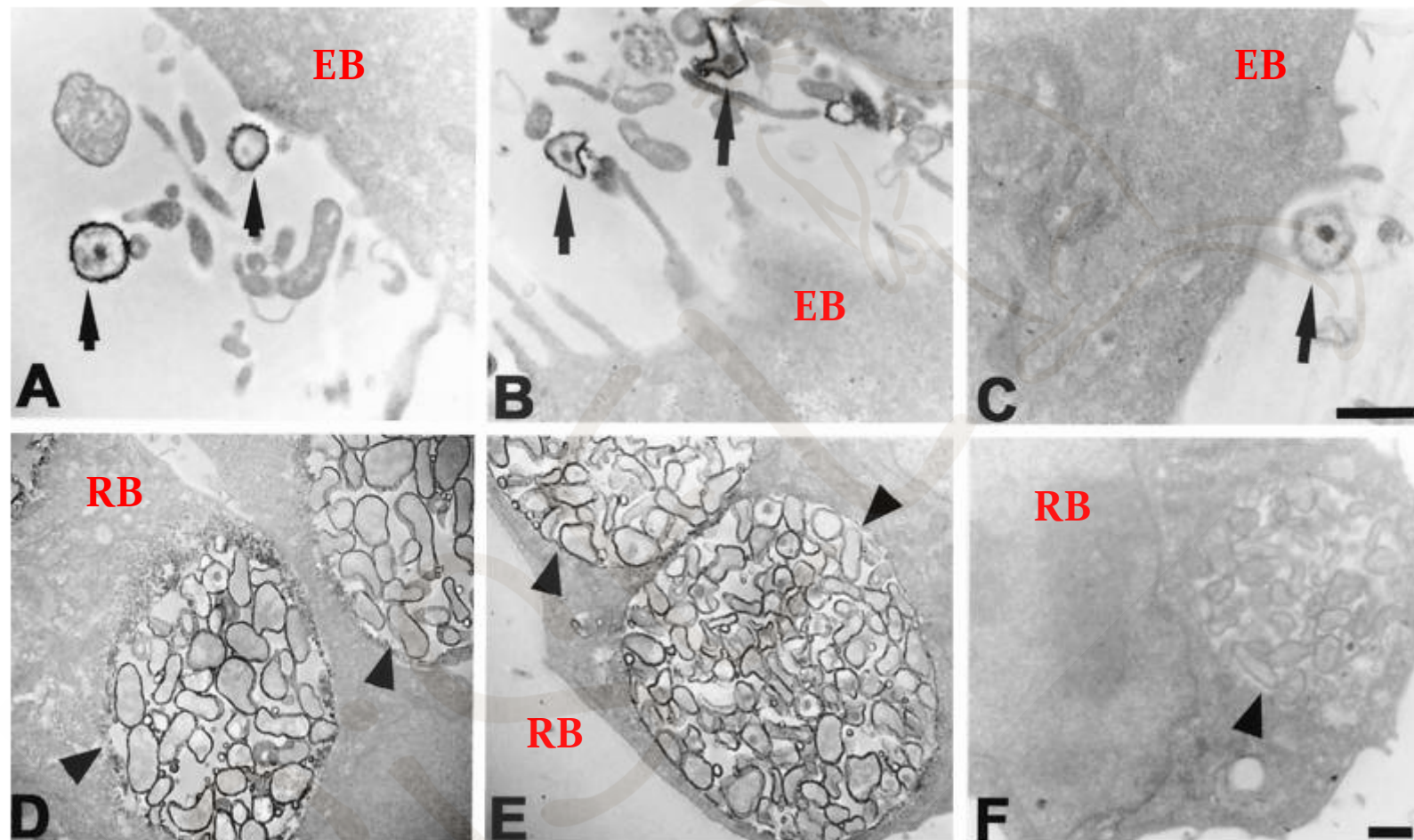


FIG. 2. Transmission electron microscopy of *C. pneumoniae* EBs (A) and RBs (D) stained with the GZD1E8 MAb indicate surface localization of the antigenic determinant. Surface staining of *C. pneumoniae* EBs (B) and RBs (E) is also shown with an anti-chlamydial LPS MAb, EVIH1. No staining of *C. pneumoniae* EBs (C) and RBs (F) was observed with anti-rickettsial MAb 8-13A4A10. Arrowheads indicate inclusions and arrows indicate EBs of *C. pneumoniae*. Bars = 0.5 μ m.

K. Wolf et al., "Chlamydia Pneumoniae Major Outer Membrane Protein Is a Surface-Exposed Antigen That Elicits Antibodies Primarily Directed against Conformation-Dependent Determinants," *Infection and Immunity* 69, no. 5 (2001): 3082-91, doi:10.1128/IAI.69.5.3082-3091.2001.

Table 1. Summary of studies that describe *C. pneumoniae* antigen/immunogen candidates with possible application in serological diagnosis

Type of study	Reference
Characterization of MOMP as an immunogen	Iijima <i>et al.</i> (1994); Jantos <i>et al.</i> (1997); Wolf <i>et al.</i> (2001)
Characterization of PorB as an immunogen	Kubo & Stephens (2000)
Characterization of CrpA as an immunogen	Melgosa <i>et al.</i> (1993); Klein <i>et al.</i> (2003)
Characterization of OmcB as an immunogen	Mygind <i>et al.</i> (1998); Stephens <i>et al.</i> (2001)
Characterization of a 76 kDa protein as an immunogen	Perez Melgosa <i>et al.</i> (1994)
Characterization of members of the PMP family as immunogens	Knudsen <i>et al.</i> (1999); Christiansen <i>et al.</i> (2000)
Identification of 263 <i>C. pneumoniae</i> proteins	Vandahl <i>et al.</i> (2001)
Identification of 53 <i>C. pneumoniae</i> antigens, 41 of which were immunogens	Montigiani <i>et al.</i> (2002)
Evaluation of six proteins (enolase, OmpH, HtrA, ArtJ, Pmp2, Pmp10) as vaccine candidates	Finco <i>et al.</i> (2005)
Evaluation of 54 kDa protein (CPn0980) as an immunogen	Campbell <i>et al.</i> (2001); Sueur <i>et al.</i> (2006)
Description of CrpA, OmcB, MOMP-VD2 and MOMP-VD3 as immunogens	Klein <i>et al.</i> (2003)
Identification of Omp11, PmpG and type III secretion system ATPase as immunogens	Park <i>et al.</i> (2009)
Studies in persistent infections	
Identification of upregulated proteins	Molestina <i>et al.</i> (2002)
Identification of altered protein expression pattern	Mukhopadhyay <i>et al.</i> (2004)
Identification of upregulated proteins	Mukhopadhyay <i>et al.</i> (2006)
Identification of eight immunogens (RpoA, MOMP, YscC, Pmp10, PorB, Pmp21-m, GroEL and CpaF-c)	Bunk <i>et al.</i> (2008)

Enrique Villegas, Antonio Sorlózano, and José Gutiérrez, "Serological Diagnosis of Chlamydia Pneumoniae Infection: Limitations and Perspectives," *Journal of Medical Microbiology* 59, no. 11 (2010): 1267–74, doi:10.1099/jmm.0.020362-0.

Comparison of *Chlamydia pneumoniae* Isolates by Western Blot (Immunoblot) Analysis and DNA Sequencing of the *omp 2* Gene

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Received 8 June 1994/Returned for modification 11 July 1994/Accepted 24 August 1994

The 60-kDa cysteine-rich outer membrane protein gene (*omp 2*) from nine *Chlamydia pneumoniae* isolates (TW-183, CM-1, CWL-050, CWL-011, IOL-207, FIL, Kajaani-6, Helsinki-12, and Parjaanonon) was amplified by PCR and sequenced from positions 1 to 580. In contrast to the sequence differences previously observed in this gene in other chlamydial species, all nine *C. pneumoniae* isolates were 100% identical. However, when sera from *C. pneumoniae* microimmunofluorescence-positive patients (with clinical signs of persistent cough or asthma) were immunoblotted against five *C. pneumoniae* isolates, distinct antigenic differences were observed. TW-183 was characterized by major bands at 35 and 43 kDa. In contrast, the other four isolates tested produced similar, though not identical, immunoblot profiles, characterized by strong bands at 18, 25, 29, 40, 46, and 53 kDa. These data support the fact that significant differences do exist between *C. pneumoniae* isolates, but unlike the case with other chlamydial species, these differences do not reside in either of the commonly studied outer membrane protein genes, *omp 1* or *omp 2*.

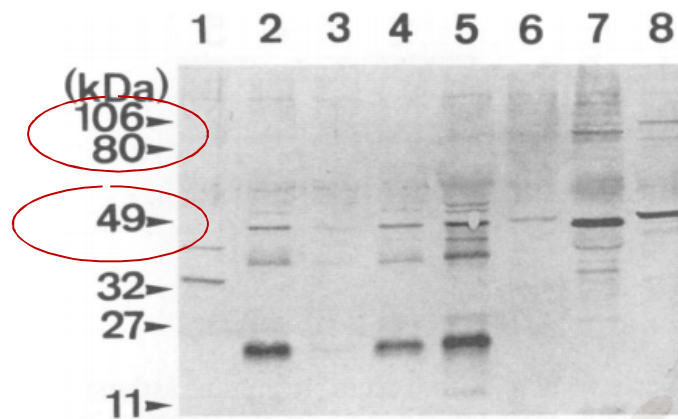


FIG. 1. Western blot with serum from a respiratory patient with a persistent cough (*C. pneumoniae*-specific MIF IgG titer of 1/256) against a range of chlamydial antigens. Lanes: 1, *C. pneumoniae* TW-183; 2, *C. pneumoniae* CM-1; 3, *C. pneumoniae* CWL-050; 4, *C. pneumoniae* CWL-011; 5, *C. pneumoniae* IOL-207; 6, *C. pecorum* (VR625); 7, *C. psittaci* (avian); 8, *C. trachomatis* (L2).

S ET AL.

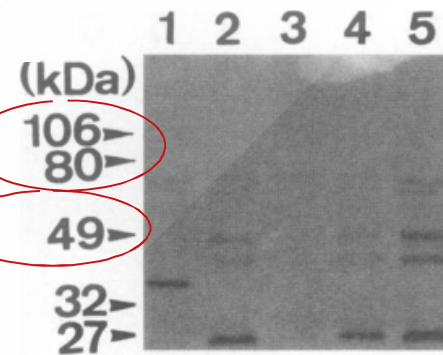


FIG. 2. Western blot with serum from an asthma patient (*C. pneumoniae*-specific MIF IgG titer of 1/1,024) against a range of *C. pneumoniae* antigens. Lanes: 1, TW-183; 2, CM-1; 3, CWL-050; 4, CWL-011; 5, IOL-207.

Imunodominantné Ag 40 (MOMP), 53, 46, 43 (73) kDa

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1994, p. 583–588
0095-1137/94/\$04.00+0
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Vol. 32, No. 3

Characterization of *Chlamydia pneumoniae* Species-Specific Proteins Immunodominant in Humans

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RINZO SOEJIMA,² AND AKIRA MATSUMOTO^{1*}

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Division of Microbiology, Hiroshima Prefectural Institute of Public Health, Hiroshima,³ Japan

Received 26 March 1993/Returned for modification 27 April 1993/Accepted 8 June 1993

Proteins of *Chlamydia pneumoniae* immunodominant in humans were characterized with the sera of 13 patients who were not likely to have been exposed to *C. trachomatis* or *C. psittaci*. The serological responses among these patients were similar on a qualitative basis, but some differences were found quantitatively. However, the serological responses of the patients who were infected with *C. pneumoniae* differed markedly from those of two patients who were infected with *C. trachomatis* and two who were infected with *C. psittaci* and those of mice that were transtracheally infected with *C. pneumoniae*. Among proteins immunodominant in the patients who were infected with *C. pneumoniae*, a 40-kDa major outer membrane protein was genus specific and 53-, 46-, and 43-kDa proteins were species specific in their reactions with the majority of the human sera used. A few sera reacted strongly with a 73-kDa protein genus specifically. Some proteins with weak immunogenicity exhibited species specificity. An antigenic analysis with human sera and murine monoclonal antibodies against the 53-kDa protein showed that the antigenicities were strictly conserved among the seven strains of *C. pneumoniae* tested. The genus-specific 73-kDa protein was solubilized with octylglucoside. All of the species-specific immunodominant proteins were solubilized with sodium dodecyl sulfate, but the genus-specific major outer membrane protein was not. These results suggest that a serological diagnosis of *C. pneumoniae* infection could be achieved species specifically by comparison of the serum responses to sodium dodecyl sulfate- and octylglucoside-soluble fractions.

GLXA produkovaný EB tesne po invadovaní hostiteľskej bunky aj po transformácii RB je exprimovaný na povrchu infikovaných aj susedných nienfikovaných buniek – marker kurentnej aj perzistentnej infekcie

**Current
Microbiology**

An International Journal
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**Cell Surface Display of the Chlamydial Glycolipid Exoantigen (GLXA)
Demonstrated by Antibody-Dependent Complement-Mediated
Cytotoxicity**

Wilmore C. Webley,¹ Gary J. Vora,² Elizabeth S. Stuart¹

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Received: 22 July 2003 / Accepted: 12 November 2003

Abstract. The chlamydial species are Gram-negative bacterial pathogens critical to human health. Their developmental cycle is associated with the formation and release of the broadly conserved glycolipid exoantigen (GLXA), which has been implicated in the chlamydial elementary body–host cell interaction. This study examines the potential surface display of this glycolipid by chlamydiae-infected cells and the ability of the GLXA they secrete to associate with the plasma membranes of uninfected cells, a prerequisite for exerting influence on them. The sequential incubation of anti-GLXA antibody and complement with *Chlamydia trachomatis* serovar K or *C. pneumoniae* AR-39-infected HeLa 229 or macrophage cells resulted in significant cellular cytotoxicity, which preceded the formation of mature elementary bodies. For uninfected cells, co-incubation of GLXA, purified from supernatants of either *C. trachomatis* or *C. pneumoniae*-infected HeLa 229 cells, followed by the successive addition of mouse anti-GLXA antibody and complement, yielded similar levels of cellular cytotoxicity. Thus, GLXA indeed is displayed on the surface of infected cells and, therefore, if antibody of appropriate specificity were present, this GLXA could serve to target these infected cells for elimination. Furthermore, released GLXA can associate with uninfected cells and therefore would be positioned to influence their behavior, especially in the context of infection.

Review

Correspondence
José Gutiérrez
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Serological diagnosis of *Chlamydia pneumoniae* infection: limitations and perspectives

Enrique Villegas,¹ Antonio Sorlózano¹ and José Gutiérrez^{1,2}

¹Departamento de Microbiología, Universidad de Granada, Granada, Spain

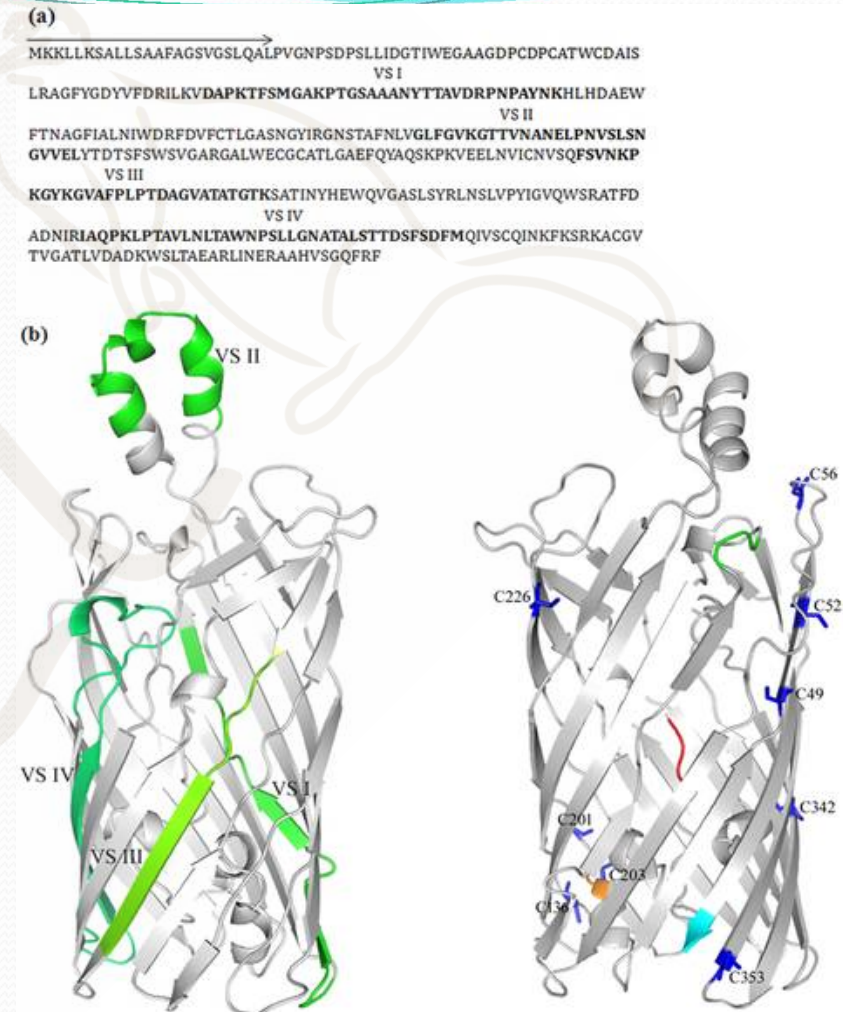
²Laboratorio de Microbiología, Hospital Universitario Virgen de las Nieves, Granada, Spain

Chlamydia pneumoniae is an obligate intracellular human pathogen responsible for a wide range of acute and chronic human diseases, including pneumonia and other respiratory diseases. Serological methods for the diagnosis of *C. pneumoniae* infection vary widely, and several authors have reported significant inter- and intra-laboratory variability in diagnostic methods and criteria. Over the past 10 years, numerous studies have focused on the identification of specific antigens for application in serodiagnosis, including the diagnosis of persistent infections. The use of proteomics may enable the development of serological diagnosis kits that offer reliable sensitivity and specificity and might even differentiate between the various stages of infection with this pathogen.

Figure 2. MOMP sequence analysis and homology model.

Figure 2. MOMP sequence analysis and homology model.

- (a) Primary sequence of MOMP from *C. pneumoniae*. The signal sequence is indicated by an arrow while the four variable segments (VS), interspersed between the five constant domains, are highlighted in bold.
- (b) *Left* - Cartoon representation of the homology model of MOMP from *C. pneumoniae*, showing the location of the four variable domains (green). VS I, III and IV are in the barrel while the protease accessible VS II is in the extracellular space. *Right* - Cysteine residues are highlighted in blue. The N- and C-termini are



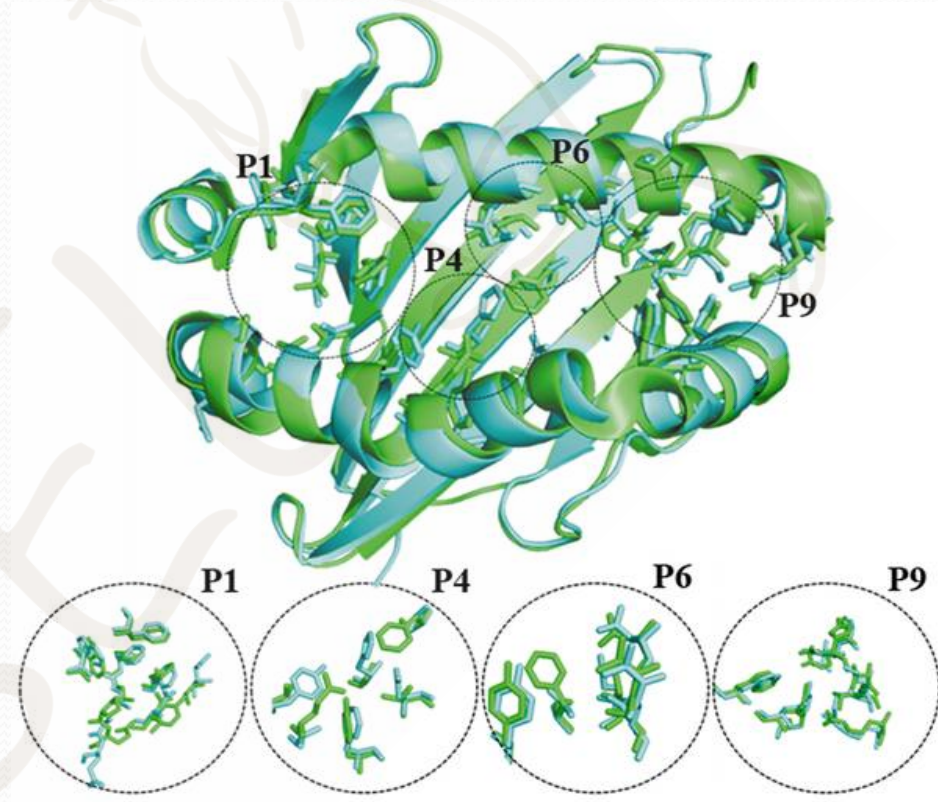
coloured red and cyan, respectively.

Atanu FO, Oviedo-Orta E, Watton KA (2013) A Novel Transport Mechanism for MOMP in Chlamydomonas reinhardtii and Its Putative Role in Immune-Therapy. PLOS ONE 8(4): e61139. <https://doi.org/10.1371/journal.pone.0061139>

Figure 4. MHC II peptide binding pocket.

Figure 4. MHC II peptide binding pocket.

Structure superposition of the binding sites of murine I-Ab (PDB code: 1MUJ) and human HLA-DR4 (PDB code: 2SEB). The binding pockets of the MHCs are designated P₁, P₄, P₆ and P₉. Pocket forming residues of I-Ab are coloured as green sticks and their corresponding HLA-DR4 residues, as identified by structural alignments, are coloured as cyan sticks.

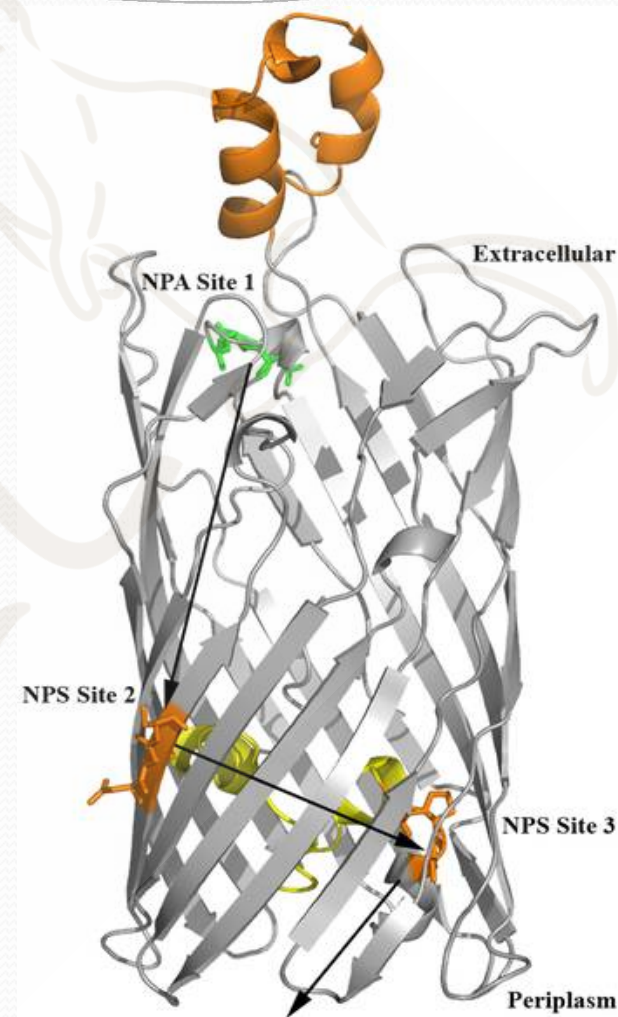


Atanu FO, Oviedo-Orta E, Watson KA (2013) A Novel Transport Mechanism for MOMP in *Chlamydomonas reinhardtii* and Its Putative Role in Immune-Therapy. PLOS ONE 8(4): e61139. <https://doi.org/10.1371/journal.pone.0061139>
<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0061139>

Figure 3. Hypothetical transport model of MOMP.

Figure 3. Hypothetical transport model of MOMP.

The model shows the relative orientations of the NPA motif (green) and the two NPS motifs (orange) including the 'hatch domain' (yellow) and the proposed mechanism for solute recognition and transport (dashed lines). The NPA motif at site 1 is on the extracellular side and may serve to recognise and orient hydrophilic molecules for transport. The two NPS motifs are oriented at juxtaposition on the inside of the barrel wall (site 2 and site 3) to coordinate binding and release of ligands into the periplasm.



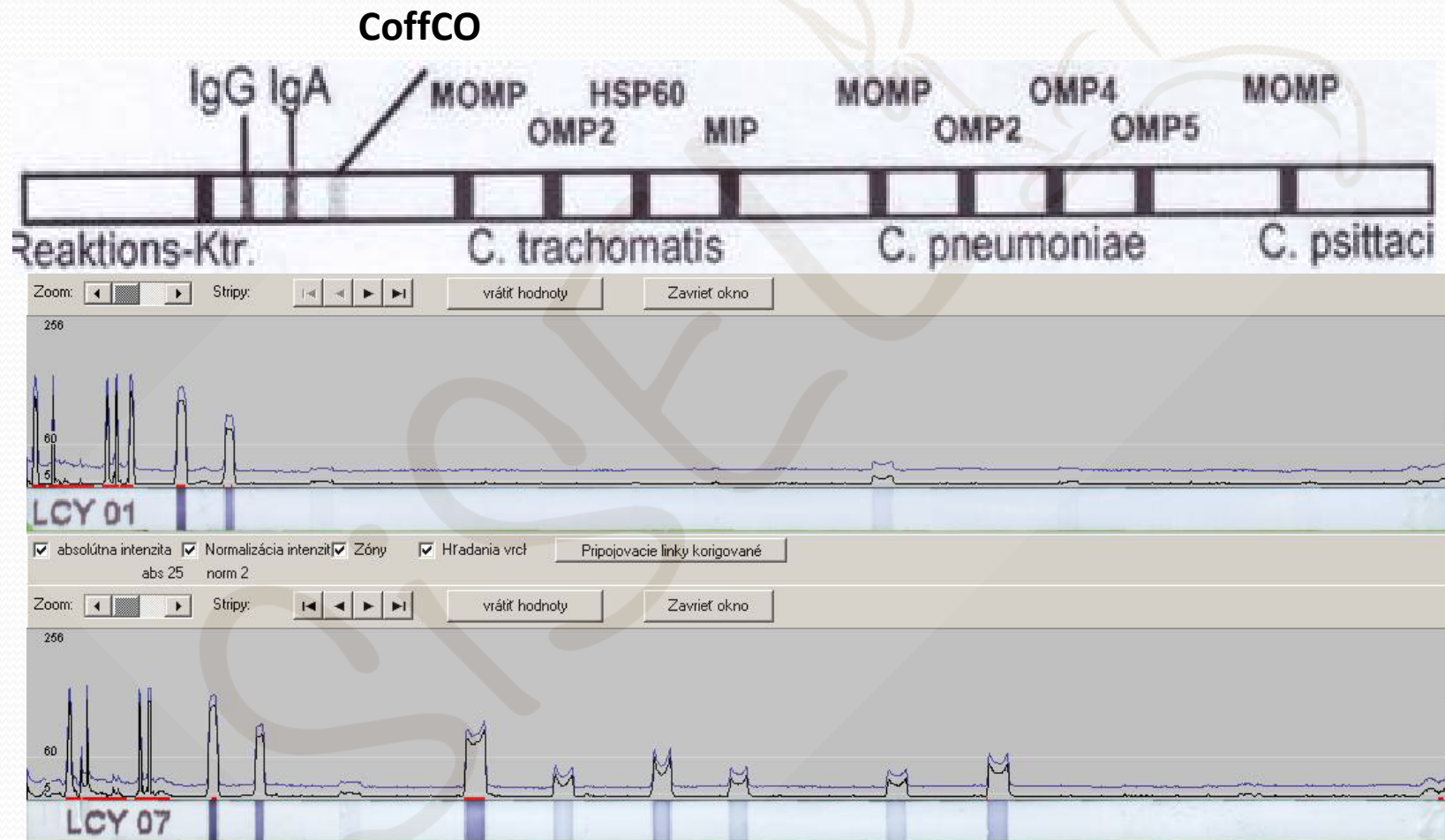
Atanu FO, Oviedo-Orta E, Watson KA (2013) A Novel Transport Mechanism for MOMP in *Chlamydomonas reinhardtii* and Its Putative Role in Immune-Therapy. PLOS ONE 8(4): e61139. <https://doi.org/10.1371/journal.pone.0061139>
<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0061139>

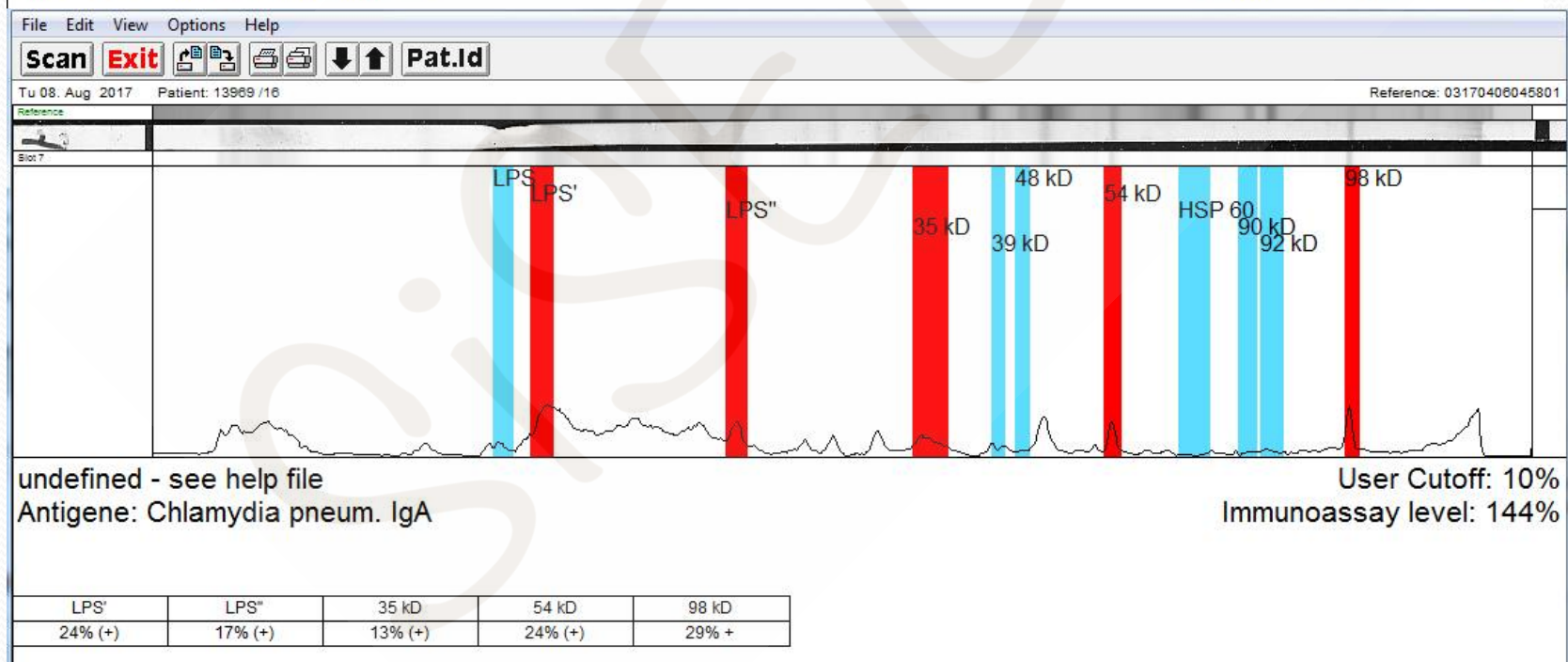
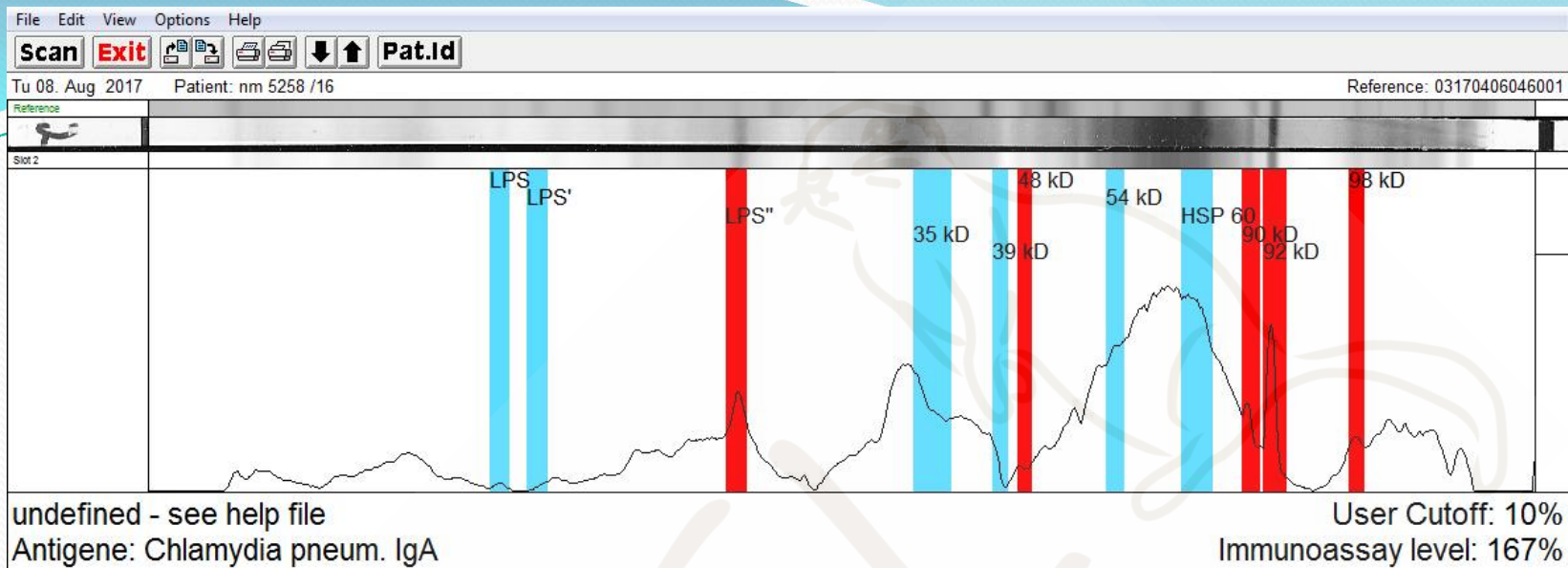
Chlamydia (Chlamydophila)

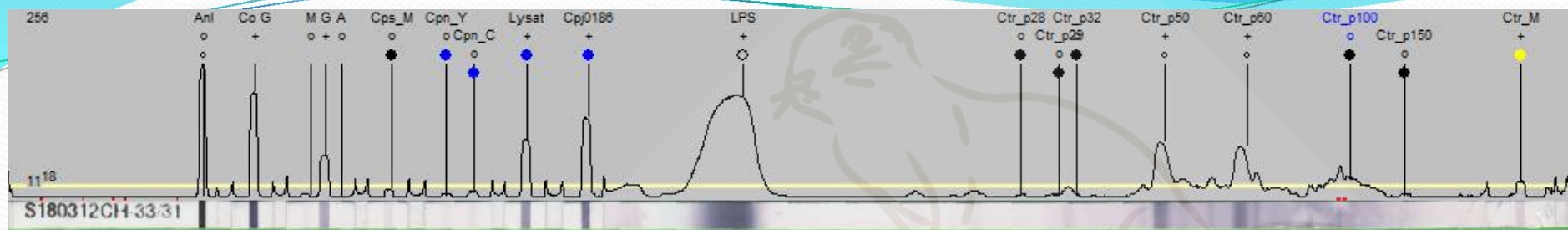
trachomatis, pneumoniae, psittaci

MOMP <i>C. trachomatis</i> <i>C. pneumoniae</i> <i>C. psittaci</i>	<p>„major outer membrane protein“ najdôležitejší imunodominantný Ag</p> <p>divergencia aminokyselinových sekvencií medzi jednt. druhmi</p> <p><i>C. pneumoniae</i>/<i>C. trachomatis</i>: 35,5% divergencia</p> <p><i>C. pneumoniae</i>/<i>C. trachomatis</i>: 27,9% divergencia</p> <p><i>C. trachomatis</i>/<i>C. psittaci</i>: 33,9% divergencia i</p>
OMP₂ <i>C. trachomatis</i> <i>C. pneumoniae</i>	<p>vysoký obsah cysteínu – univerzálny rodový marker infekcie</p>
HSP60 <i>C. trachomatis</i>	<p>potenciálny marker chron. zápalovej reakcie (hlavne <i>C. trachomatis</i>)</p>
MIP <i>C. trachomatis</i>	<p>„macrophage infectivity potentiator“ species špecifický <i>C. trachomatis</i></p>
OMP₄ <i>C. pneumoniae</i>	<p>species špecifický <i>C. pneumoniae</i></p>
OMP₅ <i>C. pneumoniae</i>	<p>species špecifický <i>C. pneumoniae</i></p>

Imunoblot *Chlamydia* spp.







☐ Absolútna intenzita
 ☐ Zóna negatívnej kontrol
 ☒ Normalizovaná intenzita
 ☒ Prúžky
 ☐ Oblasť vrcholov
 ☒ Cut-off
 pos=91,54 mm abs 32 norm 0

Korekcia štartovacej značky

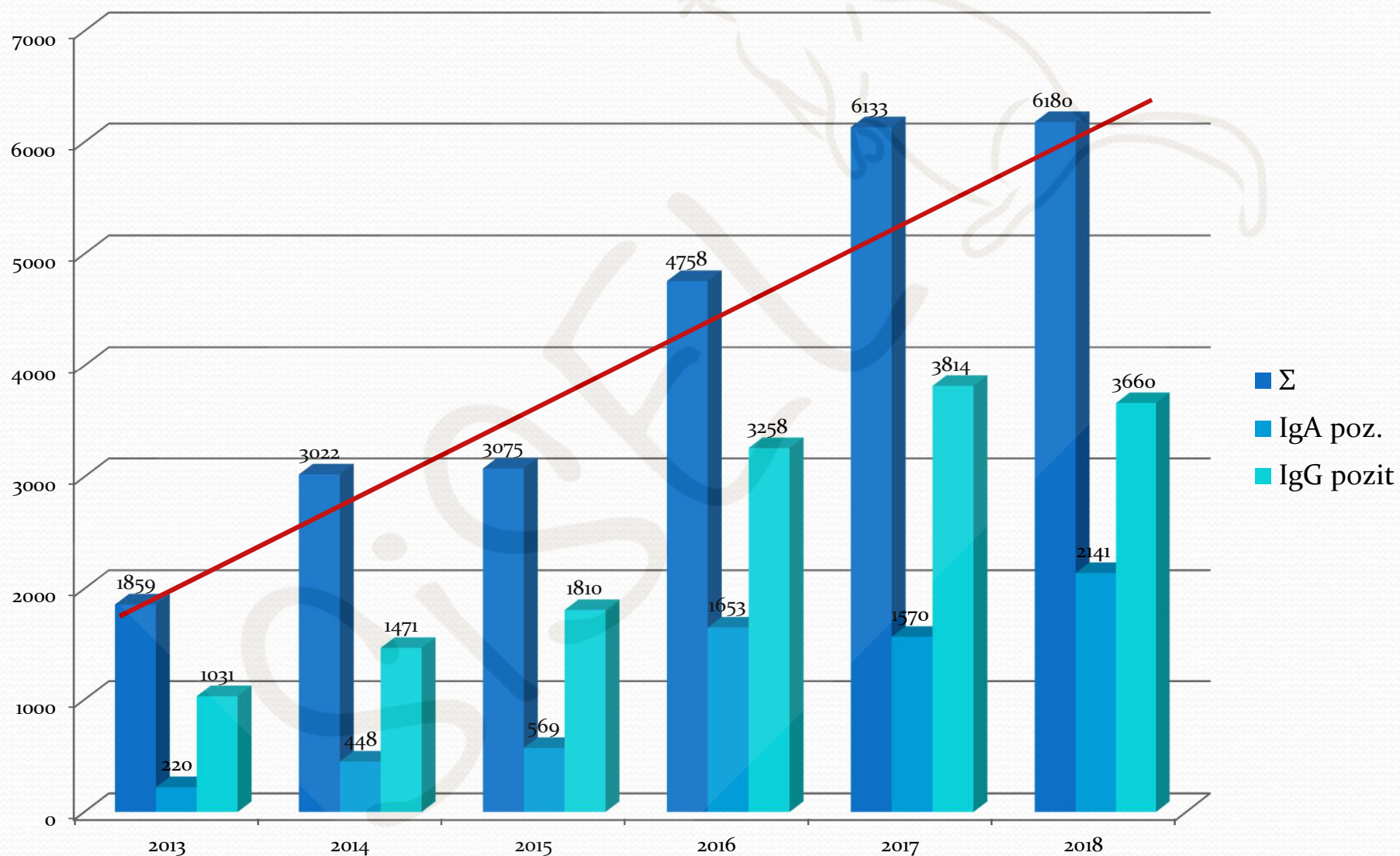
Strip číslo: 1
Test: Chlam. humanpath.EL-WB IgG ne
Dátum narodenia: Neznámy
Lab číslo:
Rodné číslo:
Prijaté: -
Vek:
Dátum expirácie:

Pacient ID: 010905067
Meno pacienta:
Šarža: S180312CH-33
Strip:
Odosielať:
Pohlavie:
Rodné meno:
Vyhodnotil:

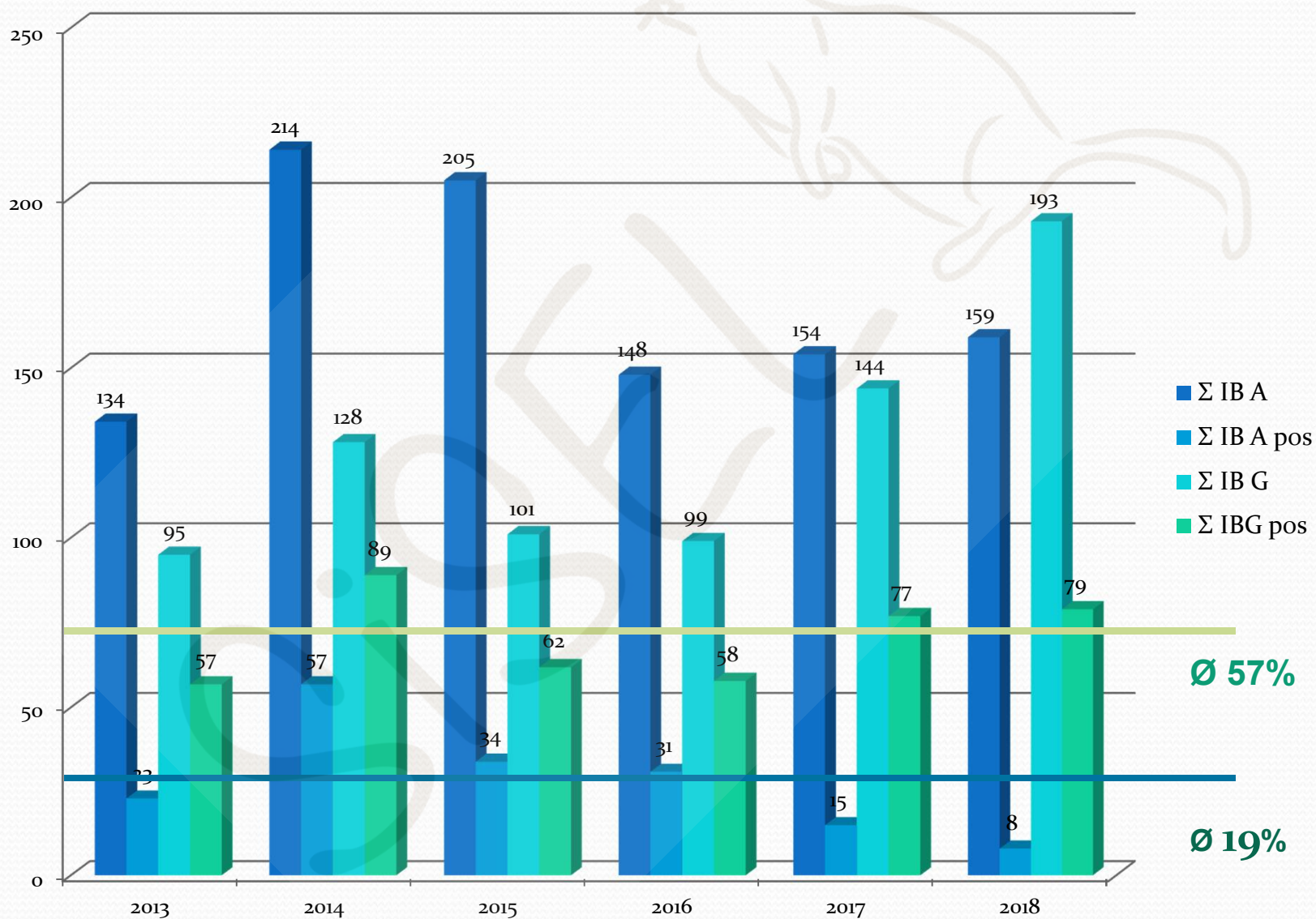
Č.	skratka	Názov	Int.	Char	Pomer
1	Ctr_M	C. trach. MOMP	22	+	
2	Ctr_p150	C. trach. p150	0	o	
3	Ctr_p100	C. trach. p100	26	o	
4	Ctr_p60	C. trach. p60	68	+	
5	Ctr_p50	C. trach. p50	74	+	
6	Ctr_p32	C. trach. p32	0	o	
7	Ctr_p29	C. trach. p29	0	o	
8	Ctr_p28	C. trach. p28	0	o	
9	LPS	LPS	137	+	
10	Cpi0186	C. pneu. Cpi0186	108	+	
11	Lysat	C. pneu. Lysat	78	+	
12	Cpn_C	C. pneu. CpaF	6	o	
13	Cpn_Y	C. pneu. YwbM	0	o	
14	Cps_M	C. psi. MOMP	10	o	
15	A	Conjugate control IgA	1	o	
16	G	Conjugate control IgG	57	+	
17	M	Conjugate control IgM	2	o	

C. trach.	pozitívny
C. pneu.	pozitívny
C. psi.	negatívny

Vyšetrenia *Chlamydia pneumoniae* ELISA v rokoch 2013 - 2018

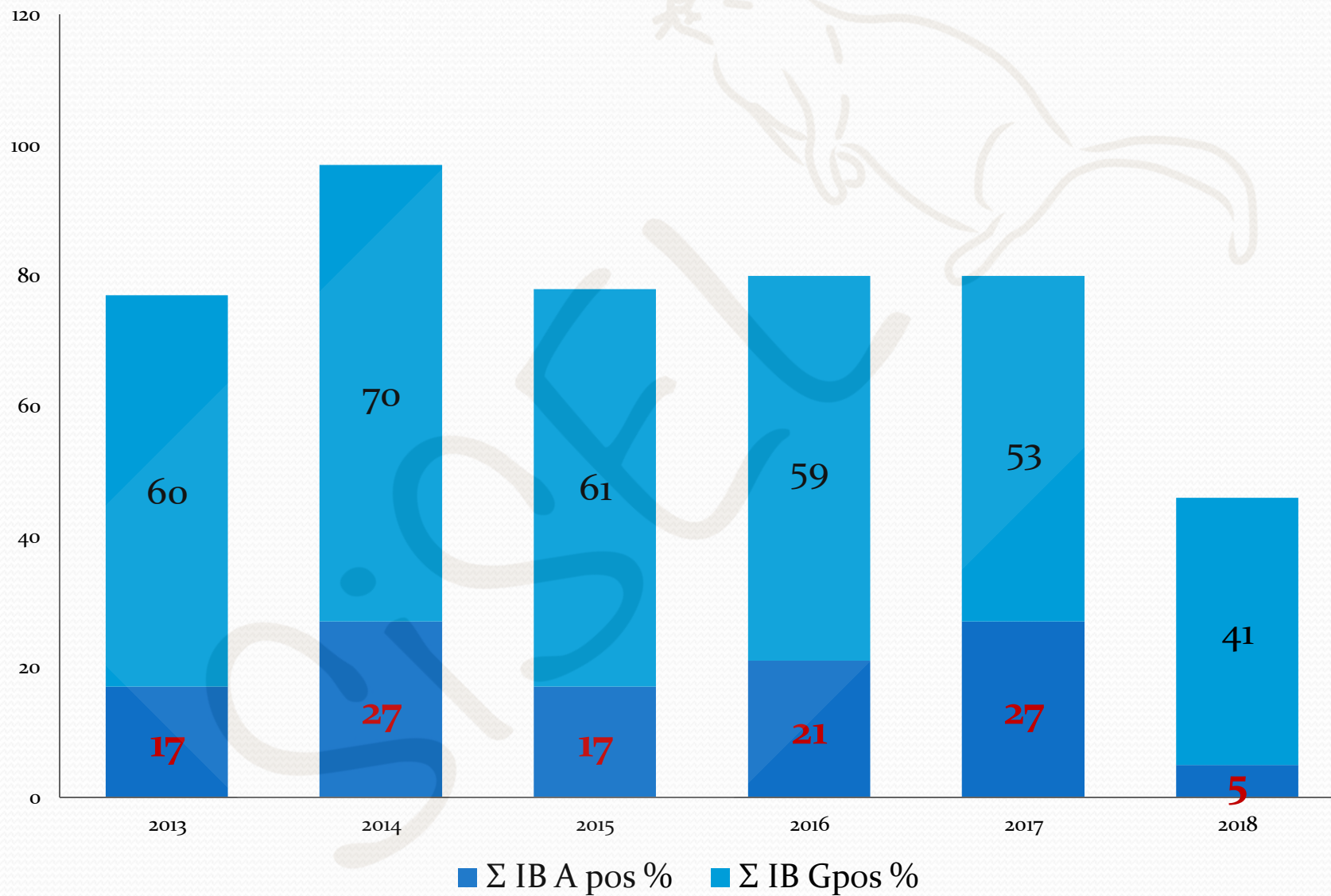


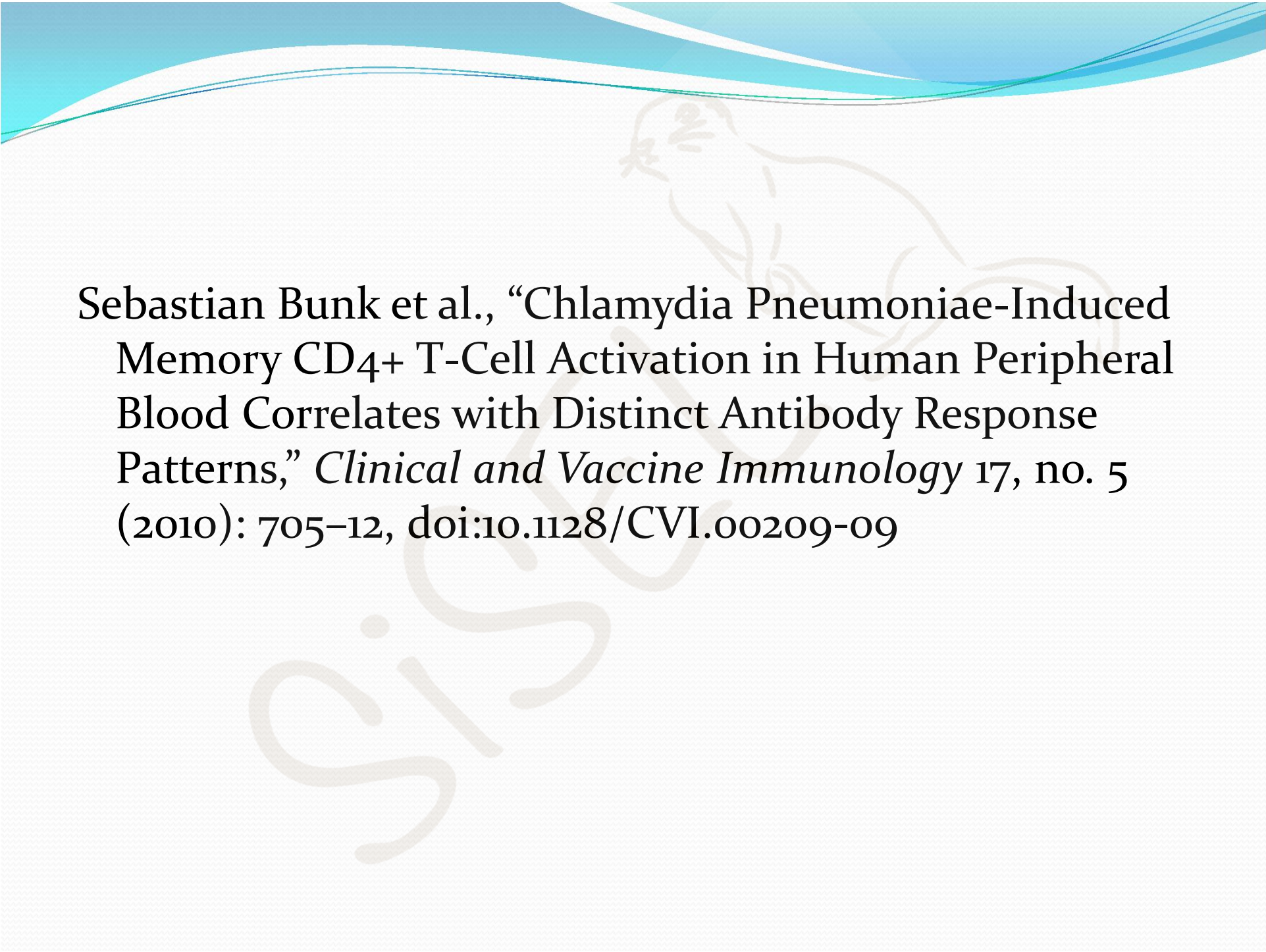
Imunoblot *Chlamydomphila pneumoniae* v rokoch 2013 - 2018



Pozitivita IgG a IgA imunoblotu *C. pneumoniae* v %

2013 - 2018





Sebastian Bunk et al., “Chlamydia Pneumoniae-Induced Memory CD4+ T-Cell Activation in Human Peripheral Blood Correlates with Distinct Antibody Response Patterns,” *Clinical and Vaccine Immunology* 17, no. 5 (2010): 705–12, doi:10.1128/CVI.00209-09

Číslo **14006**

Pacient

Rod.čís.

ovňa **2511**Odber **19.10.2015**Prech. **2015/8790**Príjem **19.10.2015**Nasl. **/**Materiál **Sérum**Oddelenie **GM**Diagnóza **M79.98 Bližšie neurčená choroba mäkkého tkaniva na inom**Vyšetrenie **CP,MP**

Komentár

Dátum:		19.10.2015	12.6.15	6.3.14	8.11.12	18.9.12	9.11.11
Číslo		14006-S	8790-S	3663-S	16421-S	13638-S	17929-S
19.10.2015	CPNIGA		0.484 negatív	0.689 negatív	0.696 negatív	0.675 negatív	0.563 negatív
19.10.2015	CPNIGG		1.936 pozitív	1.645 pozitív	2.050 pozitív	2.505 pozitív	2.969 pozitív

Číslo **14070**

Pacient

Rod.čís.

ia **24**Odber **20.10.2015**Prech. **2014/13387**Príjem **20.10.2015**Nasl. **/**Materiál **Sérum**Oddelenie **NN**Diagnóza **J20.8 Akútna bronchitída, zapríčinená iným bližšie určeným**Vyšetrenie **CP,MP**

Komentár

Dátum:		20.10.2015	25.9.14	24.5.11	5.10.10
Číslo		14070-S	13387-S	8515-S	16825-S
20.10.2015	CPNIGA		1.190 hranič	1.204 pozitív	1.451 pozitív
20.10.2015	CPNIGG		1.453 pozitív	4.149 vysoké	3.182 vysoké

Číslo **14016** Odber **19.10.2015** Príjem **19.10.2015**
 Pacient [REDACTED] Prech. **2014/15490** Nasl. **/**
 Rod.čís. [REDACTED] vek **25**

Materiál **Sérum** chlamydia trachomatis PCR... negatívna Hran. hodnoty ---
 Oddelenie **BL** Ureaplasma spp. PCR..... pozitívna ---
 Diagnóza **J41.8 Zmiešaná jednoduchá a hlienovohnisová chronická**
 Vyšetrenie **CP,CPWA,CSWA,CTWA,MP**
 Komentár [REDACTED]

Dátum:		19.10.2015	5.11.14	8.1.14	4.9.13	16.4.12	17.1.12
Číslo		14016-S	15490-S	224-S	12871-S	5861-S	864-S
20.10.2015	WCPAM		- negatívny				- negatívny
20.10.2015	WCPAO2		- negatívny				- negatívny
20.10.2015	WCPAO4		- negatívny				- negatívny
20.10.2015	WCPAO5		- negatívny				- negatívny
20.10.2015	WCPIGA		- negatívny				- negatívny
20.10.2015	WCPSAM		- negatívny				- negatívny
20.10.2015	WCPSIGA		- negatívny				- negatívny
20.10.2015	WCTAHSP		- negatívny				+ pozitívny
20.10.2015	WCTAM		+ pozitívny				+ pozitívny
20.10.2015	WCTAMIP		+ pozitívny				+ pozitívny
20.10.2015	WCPIGA		- negatívny				- negatívny
20.10.2015	WCPSAM		- negatívny				- negatívny
20.10.2015	WCPSIGA		- negatívny				- negatívny
20.10.2015	WCTAHSP		- negatívny				+ pozitívny
20.10.2015	WCTAM		+ pozitívny				+ pozitívny
20.10.2015	WCTAMIP		+ pozitívny				+ pozitívny
20.10.2015	WCTAO2		- negatívny				- negatívny
20.10.2015	WCTIGA		+ pozitívny				+ pozitívny
19.10.2015	CPNIGA		1.48 pozitív	1.164 hranič	1.652 pozitív	1.249 pozitív	1.437 pozitív
19.10.2015	CPNIGG		2.026 pozitív	2.123 pozitív	2.003 pozitív	2.672 pozitív	2.834 pozitív

Sérologické metódy - interpretácia

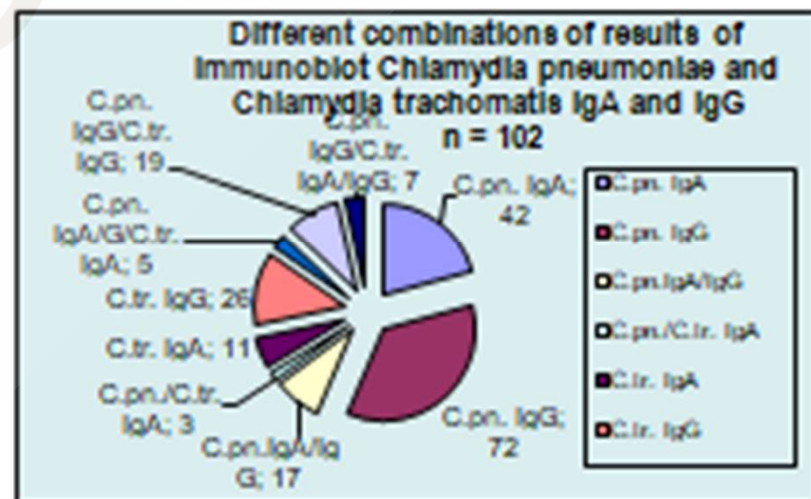
Combinations	Nº of samples
<i>C. pneumoniae</i> IgA	42
<i>C. pneumoniae</i> IgG	72
<i>C. pneumoniae</i> IgA/IgG	17
<i>C. pneumoniae</i> / <i>C. trachomatis</i> IgA	3
<i>C. trachomatis</i> IgA	11
<i>C. trachomatis</i> IgG	26
<i>C. pneumoniae</i> IgA/IgG / <i>C. trachomatis</i> IgA	5
<i>C. pneumoniae</i> IgG / <i>C. trachomatis</i> IgG	19
<i>C. pneumoniae</i> IgG / <i>C. trachomatis</i> . IgA/IgG	7

From 152 analyzed sera 42 (37,5 %) were confirmed as IgA specific against *C. pn.*, 11 (9,8 %) IgA specific against *C. tr.*, (3 against both *C. pn.* and *C. tr.*) and 72 (76,6 %) were confirmed as IgG specific against *C. pn.*, 26 (27,7 %), IgG specific against *C. tr.*, (19 against both *C. pn.* and *C. tr.*)

Added value of recombinant Immunoblot in serological diagnosis of *Chlamydophila (Chlamydia) pneumoniae* infection

Botek, R.¹, Melicharová, V.¹ Blazicková, S.^{1,2}

14th International Congress of Immunology
2010, Kobe, Japan



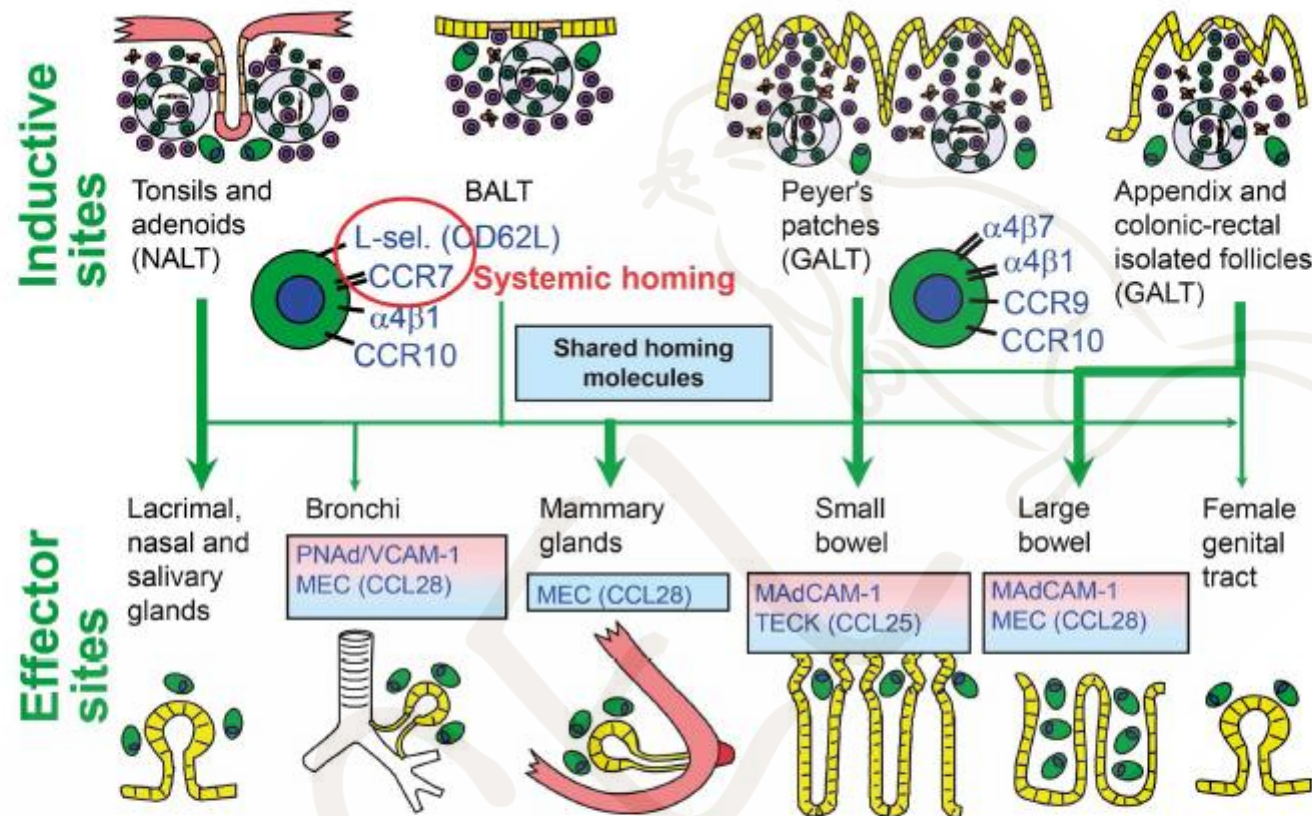


FIGURE 5 | Homing properties of human mucosal memory/effector B cells. Putative scheme for compartmentalized migration of B cells from inductive (top) to effector (bottom) sites. Depicted are more or less preferred pathways (graded arrows) presumably followed by mucosal B cells activated in nasopharynx-associated lymphoid tissue (NALT) represented by palatine tonsils and adenoids, bronchus-associated lymphoid tissue (BALT), and gut-associated lymphoid tissue (GALT) represented by Peyer's patches, appendix, and colonic-rectal

lymphoid follicles. The principal homing receptor profiles of the respective B-cell populations, and adhesion/chemokine cues directing extravasation at different effector sites, are indicated (pink and blue panels) – those operating in lactating mammary glands apparently being shared for NALT- and GALT-derived cells. Homing molecules integrating airway immunity with systemic immunity are encircled in red. Adapted from Brandtzaeg (51). MEC, mucosae-associated epithelial chemokine; TECK, thymus-expressed chemokine.

Per Brandtzaeg: SecretoryIgA:designed foranti-microbial defense, Frontiers in immunology, published: 06 August 2013 doi: 10.3389/fimmu.2013.00222



LETTERS

Chlamydia pneumoniae is frequently detected in the blood after acute lung infection

To the Editors:

The obligate intracellular bacterium *Chlamydia pneumoniae* is a common cause of acute respiratory infections with a worldwide seroprevalence of up to 70% [1]. Bacterial persistence following acute infection in the respiratory tract or in atherosclerotic blood vessels has been suggested to be involved in the pathogenesis of chronic inflammatory diseases, such as chronic obstructive pulmonary disease and atherosclerosis [2, 3]. It has been shown in animal models that after acute lung infection with *C. pneumoniae*, the pathogen is systemically distributed in the blood circulation using blood monocytes as a vector [4, 5]. However, data supporting this hypothesis in humans are still missing. From *in vitro* observations, it is known that *C. pneumoniae* infection of human blood monocytes results in a nonreplicative but viable state of the pathogen, which is refractory to antibiotic treatment [6].

NOW[®]; Inverness Medical International, Cranfield, UK). Spontaneous or induced sputum samples (10 min inhalation with 3% NaCl solution) were taken on each visit for detection of *S. pneumoniae* (blood agar culture according to the Clinical and Laboratory Standards Institute (Wayne, PA, USA) recommendations) or *C. pneumoniae* (PCR) infection. Isolation of peripheral blood monocytes (PBMC) from blinded EDTA blood samples and detection of *C. pneumoniae* by PCR were performed as described previously [6]. The study protocol was approved by the ethical committee of the University of Luebeck (Nr: 01-148). All patients were informed of the study purpose and gave written consent. Statistical analysis was performed with the SPSS software (SPSS Inc., Chicago, IL, USA) using the t-test for the analysis of nonpaired, parametrical parameters and the Mann–Whitney U-test for the

„Infections with *C. pneumoniae* account for 0.9–13% of CAP...
...sustained clearance of *C. pneumoniae* from the blood
following treatment with azithromycin was only achieved in
29% of the patients [12]. This could be due to the low
susceptibility of persistent chlamydiae to macrolide therapy.....“



LETTERS

Chlamydia pneumoniae is frequently detected in the blood after acute lung infection

TABLE 1 Patient characteristics at the time of admission

	<i>Streptococcus pneumoniae</i>	<i>Chlamydia pneumoniae</i>	p-value
Patients n	21	18	
Age yrs	62.1 ± 3.1	51.0 ± 4.1	0.036*
Males/females n	12/9	12/6	0.542
Smokers %	11 (52.4)	7 (38.9)	0.399
COPD history %	6 (28.6)	2 (11.1)	0.178
CRP mg·L⁻¹	254.47 ± 30.67	115.07 ± 23.60	0.001*
WBC n·nL⁻¹	16.02 ± 0.87	11.79 ± 1.22	0.007*

Data are presented as mean ± SEM or n (%), unless otherwise stated. COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein; WBC: white blood cells. *: p < 0.05.

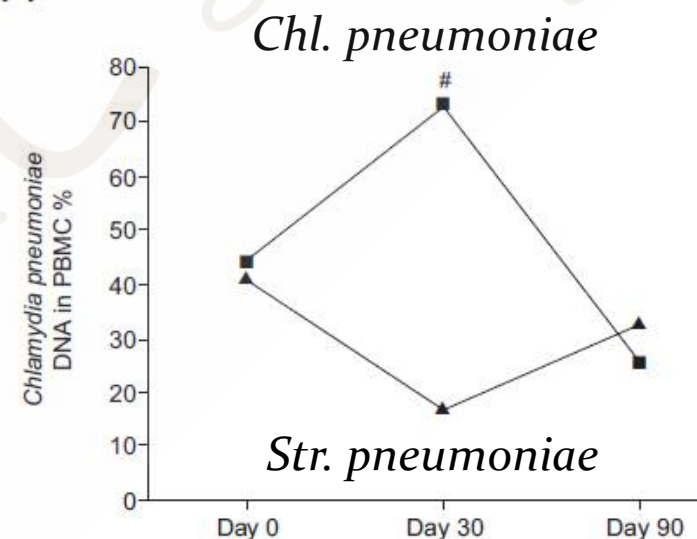


FIGURE 1. Detection frequency of *Chlamydia pneumoniae* DNA in peripheral blood monocytes (PBMC) from patients after acute respiratory infection with *C. pneumoniae* (■) or *Streptococcus pneumoniae* (▲). PBMC fractions of whole blood samples were significantly more often *C. pneumoniae* DNA positive (PCR) 30 days post infection (visit 1) in patients with acute *C. pneumoniae* infection compared with patients with *S. pneumoniae* infection. #: p = 0.007.

Clinical Investigation and Reports

Chlamydia pneumoniae Infection in Circulating Human Monocytes Is Refractory to Antibiotic Treatment

Jens Gieffers, MD; Henriette Füllgraf; Jürgen Jahn, MD; Matthias Klinger, MD; Klaus Dalhoff, MD; Hugo A. Katus, MD; Werner Solbach, MD; Matthias Maass, MD

Background—Recovery of the intracellular bacterium *Chlamydia pneumoniae* from atherosclerotic plaques has initiated large studies on antimicrobial therapy in coronary artery disease. The basic concept that antibiotic therapy may eliminate and prevent vascular infection was evaluated in vitro and in vivo by examining the antibiotic susceptibility of *C pneumoniae* in circulating human monocytes, which are thought to transport chlamydiae from the respiratory tract to the vascular wall.

Methods and Results—Blood monocytes (CD14+) from 2 healthy volunteers were obtained before and after oral treatment with azithromycin or rifampin and then inoculated with a vascular *C pneumoniae* strain and continuously cultured in the presence of the respective antibiotic. Progress of infection and chlamydial viability was assessed by immunogold-labeling and detection of *C pneumoniae*-specific mRNA transcripts. Circulating monocytes from patients undergoing treatment with experimental azithromycin for coronary artery disease were examined for *C pneumoniae* infection by cell culture. Antibiotics did not inhibit chlamydial growth within monocytes. Electron microscopy showed development of chlamydial inclusion bodies. Reverse transcription-polymerase chain reaction demonstrated continuous synthesis of chlamydial mRNA for 10 days without lysis of the monocytes. The in vivo presence of viable pathogen not eliminated by azithromycin was shown by cultural recovery of *C pneumoniae* from the circulating monocytes of 2 patients with coronary artery disease.

Conclusions—*C pneumoniae* uses monocytes as a transport system for systemic dissemination and enters a persistent state not covered by an otherwise effective antichlamydial treatment. Prevention of vascular infection by antichlamydial treatment may be problematic: circulating monocytes carrying a pathogen with reduced antimicrobial susceptibility might initiate reinfection or promote atherosclerosis by the release of proinflammatory mediators. (*Circulation*. 2001; 103:351-356.)

POROVNANIE REKOMBINANTNÝ IMUNOBLLOT VS EURIMMUN IMUNOBLLOT *CHLAMYDOPHILLA* *PNEUMONIAE*

IgA	Pozit	Negat
Recom blot	9	23
Euroimmun blot	18	14
ELISA	32	0

IgG	Pozit	Negat
RecomIblot	15	17
Euroimmun blot	28	4
ELISA	32	0

POROVNANIE REKOMBINANTNÝ IMUNOBLOT VS EURIMMUN IMUNOBLOT VS AID CHLAMYDOPHILLA PNEUMONIAE

IgA=76	Pozit	Negat	LPS
Recoml blot	6	70	
AID blot	23	53	19
ELISA	56	20	

IgA	Pozit	Negat	LPS
Recom blot	9	23	
Euroimmun blot	18	14	10
ELISA	32	0	

Chlamydophilla pneumoniae PCR

2013 - 2018

Počet vyšetřených

2606

Počet pozitivních

10 (0,4%)

2013 3

2014 0

2015 2

2016 2

2017 2

2018 1

(2019 1)

ČO NA ZÁVER

- Diagnostika *Chlamydophilla pneumoniae* na základe sérologických vyšetrení je interpretačne nemožná
- Na základe porovnania nálezov NAT a positivity IgA nemožno považovať IgA za marker aktivity infekcie, pretože často pretrvávajú aj roky
- Immunoblot s rekombinantnými antigénmi nevysvetľuje pozitivitu EIA
- Immunoblot s kombináciou rekombinantných a purifikovaných antigénov sa javí ako najvhodnejší

539

ĎAKUJEM ZA POZORNOST

