REVIEW

Atypical respiratory pathogens

Pekka Saikku

National Public Health Institute, Department in Oulu, Oulu, Finland

The main atypical pathogens in respiratory tract infections are classified on the basis of their ability to cause atypical pneumonia. This is not a well-defined clinical entity, and it is evident that atypical pathogens can sometimes cause 'typical' pneumonias and vice versa. This emphasizes the need for microbiological diagnosis, since it affects the selection of proper treatment, in which β-lactam antibiotics and aminoglycosides are not effective. Moreover, mixed infections caused by atypical and typical pathogens together are common. At this moment rapid and sensitive diagnostic methods are lacking. Besides numerous viruses, the main bacterial pathogens causing atypical pneumonias are *Mycoplasma pneumoniae*, two chlamydial species, *Chlamydia pneumoniae* and *C. psittaci*, one rickettsia, *Coxiella burnetti*, and several *Legionella* species. The majority of these pathogens cause upper respiratory tract infections more often than overt pneumonias. An atypical agent, *Chlamydia pneumoniae*, has also been associated with chronic inflammatory conditions in the cardiovascular system. The most recently discovered pathogen in atypical pneumonias is a hantavirus causing hantavirus pulmonary syndrome.

Key words: Atypical pneumonias, mycoplasmal infections, chlamydial pneumonias, Q fever, legionella infections

INTRODUCTION

'Atypical pathogens' in community-acquired respiratory tract infections are purely microbiological entities: apart from pneumonias, where the pneumococcus is the leading causative agent, the overwhelming majority of respiratory infections are caused by 'atypical pathogens' (Table 1). The list of atypical pathogens is long, and the more microbial diagnostic methods are used, the more pathogens are found. A recent example comprises the hantaviruses recently found in hantavirus pulmonary syndrome [1]. Although pneumonia and pneumonic symptoms had earlier been described in hantavirus infections, as had pulmonary edema in rickettsioses [2], no one expected a hemorrhagic fever virus to be the causative agent in severe pneumonias. The use of advanced microbial diagnostic methods established a new disease syndrome.

Corresponding author and reprint requests:
Pekka Saikku, KTL, Department in Oulu,
PO Box 310 (Aapistie 1), FIN 90101 Oulu, Finland
Tel: +358 8 537 6231 Fax: +358 8 537 6222
E-mail: pekka.saikku@ktl.fi

Accepted 29 August 1997

HISTORY

The history of atypical pathogens starts with psittacosis, caused by *Chlamydia psittaci* [3], influenza viruses [4] and Q fever, caused by rickettsia, *Coxiella burnetti* [5]. Influenza virus continues to be a great scourge of all

Table 1 Agents associated with atypical pneumonias

Agent	Pneumonia type
True bacteria	
Legionella pneumophila	Mostly epidemics
Legionella spp.	Uncommon
Mycoplasma	
Mycoplasma pneumoniae	Common
Rickettsia	
Coxiella burnetti	Zoonosis
Chlamydia	
Chlamydia pneumoniae	Common
Chlamydia psittaci	Uncommon
Chlamydia trachomatis	Infants
Viruses	
influenza A and B	Common
adenoviruses 3, 4 and 7	Common
parainfluenzas	Common
respiratory syncytial	Common
rhinoviruses	Uncommon
enteroviruses	Uncommon
coronaviruses	Uncommon
herpes viruses	Uncommon

mankind, and a possible appearance of a new killer strain is in every autumn a greater menace than the feared Ebola virus. The other two agents also continue to be important respiratory tract pathogens, but being zoonoses, are limited in their occurrence mostly to cases with animal contacts. The 1940s saw the discovery of Eaton-PPLO agent [6], *Mycoplasma pneumoniae*, which still is one of the commonest and well-recognized causes of pneumonia worldwide. Virologic research has revealed a long list of viral agents, ranging from adenoviruses, enteroviruses, parainfluenza viruses, respiratory syncytial viruses, rhinoviruses and coronaviruses finally up to hantaviruses underlying the hantavirus pulmonary syndrome [1]. Viruses are by far the most numerous causative agents in respiratory tract infections with 'atypical pathogens', and the importance of pneumococcus is the only reason justifying the division between 'typical' and 'atypical' pathogens.

CLINICAL PICTURE

Luckily, 'atypical pneumonias' are usually not clinically severe; rather, the converse is the case. Some clinical features of atypical pneumonias are presented in Table 2. In the patient history, family cases and the epidemiologic situation can aid in the diagnosis, as well as contact with a sick parrot when there is suspicion of psittacosis. The onset can be delayed and insidious. Sputum production can be minimal and polymorphonuclear leukocytes are not present. In the chest X-ray, no lobar infiltrates, but more diffuse alterations 'typical of atypical pneumonia', are seen. Leukocytosis can be absent in cases without massive pulmonary destruction, the erythrocyte sedimentation level is usually elevated, but C-reactive protein does not reach the levels found especially in severe pneumococcal pneumonias. However, none of these symptoms and signs readily differentiates an 'atypical' disease from a 'typical' one. All the values overlap, and even a lobar infiltrate in the X-ray can be caused by an atypical pathogen [7]. Without proper microbial diagnosis, the first clue to the etiology can too often be only the lack of response to the standard antimicrobial treatment used.

Table 2 Some features considered typical for atypical pneumonias

Typical patient history
Lack of purulent sputum
Typical chest X-ray finding
Absence of leukocytosis
Normal or moderately elevated C-reactive protein
Poor response to β-lactam therapy

In the following sections the main bacterial agents causing atypical respiratory tract infections are discussed, with a special emphasis on the latest bacterial addition, *Chlamydia pneumoniae*.

Mycoplasma pneumoniae

Mycoplasmal pneumonias concentrate in the younger age groups, and this is illustrated in Figure 1, comparing the prevalence of antibodies against *M. pneumoniae* and *Chlamydia pneumoniae* in the Finnish population. The clinical description of mycoplasmal infections is classical but there are some open questions. One is the lack of reliable diagnostic methods, since conventional serologic methods, cold-agglutinin and complement-fixation tests, are neither sensitive nor specific, although the former is easy to perform. A second question is, how effective is the antibiotic treatment in mycoplasmal pneumonias? Mycoplasmal diseases in general are a neglected area, and we should not rely on serology only in the diagnosis of mycoplasmal pneumonias [8].

Legionella species

The epidemic of a curious disease named afterwards as 'legionellosis' in Pittsburgh in 1976 brought a special bacterial genus to our attention [9]. Although its first member had been discovered over thirty years earlier [10], only then was it discovered to be the causative agent of several serious epidemics of respiratory infections. Relatively new also is its association with environmental constructions and air-conditioning tech-

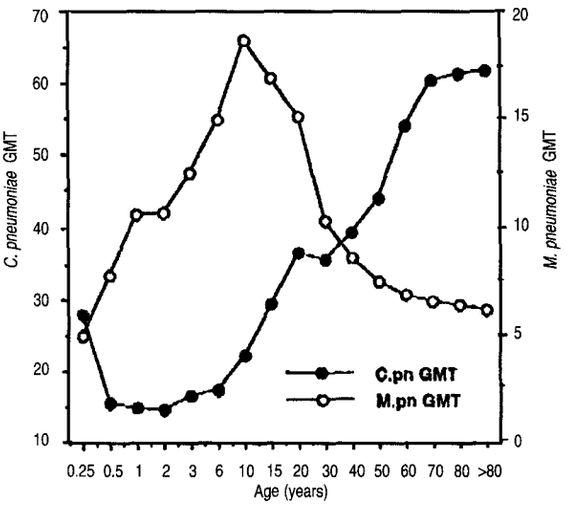

Figure 1 Geometric mean titers (GMTs) in relation to age in patients with suspected viral illnesses studied at the Department of Virology, University of Helsinki. M.pn, *Mycoplasma pneumoniae* (58 000 samples studied); C.pn, *Chlamydia pneumoniae* (20 000 samples studied).

nology. The descriptions of the importance, diagnosis and treatment of legionellosis have become established [11]. Over the years, at least 17 *Legionella* species have been associated with clinical diseases, especially with severe pneumonias, but the importance of these environmental bacteria varies considerably between different regions and settings. In some places they are causing a considerable proportion of all pneumonias [12] and are listed among the three major causes. In other areas, e.g. Finland, they are, despite an intensive search, rarities, usually imported by tourists.

Chlamydia psittaci

Respiratory tract infections caused by *Chlamydia psittaci* are directly dependent on exposure to birds carrying the pathogen. Therefore, cases are seen in connection with turkey and duck farming (chickens seem not to be important), pigeon breeding, and sick pet birds. Casual contacts with synanthropic birds are common, and ruling out a bird contact is much more difficult than finding one. There has been a debate over whether *Chlamydia pneumoniae* is a more common agent than *Chlamydia psittaci*. Even in the microimmunofluorescence (MIF) test it is sometimes difficult to differentiate these two chlamydial species from each other [13], and it demands expertise [14]. Fortunately, the treatment is the same in both chlamydial pneumonias. According to seroepidemiologic surveys, *Chlamydia pneumoniae* infections are 20–50 times more common than *Chlamydia psittaci* infections [15,16]. There is a possibility that the severity of *Chlamydia psittaci* infections could be due to the sensitization of the patient to this species by an earlier, mild *Chlamydia pneumoniae* pulmonary infection.

Chlamydia pneumoniae

The most recent addition to the long list of important atypical bacterial pathogens is *Chlamydia pneumoniae*. Like the first *Legionella* strains, the first strains of this new chlamydial agent were isolated [17] years before an epidemic in northern Finland brought them to our attention [18]. In the beginning, *Chlamydia pneumoniae* seemed to be an agent causing benign and mild disease, but later it was shown to cause—the typical feature of all *Chlamydia*—silent, slowly creeping infections, gradually leading to severe tissue damage [19].

The pathogenesis of *Chlamydia pneumoniae* infections has been studied using mouse models [20,21]. When *Chlamydia pneumoniae* is given intranasally, acute infection with polymorphonuclear leukocytes in the lungs is seen only after massive challenge doses. Otherwise a silent pneumonitis with a clear histologic picture of mononuclear inflammation around bronchioles and vessels develop without overt illness. Repeated inoculations aggravate this inflammation temporarily, but the presence of the agent is difficult to demonstrate by isolation [22]. The demonstration of nucleic acids by polymerase chain reaction (PCR) gives, however, a positive finding and cortisone treatment, after alleviating inflammation, makes isolation of the agent possible [23]. *Chlamydia pneumoniae* is also demonstrable after intranasal challenge in the blood circulation, alveolar and peritoneal macrophages, and the liver and spleen, pointing to a disseminated infection [24].

Upper respiratory tract carriage has been described [25,26], and there is a possibility that carriers do not develop antibody responses or, if they do, only after a prolonged period. Pneumonias due to *Chlamydia pneumoniae* have been described in infants [27], and from Japan there is a report of an epidemic in day-care centres [28]. However, in industrialized countries antibodies usually start to appear when children enter school [29, 30]. In a recent Finnish study on childhood pneumonias, the youngest patient with a *Chlamydia pneumoniae* infection was 7 years old, and the majority of patients were over 10 years of age (Korppi et al, unpublished data). Other respiratory syndromes associated with *Chlamydia pneumoniae* are rhinitis, sinusitis, pharyngitis, otitis, and bronchitis. A recent review of *Chlamydia pneumoniae* infections in children concentrated on respiratory tract infections [31]. However, during a *Chlamydia pneumoniae* epidemic in northern Finland, only a third of the children presenting a seroconversion were hospitalized because of respiratory tract symptoms (Uhari et al, unpublished data). The disease picture in children can thus be quite variable and demands further study.

Primary infection in young adults leads to pneumonia in about 10% of cases [32]. This is usually a mild disease, but can be prolonged with a long convalescence. In young age groups, reinfections seem not to lead to pneumonias [32]. However, reinfection pneumonias, especially in elderly patients with underlying diseases, can be very severe [33]. One possibility is that when the resistance to infection has decreased enough to allow the agent to invade the lungs, or the invading strain is different enough to be able to colonize the lungs, partial immunity can lead to hypersensitivity reactions typical of all chlamydial infections.

The role that *Chlamydia pneumoniae* plays in other acute respiratory tract infections is still under study. In adults, it has been associated with 0.5–7% of pharyngitis [34,35] cases (the last figure during an epidemic), and 5–10% of acute bronchitis cases. Sinusitis and otitis in adults have also been reported, but wider studies are so far lacking.

Mixed infections are common in *Chlamydia pneumoniae* infections. In the studies on Finnish children,

half or even the majority of patients have had a concomitant infection caused by another pathogen; virus, mycoplasma or bacterium. Similarly, during an epidemic in North Finland, nearly half of the *Chlamydia pneumoniae* pneumonias were combined with invasive pneumococcal infections [36]. *Chlamydia pneumoniae* has a ciliostatic effect [37], which in these cases may help pneumococci carried in the upper respiratory tract to invade deeper layers. These double infections were more severe than usual and the response to antibiotics effective against the pneumococcus only was poor [38]. One should not be satisfied when one pathogen is diagnosed, but always keep in mind the possibility of mixed infection.

LABORATORY DIAGNOSIS OF ATYPICAL PATHOGENS

Serology has traditionally been used to diagnose infections caused by atypical pathogens. Antigens and complete kits for antibody assays are commercially available from several sources. The most serious disadvantage is the need to demonstrate a seroconversion in the majority of the cases. This delays the diagnosis and is then of no aid to the clinician treating the acutely ill patient. Attempts have been made to overcome this delay by the demonstration of IgM antibodies in the acute phase or by using 'diagnostic' high titers in the first serum sample. The pitfalls are the lack of IgM in reinfections and the uncertainty of the diagnostic value of high titers in acute diseases caused by several atypical agents. Serology, even though inadequate, has remained the main diagnostic tool in mycoplasmal and chlamydial pneumonias as well as in Q fever.

Culture of the atypical pathogens demands special media not widely used in microbiology laboratories or, in the case of obligatory intracellular pathogens, cultured living cells in specialized units. Moreover, *Coxiella burnetti* and *Chlamydia psittaci* present dangers to laboratory workers handling the agent, and even *Chlamydia pneumoniae* has caused laboratory-acquired pneumonias [39]. In legionellosis, however, culture is a standard diagnostic procedure [40]. It should also be attempted in *Chlamydia pneumoniae* infections in order to obtain information on disease associations and strain variability of this newly recognized pathogen.

Antigen detection in respiratory tract infections has been utilized mainly in viral infections, with good success. Its use in the case of atypical bacterial pathogens has not been as rewarding. Moreover, techniques based on immunofluorescence demand experience and patience from the reader, since numbers of pathogens are often limited and their reliable identification is difficult. Lack of commercially available reagents and kits is a problem in the diagnosis of uncommon atypical pathogens. However, in legionellosis, detection of antigen in urine seems to be a reliable diagnostic method [11,41]. In pneumonias, the presence of bacterial components in the circulation, alone or in immune complexes, has been used successfully in the diagnosis of pneumococcal pneumonias [42], but has remained unstudied in the case of atypical pathogens. These types of complex are commonly, seen, however, in chronic *Chlamydia pneumoniae* infections, which lessens their diagnostic value in acute infections, especially in elderly males with arteriosclerotic lesions [43].

Nucleic acid (NA) detections seems to be the diagnostic method of the future for atypical respiratory pathogens. The commercial kit for *M. pneumoniae* direct NA detection is no longer available, but diagnostic companies are developing kits based on NA amplification for *Legionella* spp., *M. pneumoniae* and *Chlamydia pneumoniae*. These, whether based on the polymerase or ligase chain reaction, would provide a sensitive and specific diagnosis for these pathogens in 24 h. This would finally give a firm basis for a rationally targeted therapy.

TREATMENT

Table 3 shows the recommended treatment for infections caused by atypical pathogens. The therapeutic response of bacterial atypical pathogens to β-lactam antibiotics and aminoglycosides is lacking or marginal. The drugs used are tetracyclines, macrolides, and azalides. Time will tell how much the advent of newer quinolones will alter these recommendations. Prolonged treatment of 2–4 weeks has been used in legionellosis and in *Chlamydia pneumoniae* pneumonias. The possibility of mixed infections should always be kept in mind.

Table 3 Main groups of drugs currently used for atypical pathogens

Pathogen	Preferred drugs	Also effective
Legionella spp.	Erythromycin (+rifampicin)	Quinolone?
Mycoplasma pneumoniae	Erythromycin	Macrolide/azalide Quinolone
Chlamydia spp.	Tetracycline Erythromycin	Macrolide/azalide Quinolone
Coxiella burnetti	Doxycycline/ tetracycline	Quinolone
Respiratory syncytial virus	Ribavirin aerosol	

CHRONIC SEQUELAE DUE TO ATYPICAL RESPIRATORY PATHOGENS

Appropriate therapy can be efficient in preventing long-term sequelae caused by some of these atypical pathogens. Q fever endocarditis is a feared complication, which often leads to valvular operations or drug therapy for the rest of the patient's life [44]. Recently discovered *Chlamydia pneumoniae* seems to be associated with complications of unexpected severity and ubiquity. Signs of chronic *Chlamydia pneumoniae* infection have been found in both childhood [45] and adult-onset [46] asthma, chronic bronchitis [47], and sarcoidosis [48]. Most surprising is its association with arteriosclerosis, the leading cause of death in industrialized countries. Markers of chronic *Chlamydia pneumoniae* infections are found in acute myocardial infarction [49,50], and they have repeatedly been shown to be a risk factor for cardiac events [51,52]; and, finally, the pathogen has been demonstrated in atherosclerotic lesions [53,54]. Animal experiments have been positive [55,56], as have preliminary intervention trials [57,58]. It may be possible that appropriate treatment of *Chlamydia pneumoniae* respiratory infection in the acute phase prevents infection from progressing to a chronic state, which can be much more difficult to cure. This would demand acute diagnosis and targeted therapy for atypical pneumonias.

References

1. Duchin JS, Koster FT, Peters CJ et al. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. N Engl J Med 1994; 330: 949–55.
2. Donohue JF. Lower respiratory tract involvement in Rocky Mountain spotted fever. Arch Intern Med 1980; 140: 223–7.
3. Levinthal W. Die Aetiologie der Psittakosis. Klin Wochenschr 1930; 9: 654–9.
4. Smith W, Andrews CH, Laidlaw PP. A virus obtained from influenza patents. Lancet 1933; ii: 66.
5. Derrick EH. 'Q' fever, new fever entity: clinical features, diagnosis and laboratory investigation. Med J Aust 1937; 2: 281–99.
6. Eaton MD, Meiklejohn G, van Herick W. Studies on the etiology of primary atypical pneumonia: a filterable agent transmissible to cotton rat, hamsters, and chick embryo. J Exp Med 1944; 82: 329.
7. MacFarlane JT, Miller AC, Roderick Smith WH, et al. Comparative radiographic features of community acquired legionnaires' disease, pneumococcal pneumoniae, mycoplasma pneumonia, and psittacosis. Thorax 1984; 39: 28–33.
8. Skakni L, Sardet A, Just J, et al. Detection of *Mycoplasma pneumoniae* in clinical samples from pediatric patients by polymerase chain reaction. J Clin Microbiol 1992; 30: 2638–43.
9. Fraser DW, Tsai T, Orenstein W, et al. Legionnaires' disease: description of an epidemic of pneumonia. N Engl J Med 1977; 297: 1189–97.
10. McDade JE, Brenner DJ, Bozeman FM. Legionnaires' disease bacterium isolated in 1947. Ann Intern Med 1979; 90: 659–61.
11. Stout JE, Yu VL. Legionellosis. N Engl J Med 1997; 337: 682–7.
12. Fang GD, Fine M, Orloff J, et al. New and emerging etiologies for community-acquired pneumonia with implications for therapy: a prospective multicentre study of 359 cases. Medicine 1990; 69: 307–16.
13. Ozanne G, Lefebvre J. Specificity of the microimmunofluorescence assay for the serodiagnosis of *Chlamydia pneumoniae* infections. Can J Microbiol 1992; 38: 1185–9.
14. Wong KH, Skelton SK, Daugharty H. Utility of complement fixation and microimmunofluorescence assays for detecting serologic responses in patients with clinically diagnosed psittacosis. J Clin Microbiol 1994; 32: 2417–21.
15. Bourke SJ, Carrington D, Frew CE, et al. A comparison of the seroepidemiology of chlamydial infection in pigeon fanciers and farmers in the UK. J Infect 1992; 25 (suppl 1): 91–8.
16. Jantos C, Artelt P, Schiefer HG. Acute lower respiratory tract infection associated with *Chlamydia pneumoniae* in Germany. Eur J Clin Microbiol 1993; 12: 33–5.
17. Grayston JT, Kuo CC, Wang SP, Altman J. A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. N Engl J Med 1986; 315: 161–8.
18. Saikku P, Wang SP, Kleemola M, et al. An epidemic of mild pneumonia due to an unusual strain of *Chlamydia psittaci*. J Infect Dis 1985; 151: 832–9.
19. Saikku P. The epidemiology and significance of *Chlamydia pneumoniae*. J Infect 1992; 25 (suppl 1): 27–34.
20. Kaukoranta-Tolvanen SS, Laurila AL, Saikku P, et al. Experimental infection of *Chlamydia pneumoniae* in mice. Microb Pathogen 1993; 15: 293–302.
21. Yang ZP, Kuo CC, Grayston JT. A mouse model of *Chlamydia pneumonia* strain TWAR pneumonitis. Infect Immun 1993 61: 2037–40.
22. Laitinen K, Laurila A, Leinonen M, Saikku P. Experimental *Chlamydia pneumoniae* infection in mice: effect of reinfection and passive protection by immune serum. In Orfila J, Byrne GI, Chernesky MA, et al, eds. Chlamydial infections. Bologna: OCT Societá Editrice Esculapio, 1994: 545–8.
23. Laitinen K, Laurila AL, Leinonen M, Saikku P. Reactivation of *Chlamydia pneumoniae* infection in mice by cortisone treatment. Infect Immun 1996; 64: 1488–90.
24. Yang ZP, Kuo CC, Grayston JT. Systemic dissemination of *Chlamydia pneumoniae* following intranasal inoculation in mice. J Infect Dis 1995; 171: 736–8.
25. Hyman CL, Augenbraum MH, Roblin PM, et al. Asymptomatic respiratory tract infection with *Chlamydia pneumoniae* TWAR. J Clin Microbiol 1991; 29: 2082–3.
26. Hyman CL, Roblin PM, Gaydos CA, et al. Prevalence of asymptomatic nasopharyngeal carriage of *Chlamydia pneumoniae* in subjectively healthy adults: assessment by polymerase chain reaction–enzyme immunoassay and culture. Clin Infect Dis 1995; 20: 1174–8.

27. Mathews RS, Mohite A, Addy DP, Wise R. *Chlamydia pneumoniae* (TWAR) in neonates. Pediatr Infect Dis J 1991; 10: 956–7.
28. Kishimoto T, Kimura M, Kubota Y, et al. An outbreak of *Chlamydia pneumoniae* infection in households and schools. In Orfila J, Byrne GI, Chernesky ME, et al, eds. Chlamydial infections. Bologna: Societá Editrice Esculapio, 1994: 465–8.
29. Aldous MB, Grayston JT, Wang SP, Foy HM. Seroepidemiology of *Chlamydia pneumoniae* TWAR infection in Seattle Families, 1966–1979. J Infect Dis 1992; 166: 646–9.
30. Grayston JT, Campbell LA, Kuo CC, et al. A new respiratory tract pathogen: *Chlamydia pneumoniae* strain TWAR. J Infect Dis 1990; 161: 618–25.
31. Grayston JT. *Chlamydia pneumoniae* (TWAR) infections in children. Pediatr Infect Dis J 1994; 13: 675–84.
32. Kleemola M, Saikku P, Visakorpi R, et al. Epidemics of pneumonia caused by TWAR, a new *Chlamydia* organism, in military trainees in Finland. J Infect Dis 1988; 157: 230–6.
33. Troy CJ, Peeling RW, Ellis AG, et al. *Chlamydia pneumoniae* as a new source of infectious outbreaks in nursing homes. JAMA 1997; 277: 1214–18.
34. Thom DM, Grayston JT, Wang SP, et al. *Chlamydia pneumoniae*, strain TWAR, *Mycoplasma pneumoniae* and viral infections in acute respiratory disease in a university student health clinic population. Am J Epidemiol 1990; 132: 248–56.
35. Huovinen P, Lahtonen R, Ziegler T, et al. Pharyngitis in adults: the presence and coexistence of viruses and bacterial organisms. Ann Intern Med 1989; 110: 612–16.
36. Kauppinen M, Syrjälä H, Saikku P, et al. Etiology of community acquired pneumonia during a *Chlamydia pneumoniae* epidemic in northern Finland. J Infect Dis 1995; 172: 1330–5.
37. Shemer-Avni Y, Lieberman D. *Chlamydia pneumoniae*-induced ciliostasis in ciliated bronchial epithelial cells. J Infect Dis 1995; 171: 1274–8.
38. Kauppinen M, Saikku P, Kujala P, Herva E, Syrjälä H. Clinical picture of community-acquired *Chlamydia pneumoniae* requiring hospital treatment: a comparison between chlamydial and pneumococcal pneumonia. Thorax 1996; 51: 185–9.
39. Surcel H, Syrjälä H, Leinonen M, et al. Cell mediated immunity to *Chlamydia pneumoniae* measured as lymphocyte blast transformation *in vitro*. Infect Immun 1993; 61: 2196–9.
40. Zuravleff JJ, Yu VL, Shonnard J, et al. Diagnosis of Legionnaires' disease; an update of laboratory methods with new emphasis on isolation by culture. JAMA 1983; 250: 1981–5.
41. Ruf B, Shurmann D, Horbach I, et al. Frequency and diagnosis of Legionella pneumonia: a 3 year prospective study with emphasis on application of urinary antigen detection. J Infect Dis 1990; 62: 1341–7.
42. Leinonen M, Syrjälä H, Jalonen E, Kujala P, Herva E. Demonstration of pneumolysin antibodies in circulating immune complexes—a new diagnostic method for pneumococcal pneumonia. Serodiagn Immunother Infect Dis 1990; 4: 451–8.
43. Linnanmäki E, Leinonen M, Mattila K, et al. Presence of *Chlamydia pneumoniae* specific antibodies in circulating immune complexes in coronary heart disease. Circulation 1993; 87: 1130–4.
44. Levy PY, Drancourt M, Etienne J, et al. Comparison of different antibiotic regimens for therapy of 32 cases of Q fever endocarditis. Antimicrob Agents Chemother 1991; 35: 533–7.
45. Emre U, Roblin PM, Gelling M, et al. The association of *Chlamydia pneumoniae* infection and reactive airway disease in children. Arch Pediatr Adolesc Med 1994; 148: 727–32.
46. Hahn DL, Dodge RW, Golubjatnikov R. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. JAMA 1991; 266: 225–30.
47. Blasi F, Legnani D, Lombardo VM, et al. *Chlamydia pneumoniae* infection in acute exacerbations of COPD. Eur Respir J 1993; 6: 19–22.
48. Grönhagen-Riska C, Saikku P, Riska H, et al. Antibodies to TWAR—a novel type of *Chlamydia*—in sarcoidosis. In Grassi C, ed. Sarcoidosis and other granulomatous disorders. Amsterdam: Elsevier Scientific Publishers, 1988: 297–301.
49. Saikku P, Leinonen M, Mattila K, et al. Serologic evidence of an association of a novel *Chlamydia*, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet 1988; ii: 983–5.
50. Leinonen M, Linnanmäki E, Mattila K, et al. Circulating immune complexes containing *Chlamydia* lipopolysaccharide in acute myocardial infarction. Microb Pathogen 1990; 9: 67–73.
51. Saikku P, Leinonen M, Tenkanen L, et al. Chronic *Chlamydia pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki Heart Study. Ann Intern Med 1992; 116: 273–8.
52. Thom DH, Wang SP, Grayston JT, et al. *Chlamydia pneumoniae* strain TWAR antibody and angiographically demonstrated coronary artery disease. Arteriosclerosis Thrombosis 1991; 11: 547–51.
53. Shor A, Kuo CC, Patton DL. Detection of *Chlamydia pneumoniae* in coronary arterial fatty streaks and atheromatous plaques. South Afric Med J 1992; 82: 158–60.
54. Kuo C-C, Shor A, Campbell LA, et al. Demonstration of *Chlamydia pneumoniae* in atherosclerotic lesions of coronary arteries. J Infect Dis 1993; 167: 841–9.
55. Kaukoranta-Tolvanen SS, Laitinen K, Saikku P, Leinonen M. *Chlamydia pneumoniae* multiplies in human endothelial cells in vitro. Microb Pathogen 1994; 16: 313–19.
56. Laitinen K, Laurila A, Pyhälä L, Leinonen M, Saikku P. *Chlamydia pneumoniae* infection induces inflammatory changes in the aorta of rabbits. Infect Immun 1997; 65: in press.
57. Gupta S, Leatham EW, Carringdon D, Mendall MA, Kaski JC, Camm AJ. Elevated *Chlamydia pneumoniae* antibodies, cardiovascular events, and azithromycin in male survivors of myocardial infarction. Circulation 1997; 96: 404–7.
58. Gurfinkel E, Bozovich G, Daroca A, Beck E, Mautner B, Roxis Study Group. Randomised trial of roxithromycin in non-Q-wave coronary syndromes: ROXIS pilot study. Lancet 1997; 350: 404–7.