Chlamydophila (Chlamydia) pneumoniae serology and asthma in adults: A longitudinal analysis

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Background: Many cross-sectional studies have found an association between *Chlamydophila pneumoniae* infection and asthma, and a possible causative role of *C pneumoniae* infection in asthma pathogenesis has been proposed. No longitudinal studies have been undertaken to estimate the effect on asthma incidence of previous or chronic infection.
Objective: We evaluated whether serological findings suggestive for recent or chronic *C pneumoniae* infection affect asthma risk or lung function during follow-up.
Methods: We followed a population-based adult cohort for 15 years and made a clinical evaluation of persons with new persistent asthma (n = 83) and matched controls (n = 162). Serological testing was performed by microimmunofluorescence and enzyme immunoassay from both baseline and follow-up samples.
Results: Subjects with serologically diagnosed recent or chronic *C pneumoniae* infection did not run a higher risk of new asthma. An increased risk was found in subjects with allergic rhinitis, low lung function, history of smoking, and positive family background of asthma or allergy. However, chronic *C pneumoniae* infection was found to accelerate the loss of lung function significantly in subjects who contracted new nonatopic asthma (median change in FEV_1, 89.6 vs 55.9 mL/y; $P = .032$).
Conclusion: Chronic *C pneumoniae* infection promotes the development of airflow limitation in adults with nonatopic asthma. However, our results indicate that at the population level, any possible effect of *C pneumoniae* infection on asthma incidence is of minor significance. (J Allergy Clin Immunol 2005;116:1123-8.)

Key words: *asthma, atopy,* Chlamydophila pneumoniae*,* Chlamydia pneumoniae *infection, lung function, population-based, remodeling, risk, serology*

Abbreviations used
EIA: Enzyme immunoassay
PEF: Peak expiratory flow

Chlamydophila pneumoniae (formerly known as *Chlamydia pneumoniae*) is a common intracellular respiratory pathogen that may cause acute illness in both the upper and lower respiratory tracts.[1] It has been estimated that most people have 2 or 3 *C pneumoniae* infections during their lifetime.[2] This condition has been reported as a possible etiologic agent in asthma since Hahn et al[3] showed an association between *C pneumoniae* serology and asthma in 1991. Other groups in different countries have since corroborated this finding,[4,5] but contrary results have also been reported.[6]

Several observations support a possible causative role of *C pneumoniae* infection in asthma. New adult patients with asthma often report a precipitating event such as a severe respiratory infection as the onset of their illness, and acute *C pneumoniae* infection has been shown to cause asthma exacerbation.[7,8] Furthermore, members of the family *Chlamydiaceae* are known to cause chronic infections in various organs, and it has been proposed that *C pneumoniae* could also cause chronic infection in the human airways. Findings in a case-control study in which bronchial biopsy and bronchoalveolar lavage of study subjects were analyzed by using *C pneumoniae* PCR support such a concept.[9] Significant improvement in asthma symptoms and spirometry has been reported with prolonged antibiotic treatment in patients with asthma with suspected chronic infection.[10] In a randomized, controlled trial, however, only temporary benefit was found.[11]

Most previous studies concerning the possible connection between *C pneumoniae* and asthma have been made in a cross-sectional setting[5,6] or have been case series with acutely symptomatic patients or patients referred to a specific institution.[4,12] Strachan et al[13] performed a prospective study in a cohort of middle-aged men and found elevated *C pneumoniae*–specific antibodies to be associated neither with indicators of obstructive lung disease nor with progressive lung function decline.

No studies have so far been published describing serological findings before and after asthma diagnosis. We therefore conducted a 15-year follow-up of a Finnish population cohort through the national register and made a

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TABLE I. Patient characteristics by group

		New asthma during follow-up (n = 83)	Controls (n = 162)
Sex, n	Women	49	97
	Men	34	65
Age, y	Mean (range)	44 (32-56)	44 (31-58)
Smoking at baseline, %	Non	44	56
	Ex	25	18
	Current	31	26
Atopy, %	Yes	42	33
FEV_1 % predicted*	Mean (SD)	94.5 (16.1)	103.3 (12.8)

*Data missing from 3 controls.

clinical evaluation of subjects developing asthma during the follow-up and matched controls. The primary aim was to establish whether there is an increased asthma risk in subjects yielding serological findings suggestive of previous or chronic *C pneumoniae* infection.

METHODS

Approval for this study was obtained from the ethical committee at Tampere University Hospital. All subjects gave informed consent to participate.

Baseline survey

Patients and controls were selected from a population study, the Mini-Finland Health Survey, in which the material was composed of a nationally representative sample of 8000 persons age 30 years and older. The sampling method used was a 2-stage stratified cluster design. The fieldwork was carried out between January 1978 and August 1980. Lung function was measured with the Vitalograph (Vitalograph Ltd, Buckingham, United Kingdom) spirometer, and the results were corrected for body temperature and pressure, saturated with water vapor. Results of 2 technically faultless measurements were recorded, and the highest readings for FEV_1 and forced expiratory volume were taken as the final results, whether obtained at the same measurement or not. The only reason for rejecting a measurement was a technically incomplete or otherwise unsuccessful blow. Calibration was according to manufacturer's instructions. Finnish reference values[14] were used when converting results to percent predicted form. A more detailed description of the methods used has been published elsewhere.[15]

Cases and controls

The criterion for new persistent asthma was entitlement to special reimbursement of asthma medication costs from the Social Insurance Institution of Finland. Entitlement is granted if the criteria for persistent asthma are fulfilled as certified by a chest specialist. Typical history, clinical features, and course of asthma must be documented. At least 1 of the following physiological criteria is required for diagnosis: (1) a variation of $\geq$20% in diurnal peak expiratory flow (PEF) recording (reference to maximal value), (2) an increase of $\geq$15% in PEF or FEV_1 with β_2-agonist, and (3) a decrease of $\geq$15% in PEF or FEV_1 in exercise testing. Moreover, at least 6 months of continuing regular use of antiasthmatic medication must have elapsed at the time of the decision. This method of asthma case ascertainment has been evaluated earlier.[16] The follow-up through the Social Insurance Institution's register ended on December 31, 1994. Controls free of asthma and chronic obstructive pulmonary disease were selected from among participants in the Mini-Finland Health Survey. Two controls were initially matched with each case for age and sex (in 4 cases, only 1:1 matching was achieved). Baseline characteristics of the study population are presented in Table I.

TABLE II. Baseline characteristics and risk of new persistent asthma in study population by conditional logistic regression analysis*

Factor		Odds ratio	95% CI	*P* value
C pneumoniae IgG	MIFA titer $\geq$ 128	.76	.42-1.36	.359
	MIFA titer $\geq$ 512	2.73	.89-8.33	.077
	EIA	1.00	.99-1.01	.921
C pneumoniae IgA	MIFA titer $\geq$ 32	.91	.47-1.76	.780
	MIFA titer $\geq$ 64	1.42	.65-3.09	.379
	EIA	1.00	.98-1.02	.882
Chronic infection with *C pneumoniae*	MIFA IgG titer $\geq$ 128 and IgA titer $\geq$ 32	1.12	.58-2.17	.735
Inheritance	Parent with asthma	3.82	1.63-8.93	.002
	Parent with allergy	5.16	1.85-14.41	.002
Allergic rhinitis		3.98	2.16-7.33	<.001
Smoking	Ex-smoker	2.24	1.01-4.96	.048
	Current smoker	1.84	.90-3.75	.094
	Ever-smoker	1.99	1.04-3.79	.037
Lung function	FEV% < 80.0	1.65	.89-3.06	.110
	FEV_1 % predicted < 85.0	4.72	2.08-10.71	<.001

FEV%, (Forced expiratory volume in one second / forced vital capacity) × 100; *MIFA*, microimmunofluorescence.
*Number of observations, 245.

Follow-up study

Basic data and signed approval were collected by postal questionnaire. The fieldwork was performed between December 1997 and February 1998. Lung function measurements were performed as in the baseline survey. Skin prick tests were performed by specially trained nurses, applying a panel of 22 common allergen extracts (ALK A/S, Copenhagen, Denmark). A patient was considered atopic if at least 1 allergen elicited a weal with a diameter at least 3 mm larger than that of the negative control.

Serological testing

Microimmunofluorescence detection of *C pneumoniae* antibodies was performed by using *Chlamydia pneumoniae* IgG/IgM and IgA Micro-IF Test Kits (Labsystems, Helsinki, Finland). The assays were performed according to the manufacturer's instructions. IgM antibodies were screened for in a 1:16 dilution. IgG antibodies were tested in all specimens in titers of 1:32, 1:128, and 1:512, and IgA antibodies in titers of 1:8, 1:32, and 1:64, respectively. The end point of the fluorescence was marked as the titer, the highest marked as >1:512 for IgG and >1:64 for IgA. For each study object, the baseline and follow-up specimen were treated consecutively in the same batch.

TABLE III. *C pneumoniae* specific antibody titers in baseline study (% within group)

		All			Women			Men		
	Titer	New asthma n = 83	Control n = 162	*P* value	New asthma n = 49	Control n = 97	*P* value	New asthma n = 34	Control n = 65	*P* value
IgM	0	100	95.7		100	93.8		100	98.5	
	1/16	—	4.3		—	6.2		—	1.5	
IgG	0	36.1	31.5		46.9	40.2		20.6	18.5	
	1/32	31.3	30.2		30.6	34.0		32.4	24.6	
	1/128	20.5	32.1	.376*	18.4	24.7	.660*	23.5	43.1	.350*
	1/512	12.0	6.2	.112**	4.1	1.0	.220**	23.5	13.8	.225**
IgA	0	53.0	37.7		63.3	45.4		38.2	26.2	
	1/8	19.3	32.7		20.4	37.1		17.6	26.2	
	1/32	10.8	16.7	.754#	4.1	13.4	.856#	20.6	21.5	.735#
	1/64	16.9	13.0	.408##	12.2	4.1	.067##	23.5	26.2	.775##

P value calculated groupwise for IgG antibody titer at cutoff level *1/128 and **1/512 and for IgA at cutoff level #1/32 and ##1/64 with χ^2 test ($df = 1$).
P values for sex difference in patients with asthma at cutoff level 1/128 (IgG) and 1/32 (IgA) <.020 and in controls <.001 (χ^2 test, $df = 1$).

Enzyme immunoassay (EIA) detections were performed by using *Chlamydia pneumoniae* IgG, IgA, and IgM EIA (Labsystems, Helsinki, Finland). The tests were conducted as recommended by the manufacturer. For IgM, a signal/cutoff ratio was calculated. IgG and IgA results are expressed as enzyme immunounits calculated and scaled as suggested by the manufacturer. All samples were analyzed in duplicate, and for each study object, the primary and the follow-up specimen were treated in the same run.

Statistics

Statistical analysis was performed with STATA 7.0 (STATA Corp, College Station, Tex) in matched case-control pairs and groupwise analyses using SPSS 10.1 (SPSS Inc, Chicago, Ill). The statistical method selected is indicated in the results section.

RESULTS

Chlamydophila pneumoniae–specific IgM, IgG, and IgA antibody levels from the samples obtained at the baseline and follow-up were analyzed by both microimmunofluorescence and EIA. There were 7 persons with an elevated IgM antibody titer in the baseline study, 6 of whom also had elevated IgM antibody titers in the follow-up study, which would suggest that the finding was a result of a nonspecific reaction. All subjects with elevated IgM titer were controls. Omitting these did not alter subsequent results.

Baseline *C pneumoniae* serology had no significant effect on asthma risk. The analysis was first made in the original case-control setting. The results of conditional logistic regression are given in Table II, which also presents odds ratios for some known risk factors for asthma. Subsequent groupwise analysis likewise showed no significant association with *C pneumoniae* serology and new asthma. The proportions of antibody titers are set on in Table III and antibody level medians measured by EIA in different groups in Table IV. Because of the significant sex difference in serology (see Control of Confounding Factors), we also analyzed the association of asthma with serology separately in male and female subjects.

To assess the possible weakening effect of the considerably long follow-up period, we performed these analyses separately for new patients with asthma diagnosed in the first half of the follow-up period. The results were as above; no significant differences were observed between subjects who contracted asthma and controls (data not shown).

Seroconversion and antibody level changes

The proportions of subjects whose microimmunofluorescence titer converted from negative to positive during follow-up (= seroconversion) did not differ between the study groups. This was observed with IgG and IgA in both sexes. Different cutoff points gave similar results (data not shown). Change in specific antibody levels as measured by EIA between baseline and follow-up study was also calculated. Both conditional logistic regression and groupwise comparisons failed to show a significant association of these parameters with new asthma (Table IV).

Control of confounding factors

Because significant sex differences in *C pneumoniae* antibody levels were observed by both microimmunofluorescence and EIA methods (Tables III and IV), the association between asthma and serology was analyzed separately in male and female subjects. Different smoking habits or age distribution (Table I) did not explain the observed sex difference. There were no significant sex differences within the study groups in the mean change in antibody levels during follow-up. Correspondingly, no significant sex difference was found in the amount of seroconversion by the microimmunofluorescence method. Neither atopy nor smoking status at baseline had any significant effect on the association between new asthma and antibody levels. Moreover, smoking status in the baseline study had no significant effect on antibody level changes during follow-up.

TABLE IV. Baseline *C pneumoniae* specific antibody levels and level changes during follow-up measured by EIA*

		New asthma	Control	*P* value
All subjects		**(n = 83)**	**(n = 162)**	
IgG	Median	71.5	75.0	.919
	QR	35.0, 132.0	40.6, 132.6	
IgA	Median	12.0	12.0	.666
	QR	5.5, 22.5	7.0, 22.6	
d-IgG	Median	−1.5	2.0	.518
	QR	−13.5, 17.5	−12.5, 19.6	
d-IgA	Median	.0	1.8	.224
	QR	−2.0, 4.5	−2.0, 6.0	
Women		**(n = 49)**	**(n = 97)**	
IgG	Median	57.5	57.5	.751
	QR	26.0, 92.3	32.5, 101.3	
IgA	Median	8.0	10.0	.563
	QR	4.5, 18.0	6.0, 16.0	
d-IgG	Median	−1.0	4.5	.431
	QR	−11.0, 21.8	−9.5, 22.3	
d-IgA	Median	.5	2.0	.443
	QR	−1.5, 3.8	−2.0, 6.3	
Men		**(n = 34)**	**(n = 65)**	
IgG	Median	105.0	109.5	.915
	QR	55.9, 182.6	59.0, 181.5	
IgA	Median	17.3	21.0	.637
	QR	8.9, 39.0	9.8, 37.5	
d-IgG	Median	−3.3	−2.5	.930
	QR	−14.8, 11.5	−20.3, 14.5	
d-IgA	Median	−.3	1.5	.365
	QR	−4.1, 8.3	−1.8, 7.5	

d-IgG (d-IgA), Change in *C pneumoniae* specific IgG (IgA) antibody levels during follow-up; *QR,* quartile range.
*Results expressed as enzyme immunounits. *P* values calculated by Mann-Whitney *U* test. *P* values for sex difference at baseline in patients with asthma < .005 and in controls < .001. No significant sex difference in change values.

Lung function decline during follow-up

Subjects who contracted new asthma had faster decline in FEV_1 (median, 57.9; quartile range, 44.9-75.4 mL/y) than controls (45.9; 34.7-57.6 mL/y; $P < .001$, Mann-Whitney *U*). The effect of smoking was significant in both patients with asthma and controls (median: patients with asthma, smokers 70.5 vs nonsmokers 54.1 mL/y; $P = .031$; controls, 57.5 vs 42.9 mL/y; $P < .001$). Therefore, we further analyzed the effect of *C pneumoniae* serology in nonsmoking subjects.

Patients with asthma with serological evidence suggesting chronic *C pneumoniae* infection (microimmunofluorescence IgG ≥ 128 and IgA ≥ 64) had significantly faster rate of decline in FEV_1 than other patients with asthma (median, 63.0 vs 51.4 mL/y; $P = .036$; Mann-Whitney *U*) and controls (Fig 1). When atopic and nonatopic patients with asthma were analyzed separately, it was found that *C pneumoniae* serology had a significant effect only in the nonatopic group (median, 89.6 vs 55.9 mL/y; $P = .032$; Mann-Whitney *U*; Fig 2). In controls, neither *C pneumoniae* serology (Fig 1) nor atopy had an effect on lung function decline.

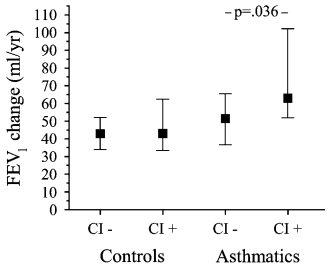

FIG 1. Chronic *C pneumoniae* infection at the baseline and decline in FEV_1 during follow-up in nonsmoking patients with asthma and controls. Values are median *(■)* and quartile range *(whiskers),* given in milliliters per year. Criteria for chronic *C pneumoniae* infection *(CI +)* = microimmunofluorescence IgG titer ≥ 128 and IgA titer ≥ 32.[5] *P* values calculated with Mann-Whitney *U* test.

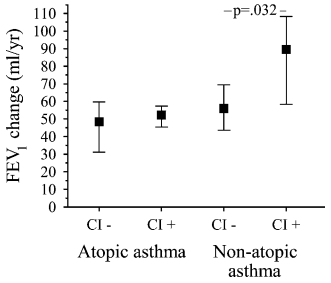

FIG 2. Chronic *C pneumoniae* infection at the baseline and decline in FEV_1 during follow-up in nonsmoking atopic and nonatopic patients with asthma. Values are median *(■)* and quartile range *(whiskers),* given in milliliters per year. Criteria for chronic *C pneumoniae* infection *(CI +)* = microimmunofluorescence IgG titer ≥ 128 and IgA titer ≥ 32.[5] *P* values calculated with Mann-Whitney *U* test.

DISCUSSION

This case-control study was made among subjects drawn from a population-based adult cohort. Our primary aim was to establish whether serologically diagnosed *C pneumoniae* infection is a risk factor for new persistent asthma in adulthood. Case ascertainment during follow-up was performed through the national register, which relies on diagnoses made by chest physicians on clinical or physiologic criteria and indicating a persistent form of asthma. This definition of asthma—the clinical assessment—is the standard applied to validate other definitions used in epidemiological studies.[17] The positive predictive value of our definition is very high compared with those used in most epidemiological studies.[16] Because the level of reimbursement of medication costs is relatively high (75% of total costs), it may be assumed that all subjects fulfilling the criteria are registered. On the other hand, the sensitivity of our definition is lower than that of most other definitions of asthma, because it includes only persistent cases. Consequently, our results apply to the probability of new persistent asthma.

Several authors have discussed the possibility of chronic *C pneumoniae* infection in the human airways. Evidence from a study in which biopsy specimens and

bronchoalveolar lavage of study subjects were analyzed support the existence of such a condition. Martin et al[9] showed that *C pneumoniae* specific PCR is positive in some patients with asthma without signs of acute infection, whereas in controls, no positive findings were detected. Elevated antibody titers, especially IgA titers, have been considered a reliable marker of chronic infection with other bacteria.[18,19] It has also been noted that the IgA response is often a predominant feature in *C pneumoniae* reinfection.[20] A cross-sectional association of asthma and *C pneumoniae* serology has been shown in several studies. Cook et al[12] reported that patients with severe chronic asthma had a higher prevalence of specific antibodies than controls. In another study, the association was strongest in nonatopic long-standing asthma.[4] A significant association between chronic *C pneumoniae* infection and asthma emerged in a recent study in which a high IgG titer combined with a high IgA titer was used as the criterion for chronic infection.[5] However, the cross-sectional design of these studies furnishes no information on the temporal relation of asthma and raised antibody titers. These observations have nonetheless led some researchers to conclude that previous or chronic infection causes persistent asthma, and it has even been proposed that *C pneumoniae* could underlie the substantial increase in the prevalence of asthma during the last few decades.[21,22]

Our first main finding was that elevated *C pneumoniae*–specific antibody titers did not increase the probability of new persistent asthma. This finding was consistent at all cutoff levels of microimmunofluorescence and equally by the EIA method. Moreover, high IgA together with high IgG titer as criteria gave a similar result. Our observation is in line with the findings in a previous longitudinal study performed in an adult male cohort, in which no association was found between elevated antibody titers and indicators of chronic nonspecific lung disease.[13]

Smoking has previously been found to elevate *C pneumoniae*–specific antibody levels.[23] In our study population, smoking was more common among subjects who contracted new asthma. This should have enhanced the suspected effect of *C pneumoniae* on asthma risk. Therefore, we consider it unlikely that any difference in the smoking habits would have corrupted our findings. Other factors previously shown to favor the prevalence of elevated *C pneumoniae*–specific antibody titers are male sex and increasing age.[23,24] The sex difference is not related to smoking status.[25] In our study, antibody levels were significantly higher in male subjects in both study groups.

It must be borne in mind that our results are not necessarily applicable to childhood asthma, and it is possible that *C pneumoniae* infection is a causative factor in some asthma subgroup. A definition of asthma associated with infection has indeed been proposed.[26] However, although patients often name respiratory infection as a starting point in their disease, they may have had asthma symptoms long beforehand but do not recall them.[27]

High titers of specific antibodies were associated with a higher asthma symptom score, lower FEV_1, and use of high-dose inhaled steroids in a preintervention analysis of antibiotic treatment study.[28] This could imply that the cross-sectional association between *C pneumoniae* serology and asthma is related to converted immune defense of patients with asthma or a subgroup of patients with asthma. Many different host factors may modulate the effects of infection in the airways. Recently, it was shown that children with asthma with variant alleles in gene coding for mannose-binding lectin more often had IgG antibodies against *C pneumoniae* than controls with variant alleles.[29] *C pneumoniae* might thus be seen primarily as an inciter of inflammation rather than an initiator of the disease.[22] A similar relationship might also prevail in chronic bronchitis, in which *C pneumoniae* infection is associated with a higher rate of exacerbations.[30]

Our second main finding was that serological findings suggesting chronic *C pneumoniae* infection were associated with the decline in FEV_1 in subjects who contracted new asthma. The accelerated development of airflow limitation was restricted to nonatopic patients with asthma. This finding corroborates findings of a cross-sectional study.[31] In our study, serological findings preceded asthma diagnosis. It has been shown that *C pneumoniae* infection promotes remodeling both *in vitro*[32] and in an animal model.[33] Moreover, chronic *Chlamydia trachomatis* infection plays a role in tissue scarring in other organs.[34,35] Therefore, it seems that serologically diagnosed chronic infection promotes airway remodeling, but the reason it is seen only in nonatopic patients with asthma is unclear. Previously reported cross-sectional associations between *C pneumoniae* serology and severe[12,28] and nonatopic[4] asthma may also be related to this phenomenon. In future studies (in both children and adults), histological specimens together with direct detection of the organism (ie, PCR) in the lung could give us a more definitive insight into these matters.

In summary, these findings in the adult population do not support the view that *C pneumoniae* infection could be the cause of the observed increase in the prevalence of asthma. Moreover, it remains to be shown whether *C pneumoniae* is a true risk factor in asthma. However, the specific immunological features of nonatopic asthma in association with chronic *C pneumoniae* infection favor remodeling in the human airways.

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REFERENCES

1. Marrie TJ. Chlamydia pneumoniae. Thorax 1993;48:1-4.
2. Grayston JT, Campbell LA, Kuo CC, Mordhorst CH, Saikku P Thom DH, et al. A new respiratory tract pathogen: Chlamydia pneumoniae strain TWAR. J Infect Dis 1990;161:618-25.
3. 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.

4. Von Hertzen L, Toyryla M, Gimishanov A, Bloigu A, Leinonen M, Saikku P, et al. Asthma, atopy and Chlamydia pneumoniae antibodies in adults. Clin Exp Allergy 1999;29:522-8.
5. Gencay M, Rudiger JJ, Tamm M, Soler M, Perruchoud AP, Roth M. Increased frequency of Chlamydia pneumoniae antibodies in patients with asthma. Am J Respir Crit Care Med 2001;163:1097-100.
6. Routes JM, Nelson HS, Noda JA, Simon FT. Lack of correlation between Chlamydia pneumoniae antibody titers and adult-onset asthma. J Allergy Clin Immunol 2000;105:391-2.
7. Allegra L, Blasi F, Centanni S, Cosentini R, Denti F, Raccanelli R, et al. Acute exacerbations of asthma in adults: role of Chlamydia pneumoniae infection. Eur Respir J 1994;7:2165-8.
8. Miyashita N, Kubota Y, Nakajima M, Niki Y, Kawane H, Matsushima T. Chlamydia pneumoniae and exacerbations of asthma in adults. Ann Allergy Asthma Immunol 1998;80:405-9.
9. Martin RJ, Kraft M, Chu HW, Berns EA, Cassell GH. A link between chronic asthma and chronic infection. J Allergy Clin Immunol 2001; 107:595-601.
10. Hahn DL, Bukstein D, Luskin A, Zeitz H. Evidence for Chlamydia pneumoniae infection in steroid-dependent asthma. Ann Allergy Asthma Immunol 1998;80:45-9.
11. Black PN, Blasi F, Jenkins CR, Scicchitano R, Mills GD, Rubinfeld AR, et al. Trial of roxithromycin in subjects with asthma and serological evidence of infection with Chlamydia pneumoniae. Am J Respir Crit Care Med 2001;164:536-41.
12. Cook PJ, Davies P, Tunnicliffe W, Ayres JG, Honeybourne D, Wise R. Chlamydia pneumoniae and asthma. Thorax 1998;53:254-9.
13. Strachan DP, Carrington D, Mendall M, Butland BK, Yarnell JW, Elwood P. Chlamydia pneumoniae serology, lung function decline, and treatment for respiratory disease. Am J Respir Crit Care Med 2000;161:493-7.
14. Viljanen AA, Halttunen PK, Kreus KE, Viljanen BC. Spirometric studies in non-smoking, healthy adults. Scand J Clin Lab Invest Suppl 1982;159: 5-20.
15. Von Hertzen L, Reunanen A, Impivaara O, Malkia E, Aromaa A. Airway obstruction in relation to symptoms in chronic respiratory disease—a nationally representative population study. Respir Med 2000;94:356-63.
16. Karjalainen A, Kurppa K, Martikainen R, Klaukka T, Karjalainen J. Work is related to a substantial portion of adult-onset asthma incidence in the Finnish population. Am J Respir Crit Care Med 2001;164:565-8.
17. Pekkanen J, Pearce N. Defining asthma in epidemiological studies. Eur Respir J 1999;14:951-7.
18. Brett MM, Ghoneim AT, Littlewood JM. Serum IgA antibodies against Pseudomonas aeruginosa in cystic fibrosis. Arch Dis Child 1990;65: 259-63.
19. Buck GE, Gourley WK, Lee WK, Subramanyam K, Latimer JM, DiNuzzo AR. Relation of Campylobacter pyloridis to gastritis and peptic ulcer. J Infect Dis 1986;153:664-9.
20. Ekman MR, Grayston JT, Visakorpi R, Kleemola M, Kuo CC, Saikku P. An epidemic of infections due to Chlamydia pneumoniae in military conscripts. Clin Infect Dis 1993;17:420-5.
21. Hahn DL. Chlamydia pneumoniae infection and asthma. Lancet 1992; 339:1173-4.
22. Von Hertzen L. Role of persistent infection in the control and severity of asthma: focus on Chlamydia pneumoniae. Eur Respir J 2002;19:546-56.
23. Karvonen M, Tuomilehto J, Pitkaniemi J, Naukkarinen A, Saikku P. Importance of smoking for Chlamydia pneumoniae seropositivity. Int J Epidemiol 1994;23:1315-21.
24. Saikku P. The epidemiology and significance of Chlamydia pneumoniae. J Infect 1992;25(suppl 1):27-34.
25. Von Hertzen L, Surcel HM, Kaprio J, Koskenvuo M, Bloigu A, Leinonen M, et al. Immune responses to Chlamydia pneumoniae in twins in relation to gender and smoking. J Med Microbiol 1998;47:441-6.
26. Hahn DL, Peeling RW, Dillon E, McDonald R, Saikku P. Serologic markers for Chlamydia pneumoniae in asthma. Ann Allergy Asthma Immunol 2000;84:227-33.
27. Dodge R, Cline MG, Lebowitz MD, Burrows B. Findings before the diagnosis of asthma in young adults. J Allergy Clin Immunol 1994;94: 831-5.
28. Black PN, Scicchitano R, Jenkins CR, Blasi F, Allegra L, Wlodarczyk J, et al. Serological evidence of infection with Chlamydia pneumoniae is related to the severity of asthma. Eur Respir J 2000;15:254-9.
29. Nagy A, Kozma GT, Keszei M, Treszl A, Falus A, Szalai C. The development of asthma in children infected with Chlamydia pneumoniae is dependent on the modifying effect of mannose-binding lectin. J Allergy Clin Immunol 2003;112:729-34.
30. Blasi F, Damato S, Cosentini R, Tarsia P, Raccanelli R, Centanni S, et al. Chlamydia pneumoniae and chronic bronchitis: association with severity and bacterial clearance following treatment. Thorax 2002;57:672-6.
31. ten Brinke A, van Dissel JT, Sterk PJ, Zwinderman AH, Rabe KF, Bel EH. Persistent airflow limitation in adult-onset nonatopic asthma is associated with serologic evidence of *Chlamydia pneumoniae* infection. J Allergy Clin Immunol 2001;107:449-54.
32. Rödel J, Woytas M, Groh A, Schmidt K-H, Hartmann M, Lehmann M, et al. Production of basic fibroblast growth factor and interleukin 6 by human smooth muscle cells following infection with *Chlamydia pneumoniae*. Infect Immun 2000;68:3635-41.
33. McIntosh JC, Simecka JW, Ross SE, Davis JK, Miller EJ, Cassell GH. Infection-induced airway fibrosis in two rat strains with differential susceptibility. Infect Immun 1992;60:2936-42.
34. Moore DE, Spadoni LR, Foy HM, Wang SP, Daling JR, Kuo CC, et al. Increased frequency of serum antibodies to *Chlamydia trachomatis* in infertility due to distal tubal disease. Lancet 1982;2:574-7.
35. Mabey DC, Robertson JN, Ward ME. Detection of *Chlamydia trachomatis* by enzyme immunoassay in patients with trachoma. Lancet 1987;2: 1491-2.