

Serotypes of *Chlamydia trachomatis* and Risk for Development of Cervical Squamous Cell Carcinoma

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HUMAN PAPILLOMAVIRUS (HPV) infection is the leading cause of cervical neoplasia.¹⁻³ Additional risk factors include other sexually transmitted infections (STIs) and smoking. The evidence linking oncogenic HPV types and cervical carcinoma is very strong and consistent. Human papillomavirus DNA-based cohort studies^{4,5} have confirmed the seroepidemiologic findings^{6,7} that past HPV infection predisposes women to developing cervical carcinoma. Longitudinal seroepidemiologic studies have also provided evidence that *Chlamydia trachomatis* infection is an independent risk factor for the development of invasive

Context Human papillomavirus (HPV) infection has been established as a cause of cervical cancer. Epidemiologic studies suggest that *Chlamydia trachomatis* infection also confers increased risk for cervical squamous cell carcinoma (SCC). Whether this risk is serotype-specific is unknown.

Objective To study the association between exposure to different *C trachomatis* serotypes and subsequent development of cervical SCC.

Design and Setting Longitudinal, nested case-control study within a cohort of 530 000 women who provided samples to serum banks in Finland, Norway, and Sweden. The data files were linked to respective national cancer registries.

Subjects One hundred twenty-eight women who had developed invasive cervical SCC at least 12 months following serum donation. Each case had 3 matched controls.

Main Outcome Measure Risk for the development of cervical SCC by IgG antibodies to 10 different *C trachomatis* serotypes, adjusted for antibodies to HPV types 16, 18, and 33 and for serum cotinine levels.

Results Of specific *C trachomatis* serotypes, serotype G was most strongly associated with SCC (adjusted odds ratio [OR], 6.6; 95% confidence interval [CI], 1.6-27.0). Other serotypes associated with SCC were I (OR, 3.8; 95% CI, 1.3-11.0) and D (OR, 2.7; 95% CI, 1.3-5.6). Presence of serum IgG antibodies to more than 1 serotype increased the adjusted ORs for SCC ($P < .001$ for trend).

Conclusions *Chlamydia trachomatis* serotype G is most strongly associated with subsequent development of cervical SCC. Increasing numbers of exposures to different *C trachomatis* serotypes also increases risk. Our results strengthen the evidence that there is a link between past *C trachomatis* infection and cervical SCC.

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cervical squamous cell carcinoma (SCC).⁶⁻⁸ Cervical chlamydial infection can persist for long periods of time.⁹ Similarly, elevated antichlamydial antibody titers persist for several years.¹⁰ Microimmunofluorescence (MIF) testing is still the gold standard for chlamydia serology, and researchers also can use MIF for serotyping.^{11,12} We found a link between the presence of serum antibodies to *C trachomatis* and the subsequent development of cervical SCC.⁸ This study was conducted to determine whether this association is serotype-specific.

METHODS

Serum Banks and Cancer Registries

The serum banks and cancer registries we used have been described in detail.^{7,8} Our study used a joint cohort of 3 population-based serum banks to which a total of 530 000 women have donated blood samples. The Finnish Maternity Cohort¹³ has collected samples since 1983 from

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See also p 81 and Patient Page.

more than 98% of pregnant women in Finland. The Janus project¹⁴ collected samples from 1973-1987 and from 1987-1991 during routine health or screening examinations in various counties in Norway. The Västerbotten project¹⁵ has collected samples since 1986 from about 65% of adults during a health promotion project in northern Sweden.

The Finnish Cancer Registry and the Cancer Registry of Norway are nationwide, while the Regional Cancer Registry at the Oncological Centre in Umeå covers the 4 northernmost counties of Sweden.¹⁶ These registries achieve almost 100% capture by using reports from hospitals, pathology laboratories, and physicians.

Identification of Cases and Controls

Women with cervical carcinoma were identified by linking the data files of the population-based serum banks with the nationwide cancer registries, as described.^{7,8} By the end of 1994 and following histological review, 181 women with invasive cervical carcinoma were identified. For each case, the earliest pre-diagnostic serum sample was chosen and 3 matched controls free of cancer at the time of case diagnosis were randomly selected. Controls were matched for sex, age at serum sampling (± 2 years), storage time of serum samples (± 2 months), country, and (in Norway) county. Serum sample volume was insufficient in 10 controls. Thus, the final number of controls was 533. Permission to link the Finnish Maternity Cohort serum bank and the cancer registry was obtained by the Data Inspection Board of Finland, the ethics committees of the National Research and Development Center for Health and Welfare (STAKES), Finland, and the University Hospital, Helsinki, Finland. In Norway and Sweden, all serum bank donors provided informed consent.

Chlamydia Serology

Serum IgG antibodies to *C trachomatis* and *Chlamydia pneumoniae* were measured by MIF testing as described.^{7,8} Titers of ≥ 16 were considered positive for

C trachomatis. Titers of ≥ 32 were considered positive for *C pneumoniae*, which was used as a control antigen. Serum IgG antibodies to *C trachomatis* were also measured by 2 types of enzyme-linked immunosorbent assay (ELISA): an elementary body (EB) ELISA (Chlamydia IgG, Labsystems Co, Helsinki, Finland), and a peptide ELISA (Chlamydia trachomatis IgG, Labsystems Co). The latter applies *C trachomatis* major outer membrane protein (MOMP) variable domain IV synthetic peptide as the antigen.¹⁷

Microimmunofluorescence was used to further analyze all *C trachomatis* antibody-positive serum samples for antibodies against the following *C trachomatis* serotypes: B, D, E, F, G, and J (American Type Culture Collection, Rockville, Md); and C, H, I, and K (Washington Research Foundation, Seattle, Wash). Elementary body antigens were prepared from *C trachomatis* serotypes B, D, E, F, G, and J (grown in McCoy cells), and from serotypes C, H, I, and K (grown in HeLa-229 cells), and purified using conventional techniques.¹⁸ Elementary body aliquots were stored at -70°C prior to use. Type-specificity of the different antigen preparations was confirmed by *C trachomatis* type-specific monoclonal antibodies. Immunoglobulin G antibodies to single serotypes (B, D, E, F, G, J, C, H, I, and K) were measured by MIF as described above. The serum samples were analyzed at 2-fold dilutions.

C trachomatis DNA

For this study, diagnostic-phase tumor biopsy specimens were available from 85 (47%) of the 181 cases. Two to three 10- μm sections were used for DNA extraction from paraffin-embedded biopsy specimens.¹⁹ For each block, the microtome was cleaned with alcohol and a new blade used. As a contamination control, sections were taken from empty paraffin blocks between each specimen. To remove the paraffin, the sections were rinsed with xylene and centrifuged (2 times). To remove the xylene, the sections were then rinsed with 96% alcohol, centrifuged, and decanted

(3 times). Acetone was then added and the sections incubated at 56°C for 1 hour, after which 100 μL of proteinase K (200 $\mu\text{L}/\text{mL}$) was added for an overnight incubation at 56°C . The next day the tubes were centrifuged, decanted, and incubated at 95°C for 15 minutes. Finally, the tubes were centrifuged and the DNA concentration was measured by a spectrophotometer. Seventy-nine (93%) of the 85 cervical biopsy specimens analyzed showed successful amplification of the human DQA gene. The presence of *C trachomatis* DNA was determined using the automated Cobas Amplicor *Chlamydia trachomatis* test (Roche Molecular Diagnostics, Branchburg, NJ) from all cases that showed successful amplification of the human HLA DQA gene. The test uses primers CP24 and CP27 to define a DNA sequence of approximately 207 nucleotides within the cryptic plasmid of *C trachomatis*. An internal control was added to the Cobas Amplicor test to identify processed specimens containing substances that interfere with amplification.

HPV Serology and Serum Cotinine Analyses

Immunoglobulin G antibodies to HPV types 16, 18, and 33 were determined by ELISA using viruslike particle capsid antigens, as described in other studies.^{7,8} Human papillomavirus ELISA has been extensively validated in previous studies.^{20,21} Serum cotinine was used as a surrogate marker for smoking; levels were measured by radioimmunoassay.^{7,8}

Statistical Analyses

Odds ratios (ORs) with 95% confidence intervals (CIs) and 2-sided *P* values were estimated by conditional logistic regression for matched case-control sets.²² The effect of smoking and infection with HPV-16, HPV-18, or HPV-33 was considered by adjusting for serum cotinine levels and serum antibodies to any of these HPV types. Test for trend was calculated by modeling the number of serotypes as 1 quantitative variable, assuming scores 0 to 4 for exposure to 0, 1, 2, 3, or 4 or more serotypes.

Table 1. Risk for Cervical Carcinoma Associated With the Presence of IgG Antibodies to *Chlamydia trachomatis* or *Chlamydia pneumoniae**

Antibodies	Cases, %			Controls, %			Crude OR			Adjusted OR SCC and Lagtime >12 mo (95% CI)†
	All (n = 181)	SCC (n = 150)	SCC and Lagtime >12 mo (n = 128)	All (n = 533)	SCC (n = 442)	SCC and Lagtime >12 mo (n = 377)	All	SCC	SCC and Lagtime >12 mo	
<i>C trachomatis</i>										
EB-ELISA IgG	66	68	68	55	52	50	1.9	2.4	2.7	2.1 (1.3-3.5)
Peptide-ELISA IgG	49	52	55	37	39	38	1.7	1.7	2.1	1.5 (0.9-2.3)
MIF (any serotype)	27	31	33	13	14	13	2.4	2.6	3.3	2.5 (1.4-4.3)
B	3.8	4.6	5.4	0.9	0.9	1.1	3.9	4.9	4.9	4.1 (1.0-18.0)
D	14	15	17	5.0	4.9	5.0	2.8	3.3	3.7	2.7 (1.3-5.6)
E	11	13	13	6.0	5.8	5.3	1.9	2.3	2.7	2.0 (0.9-4.3)
F	2.7	3.3	3.1	1.7	1.6	1.6	1.5	1.9	1.8	2.2 (0.5-9.4)
G	5.5	6.6	7.8	1.7	1.3	1.3	3.5	5.5	6.9	6.6 (1.6-27.0)
J	3.8	4.6	4.7	1.3	1.3	1.6	2.9	3.4	3.0	2.9 (0.9-9.5)
C	0.5	0.7	0.8	0.6	0.7	0.8	1.0	1.0	1.0	1.3 (0.1-13.0)
H	2.7	3.3	3.1	0.6	0.7	0.8	4.8	4.8	4.0	3.6 (0.8-17.0)
I	6.0	6.6	7.0	2.1	2.0	1.8	3.0	3.1	3.7	3.8 (1.3-11.0)
K	0	0	0	0.7	0.9	1.1	0	0	0	0
<i>C pneumoniae</i>										
MIF	54	53	50	50	51	52	1.2	1.1	0.9	0.9 (0.6-1.4)

*SCC indicates squamous cell carcinoma; lagtime, time between serum donation and diagnosis of SCC; OR, odds ratio; CI, confidence interval; EB, elementary body; ELISA, enzyme-linked immunosorbent assay; and MIF, microimmunofluorescence.

†Adjusted for serum antibodies to human papillomavirus types 16, 18, and 33 and for serum cotinine.

RESULTS

Of the 181 patients with invasive cervical carcinoma, 48 were from Finland, 129 from Norway, and 4 from Sweden. Of all carcinomas, 109 (60%) were localized, and 62 (34%) were metastatic. In 10 cases (6%) the stage was unknown. The mean age of the patients at diagnosis was 44 years (range, 23-64 years), and the mean time between serum donation and diagnosis was 56 months (range, 1-221 months). Of all patients, 150 had cervical SCC, of whom 128 had a time between serum donation and diagnosis (lag time) of at least 12 months (TABLE 1).

The overall prevalence rates of serum IgG antibodies to *C trachomatis* among all cases and controls were 27% and 13%, respectively (Table 1). The corresponding case and control rates for HPV-16, HPV-18, or HPV-33 were 37% and 18%, respectively, and for cotinine, 50% and 39%. Antibodies to *C trachomatis* were analyzed by 3 methods: MIF and 2 commercially available ELISAs (Table 1). In general, ELISAs were more sensitive than MIF. Among all invasive carcinomas (n=181), antibodies measured by the ELISAs yielded low point estimates for the risk of cervical carcinoma (EB ELISA:

OR, 1.5, 95% CI, 1.0-2.4; peptide ELISA: OR, 1.3, 95% CI, 0.9-1.9; both adjusted for HPV types 16, 18, and 33 and serum cotinine). Antibodies measured by MIF were associated with higher risk (adjusted OR, 1.8; 95% CI, 1.1-2.8). Serum IgG antibodies to *C pneumoniae* were not associated with cervical carcinoma (adjusted OR, 1.2; 95% CI, 0.8-1.7).

The highest point estimates were found for SCC diagnosed 12 or more months after serum donation. Within that group (n=128), serotype G was most strongly associated with SCC (adjusted OR, 6.6; 95% CI, 1.6-27.0) (Table 1). Other serotypes also associated with SCC were I (OR, 3.8; 95% CI, 1.3-11.0) and D (OR, 2.7; 95% CI, 1.3-5.6) (Table 1). In addition, there was a borderline association with serotype B (OR, 4.1; 95% CI, 1.0-18.0). Exposure to more than 1 serotype increased the risk for cervical SCC ($P<.001$ for trend) (TABLE 2).

Chlamydia trachomatis DNA was detected by polymerase chain reaction (PCR) in 4 (5%) of the 79 cases analyzed. One of the DNA-positive cases was antibody positive for multiple serotypes (D, E, F, H, I, J), whereas the other cases were *C trachomatis* seronegative.

COMMENT

To the best of our knowledge, this is the first study providing longitudinal seroepidemiologic evidence of an association between exposure to specific serotypes of *C trachomatis* and cervical SCC. Presence of serum IgG antibodies to *C trachomatis* serotype G was associated with the highest risk. Immunoglobulin G antibodies to more than 1 serotype of *C trachomatis* increased the risk for subsequent development of SCC.

Microimmunofluorescence is the method of choice for *C trachomatis* serotyping.¹² So far, 18 different serotypes (or serovars) have been described.^{11,23} Distribution of the genital serotypes varies from one area to another, suggesting that some serotypes have biological advantage over others in defined populations.²⁴ Serotypes D and E represent approximately 50% of all isolates, followed by F and G serotypes, which represent 15% to 40%; other serotypes represent less than 10% each.²⁵⁻²⁷ Serotypes E and G have been found more often in women than men, whereas serotype D has been found more frequently in men than in women.^{28,29}

In another seroepidemiologic study from Finland, antibodies to the GFK se-

Table 2. Risk for Cervical Carcinoma Associated With Exposure to 1 or More *Chlamydia trachomatis* Serotypes*

No. of Serotypes	Cases, %			Controls, %			Crude OR			Adjusted OR SCC and Lagtime >12 mo (95% CI)†
	All (n = 181)	SCC (n = 150)	SCC and Lagtime >12 mo (n = 128)	All (n = 533)	SCC (n = 442)	SCC and Lagtime >12 mo (n = 377)	All	SCC	SCC and Lagtime >12 mo	
0	78.7	76.2	73.5	92	92.1	92.3	1.0	1.0	1.0	1.0
1	8.8	9.3	10	3.2	3.6	3.2	3.2	3.0	3.9	2.2 (0.8-6.1)
2	6.0	6.6	7.8	2.2	2.5	2.4	3.6	3.7	5.1	6.0 (1.6-23.0)
3	2.7	3.3	3.9	0.6	0.4	0.5	4.5	6.7	6.7	4.2 (0.7-26.0)
≥4	3.8	4.6	4.8	2.0	1.4	1.6	2.7	3.7	4.0	4.2 (0.9-19.0)‡

*SCC indicates squamous cell carcinoma; OR, odds ratio; and CI, confidence interval.

†Adjusted for serum antibodies to human papillomavirus types 16, 18, and 33 and for serum cotinine.

‡ $P < .001$.

rototype pool were more common in women who developed SCC than in controls.⁶ Similarly, serotype G was the strongest risk factor for SCC in the present study. Serotype G has also been associated with symptomatic infections and upper genital tract infections.^{30,31} Serotype D was also associated with SCC in this study. When different serotypes of *C trachomatis* were inoculated intravaginally into mice, the duration of infection with D and E was longest and induced the highest antibody titers.³² Thus, specific *C trachomatis* serotypes might be more virulent, perhaps less sensitive to appropriate antimicrobial treatment,³³ and could play a role in carcinogenesis. By analogy, chronic inflammation associated with persistent infection by *Helicobacter pylori* strain CagA+ is a known risk factor for the development of gastric carcinoma and lymphoma.^{34,35}

Chlamydia trachomatis antibodies and multiple serotypes of *C trachomatis* have been detected in women with several sex partners and in women with upper genital tract infections.^{20,26,36,37} The presence of mixed infections implies that infection with one serotype does not induce protective immunity against subsequent infection caused by another serotype.³⁶ Multiple exposures might increase the risk of ultimately acquiring infections caused by the cancer-associated serotypes, serotype G in particular (in this study, all but 1 case with antibodies to ≥4 serotypes had antibodies to serotype G). On the other hand, mixed serotypes are not common in patients with recurrent *C trachomatis* infections. Broadly reactive antigens of

C trachomatis may simply result in humoral immune response against conserved cross-reactive epitopes.³¹ Therefore, antibodies to multiple serotypes discovered in patients with cervical SCC may also suggest chronic infection by a single serotype. On the basis of this study we cannot distinguish between these 2 possibilities.

Schlott et al³⁸ detected *C trachomatis* DNA by in situ PCR in 40% of cervical carcinoma tissue samples, but not in the carcinomatous cells.³⁸ We found only 5% of the biopsy specimens positive for *C trachomatis* DNA. The reason for this discrepancy is not known, but assay-specific methodological differences are a plausible explanation. Furthermore, the plasmid primers used for our PCR may not be optimal for the detection of chronic *C trachomatis* infection. The target cells for *C trachomatis* are endocervical glandular cells,³⁹ which might not be present in the diagnostic tumor biopsy specimen. Finally, our previous seroepidemiologic^{6,8} and longitudinal DNA studies (K.-L. Wallin, PhD, F. Wiklund, MSc, T. Luostarinen, MSc, et al, unpublished data, 2000) have indicated that exposure to *C trachomatis* takes place several years or even decades before the diagnosis of cervical SCC. It is possible that the few tissue specimens positive for *C trachomatis* DNA at the time of the cancer diagnosis only represent the minimum number of *C trachomatis* infections associated with cervical carcinoma.

Evidence based on longitudinal studies, such as the present study, is always stronger than that based on cross-sectional surveys in which selection bias

is difficult to rule out. Our study was nested in a population-based, well-defined cohort, suggesting that bias due to differential misclassification was also highly unlikely. Use of serum banks and cancer registries with almost 100% reporting coverage provides an ideal setting for etiologic studies with true cancer as the end point.

The development of SCC takes several years, probably decades. The link between bacterial infections and carcinogenesis is not clear, but genetic damage and neoplastic changes can be induced in vitro by coculturing cells with activated inflammatory cells.⁴⁰ Release of nitric oxide occurs in *C trachomatis* infections.⁴¹ Recent studies have also shown that *C trachomatis* inhibits host cell apoptosis by specific mechanisms.⁴² In chronic chlamydial infections, these mechanisms could initiate or promote cervical carcinogenesis. The serotype-specific differences, and the fact that the risk was higher in women exposed to more than 1 serotype, strengthen the evidence for the role of *C trachomatis* in cervical carcinogenesis. It is tempting to speculate on the potential molecular mechanisms explaining this association. Future studies should address the question of whether there are any specific determinants related to serotype G that may be directly or indirectly carcinogenic.

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REFERENCES

- IARC. *Human Papillomaviruses*. Lyon, France: International Agency for Research on Cancer; 1995. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 64.
- Koutsky L. Epidemiology of genital human papillomavirus infection. *Am J Med*. 1997;102:3-8.
- Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol*. 1999;189:12-19.
- Wallin K-L, Wiklund F, Ångström T, et al. Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. *N Engl J Med*. 1999;341:1633-1638.
- Josefsson AM, Magnusson PKE, Ylitalo N, et al. Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma in situ. *Lancet*. 2000;355:2189-2193.
- Lehtinen M, Dillner J, Knekt P, et al. Serologically diagnosed infection with human papillomavirus type 16 and risk for subsequent development of cervical carcinoma. *BMJ*. 1996;312:537-539.
- Dillner J, Lehtinen M, Bjørge T, et al. Prospective seroepidemiologic study of human papillomavirus infection as a risk factor for invasive cervical cancer. *J Natl Cancer Inst*. 1997;89:1293-1299.
- Koskela P, Anttila T, Bjørge T, et al. *Chlamydia trachomatis* infection as a risk for invasive cervical carcinoma. *Int J Cancer*. 2000;85:35-39.
- Beatty WL, Morrison RP, Byrne GI. Persistent *Chlamydiae*. *Microbiol Rev*. 1994;58:686-699.
- Puolakkainen M, Vesterinen E, Purola E, et al. Persistence of chlamydial antibodies after pelvic inflammatory disease. *J Clin Microbiol*. 1986;23:924-928.
- Wang SP, Grayston JT. Immunologic relationship between genital TRIC, lymphogranuloma venereum, and related organism in a new microtiter indirect immunofluorescence test. *Am J Ophthalmol*. 1970;70:367-374.
- Newhall WJ, Terho P, Wilde CE, et al. Serovar determination of *Chlamydia trachomatis* isolates by using type-specific monoclonal antibodies. *J Clin Microbiol*. 1986;23:333-338.
- Bardy AH, Seppälä T, Lillsunde P, et al. Objectively measured tobacco exposure during pregnancy. *Br J Obstet Gynaecol*. 1993;100:721-726.
- Jellum E, Andersen A, Lund-Larsen P, et al. Experiences of the Janus Serum Bank in Norway. *Environ Health Perspect*. 1995;103:85-88.
- Dillner J, Lenner P, Lehtinen M, et al. A population-based seroepidemiological study of cervical cancer. *Cancer Res*. 1994;54:134-141.
- Teppo L, Pukkala E, Lehtonen M. Data quality and quality control of a population-based cancer registry: experience in Finland. *Acta Oncol*. 1994;33:365-369.
- Närvänen A, Puolakkainen M, Hao W, Kino K, Suni J. Detection of antibodies to *Chlamydia trachomatis* with peptide based species specific EIA. *Infect Dis Obstet Gynecol*. 1997;5:349-354.
- Miyashita N, Matsumoto A. Establishment of a particle-counting method for purified elementary bodies of *Chlamydia* and evaluation of sensitivities of the IDEIA *Chlamydia* kit and DNA probe by using the purified elementary bodies. *J Clin Microbiol*. 1992;30:2911-2916.
- Lie AK, Schjeldstad FE, Hagen B, et al. Comparison of light microscopy, in situ hybridization and polymerase chain reaction for detection of human papillomavirus in histological tissue of cervical intraepithelial neoplasia. *APMIS*. 1997;105:115-120.
- Dillner J, Kallings I, Brihmer C, et al. Seropositivities to human papillomavirus types 16, 18, or 33 capsids and to *Chlamydia trachomatis* are markers of sexual behavior. *J Infect Dis*. 1996;173:1394-1398.
- Andersson-Ellström A, Dillner J, Hagmar B, et al. Comparison of development of serum antibodies to HPV16 and HPV33 and acquisition of cervical HPV DNA among sexually experienced and virginal young girls. *Sex Transm Dis*. 1996;23:234-238.
- Breslow N, Day N. *Statistical Methods in Cancer Research*. Lyon, France: International Agency for Research on Cancer; 1980. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 32.
- Wang SP, Grayston JT. Three new serovars of *Chlamydia trachomatis*: Da, Ia and L2a. *J Infect Dis*. 1990;163:403-405.
- Workowski KA, Suchland RJ, Pettinger MB, Stamm WE. Association of genital infection with specific *Chlamydia trachomatis* serovars and race. *J Infect Dis*. 1992;166:1445-1449.
- Kuo CC, Wang SP, Holmes KK, Grayston T. Immunotypes of *Chlamydia trachomatis* isolates in Seattle, Washington. *Infect Immun*. 1983;41:865-868.
- Saikku P, Wang SP. *Chlamydia trachomatis* immunotypes in Finland. *APMIS*. 1987;95:131-134.
- Moncan T, Eb F, Orfila J. Monoclonal antibodies in serovar determination of 53 *Chlamydia trachomatis* isolates from Amiens, France. *Res Microbiol*. 1990;141:695-701.
- Barnes RC, Rompalo AM, Stamm WE. Comparison of *Chlamydia trachomatis* serovars causing rectal and cervical infections. *J Infect Dis*. 1987;156:953-958.
- Poole E, Lamont I. *Chlamydia trachomatis* serovar differentiation by direct sequence of the variable segment 4 region of the major outer membrane protein gene. *Infect Immun*. 1992;60:1089-1094.
- Lan J, Melgers CJ, Walboomers JM, et al. Prevalence and serovar distribution of asymptomatic cervical *Chlamydia trachomatis* infections as determined by highly sensitive PCR. *J Clin Microbiol*. 1995;33:3194-3197.
- Dean D, Oudens E, Bolan G, et al. Major outer membrane protein variants of *Chlamydia trachomatis* are associated with severe upper genital tract infection and histopathology in San Francisco. *J Infect Dis*. 1995;172:1013-1022.
- Ito JIR, Lyons JM, Airo-Brown LP. Variation in virulence among oculo-genital serovars of *Chlamydia trachomatis* in experimental genital tract infection. *Infect Immun*. 1990;58:2021-2023.
- Dean D, Suchland R, Stamm W. Apparent long term persistence of *Chlamydia trachomatis* cervical infections: analysis by OMP1 genotyping. In: Stephens RS, ed. *Chlamydial Infections: Proceedings of the Ninth International Meeting on Human Chlamydial Infections*; 1998:39-42.
- Eck M, Schmausser B, Haas R, et al. MALT-type lymphoma of the stomach is associated with *Helicobacter pylori* strains expressing the CagA protein. *Gastroenterology*. 1997;112:1482-1486.
- Chow WH, Blaser M, Blot W, et al. An inverse relation between cagA strains of *Helicobacter pylori* infection and risk of esophageal and gastric cardia adenocarcinoma. *Cancer Res*. 1998;58:588-590.
- Barnes RC, Suchland RJ, Wang S-P, Kuo CC, Stamm WE. Detection of multiple serovars of *Chlamydia trachomatis* in genital infections. *J Infect Dis*. 1985;152:985-989.
- Lin JS, Donegan P, Heeren T, et al. Transmission of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* among men with urethritis and their female sex partners. *J Infect Dis*. 1998;178:1707-1712.
- Schlott T, Ruda G, Hoppert M, et al. The in situ polymerase chain reaction for detection of *Chlamydia trachomatis*. *J Histochem Cytochem*. 1998;46:1017-1023.
- Paavonen J, Stevens CE, Wölnner-Hanssen P, et al. Colposcopic manifestations of cervical and vaginal infections. *Obstet Gynecol Surv*. 1988;43:373-381.
- Rosin MP, Anwas WA, Ward AJ. Inflammation, chromosomal instability, and cancer. *Cancer Res*. 1994;54:1929-1933.
- Mayer JM, Woods ML, Vavrin Z, Hibbs JB. Gamma interferon-induced nitric oxide production reduces *Chlamydia trachomatis* infectivity in McCoy cells. *Infect Immun*. 1993;61:491-497.
- Fan B, Lu H, Hu H, et al. Inhibitor of apoptosis in *Chlamydia*-infected cells. *J Exp Med*. 1998;187:487-496.