Human Leukocyte Antigen Class II DQ Alleles Associated With *Chlamydia trachomatis* Tubal Infertility

CRAIG R. COHEN, MD, MPH, SAMUEL S. SINEI, MBChB, MMed, ELIZABETH A. BUKUSI, MBChB, MMed, JOB J. BWAYO, MBChB, PhD, KING K. HOLMES, MD, PhD, AND ROBERT C. BRUNHAM, MD

***Objective*: To investigate epidemiologic tubal infertility risk factors and the relationship between HLA class II alleles and *Chlamydia trachomatis* tubal infertility.**

***Methods*: Forty-seven women with tubal infertility and 46 fertile controls were studied in Nairobi, Kenya. A questionnaire was administered and serum collected for measurement of *C trachomatis* antibodies. HLA class II molecular typing was done with DNA extracted from peripheral blood lymphocytes. The prevalence of *C trachomatis* microimmunofluorescence antibody, chlamydia heat shock protein 60 antibody, and HLA class II alleles was compared among cases of tubal infertility and fertile controls.**

***Results*: Women with tubal infertility more often had histories of pelvic inflammatory disease (15% versus 0%; odds ratio [OR] 16; 95% confidence interval [CI] 5.5, 47) histories of spontaneous abortion (34% versus 7%; OR 6.7; 95% CI 2.8, 16), and antibodies to *C trachomatis* (53% versus 26%; OR 3.2; 95% CI 1.3, 7.7) than controls. Among infertile women, DQA*0101 and DQB*0501 alleles were positively associated with *C trachomatis* tubal infertility (OR 4.9; 95% CI 1.3, 18.6, and OR 6.8; 95% CI 1.6, 29.2, respectively). DQA*0102 was negatively associated with *C trachomatis* tubal infertility (OR 0.2; 95% CI 0.005, 0.6).**

***Conclusion*: *Chlamydia trachomatis* infection is an important cause of tubal infertility in Nairobi. The association of specific HLA class II alleles with *C trachomatis* microimmunofluorescence seropositivity among women with tubal infertility suggests that the DQ locus might modify susceptibility to and pathogenicity of *C trachomatis* infection. (Obstet Gynecol 2000;95:72–7. © 2000 by The American College of Obstetricians and Gynecologists.)**

Chlamydia trachomatis is the most common cause of pelvic inflammatory disease in the United States[1] and is an important cause of female infertility worldwide.[2,3] Our understanding of the immunopathologic pathway associated with genital *C trachomatis* infection and its sequelas remains incomplete. Studies in macaques showed that single episodes of *C trachomatis* salpingitis usually are self-limited, whereas repeated infection eventually produces severe tubal scarring, suggesting that acquired immune responses to chlamydial antigens are important in pathogenesis.[4] Human leukocyte antigen class II molecules, ie, DQ, DR, and DP, present peptides to CD4 T cells and restrict the range of cellular and antibody responses to antigens. In vitro studies of peripheral blood mononuclear cells from women with and without trachoma, an ocular infection due to *C trachomatis*, suggest that CD4 Th-2 cells and their cytokines affect the pathogenesis of trachomatous scarring and are associated with HLA-DQ regulation of Th1/Th2 response.[5] Other research suggested that genetic and immunologic risk factors are associated with the risk of *C trachomatis* salpingitis[6] and subsequent tubal damage.[7,8]

The development of tubal scarring resulting from *C trachomatis* initially depends on infection of the female lower genital tract. Once the lower genital tract is infected, the infection must ascend to cause salpingitis and tubal scarring.[9] We hypothesized that each step in the causal pathway from infection to tubal fibrosis might be associated with specific genetic and immuno-

From the Departments of Obstetrics and Gynecology and Medicine, University of Washington, Seattle, Washington; Departments of Obstetrics and Gynecology and Medical Microbiology, University of Nairobi, Nairobi, Kenya; Kenya Medical Research Institute, Nairobi, Kenya; and Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada.

Supported in part by grants from the National Institutes of Health (D43 TW0007, AI34616, and AI31448). CRC was a fellow in the International AIDS Research and Training Program supported by the Fogarty International Foundation, Grant T22 TW00001.

Linda Cles, Aggie Clark, and Dr. Walter Stamm performed the Chlamydia trachomatis *microimmunofluorescence and ligase chain reaction assays, and Dr. Lakshmi Gaur performed HLA class II typing.*

Table 1. Demographics in Patients and Controls by Antibody Serostatus

Variable	Tubal infertility		Tubal ligation	
	Microimmunofluorescence positive (n = 25)	Microimmunofluorescence negative (n = 22)	Microimmunofluorescence positive (n = 12)	Microimmunofluorescence negative (n = 34)
Age (mean y, SD)	27.8 (2.4)	28.5 (4.1)	34.3 (5.3)	33.8 (5.3)
Marital status				
Single	9 (36%)	6 (27%)	1 (8%)	4 (12%)
Married	15 (60%)	16 (73%)	11 (92%)	27 (79%)
Divorced or widowed	1 (4%)	0	0	3 (9%)
Lifetime number of sexual partners (median, range)	3 (2–16)	3 (1–9)*	2 (1–6)	2.0 (1–7)
Age of first intercourse (mean y, SD)	17.1 (1.7)	17.7 (3.1)	17.0 (3.0)	17.9 (2.8)
No. of prior pregnancies (median, range)	1 (0–2)	0 (0–6)	5.5 (4–10)	5.8 (2.3)
History of therapeutic abortion	1 (4%)	1 (5%)	0	1 (3%)
History of spontaneous abortion	7 (28%)	5 (23%)	1 (8%)	13 (38%)
History of IUD	1 (4%)	0	5 (42%)	14 (41%)
History of oral contraceptives	4 (16%)	1 (5%)	8 (67%)	20 (59%)
No. of mo attempting to conceive (mean, SD)	59.1 (29.2)	50.1 (26.8)	NA	NA
History of abdominal surgery	8 (32%)	7 (32%)	2 (17%)	6 (18%)
History of PID	5 (20%)	2 (9%)	0	0
History of antibiotics for abdominal pain	22 (88%)	16 (73%)	1 (8%)	6 (18%)
History of any STD	9 (36%)	12 (55%)	3 (25%)	4 (12%)
AFS score (mean, SD)	4.6 (1.3)	4.3 (1.6)	0.1 (0.3)	0.4 (0.7)
Postrepair conception rate	5 (20%)	6 (27%)	NA	NA
Duration to conception (median days, range)	180 (35–350)	168 (14–390)	NA	NA

SD = standard deviation; IUD = intrauterine device; NA = not applicable; PID = pelvic inflammatory disease; STD = sexually transmitted disease; AFS = American Fertility Society.
* $P < .05$.

logic risk factors. We studied women with tubal factor infertility in Nairobi, Kenya, to investigate epidemiologic and HLA class II genetic factors associated with altered susceptibility to *C trachomatis* infection and disease.

Materials and Methods

Between May 1995 and May 1996, we recruited women who presented to the gynecology clinic at Kenyatta National Hospital in Nairobi with histories of at least 12 months of infertility, regular menses, distal tubal occlusion on hysterosalpingogram, and sexual partners with normal semen analyses. Human immunodeficiency virus (HIV)-1 counseling and testing were done as part of infertility work-ups. Although HIV-1 testing was not mandatory, most accepted and those who were HIV-1 infected were not offered tubal surgery. For controls we recruited women at the same institution who were to have minilaparotomies for tubal ligation.

Informed consent was obtained from each participant. The Institutional Review Board of the University of Washington, and the Kenyatta National Hospital Ethical and Research Committee, Nairobi, Kenya, approved the protocol. After written informed consent was obtained, women completed questionnaires that included sexual, infertility, and sexually transmitted disease (STD) information. Subjects had laparoscopic tubal reconstructive surgery, whereas controls had minilaparotomy tubal ligation. Cervical, endometrial, and fallopian tube specimens were analyzed for *Neisseria gonorrhoeae* culture and *C trachomatis* ligase chain reaction. Blood was obtained for *C trachomatis* microimmunofluorescence serology, chlamydial heat shock protein serology, and molecular HLA class II genotyping.

At surgery, pelvic adhesive disease was staged ac-

Table 2. Microbiologic and Serologic Findings in Patients and Controls by Antibody Serostatus

Variable	Tubal infertility		Tubal ligation	
	Microimmunofluorescence positive (n = 25)	Microimmunofluorescence negative (n = 22)	Microimmunofluorescence positive (n = 12)	Microimmunofluorescence negative (n = 34)
Neisseria gonorrhoeae culture	0	0	0	0
Chlamydia trachomatis ligase chain reaction	1 (4)	1 (5)	0	0
C trachomatis microimmunofluorescence titer (median, range)	32 (16–256)	0 (0–8)	16 (16–128)	0 (0–8)
C trachomatis microimmunofluorescence antibody to				
B serogroup	5 (22%)	NA	2 (17%)	NA
C serogroup	7 (30%)	NA	5 (42%)	NA
Both serogroups	11 (48%)	NA	5 (42%)	NA
CHSP-60 antibody ≥0.2 optical density	11 (44%)	1 (5%)*	3 (25%)	6 (18%)

NA = not applicable.
* $P < .05$.

cording to a modified American Fertility Society adnexal adhesion classification system.[10] Postoperatively, women were discharged with doxycycline 100 mg twice daily for 1 week and observed to determine fertility outcome for up to 2 years after reconstructive surgery.

Neisseria gonorrhoeae was cultured using Thayer Martin media in 5% CO_2. *Chlamydia trachomatis* was detected (ligase chain reaction; Abbott, North Chicago, IL) and antibody against *C trachomatis* measured using the microimmunofluorescence assay of Wang et al.[11] Women with serovar-specified immunoglobulin IgM or IgG titer of at least 1:16 were considered to have a positive antibody response to *C trachomatis*. Sera were tested at 1:500 dilution for antibody to chlamydia heat shock protein-60 enzyme-linked immunosorbent assay (ELISA) and considered positive if absorbance was at least 0.2 optical density (OD) units.[7]

Deoxyribonucleic acid was isolated from peripheral blood leukocytes by an automatic extractor (Model 340 A; Applied Biosystems, Foster City, CA) for HLA class II typing done by reverse dot blot sequence–specific oligonucleotide method. The second exons of the DQA1, DQB1, and DRB1 were amplified by polymerase chain reaction (PCR)[12] and were labeled by incorporation of digoxigenin-labeled deoxyuridine triphosphate during the PCR reaction. Labeled PCR products were hybridized to allele-specific probes selected from the second exon of DQA1, DQB1, and DRB1, which were immobilized on nylon membranes. Positive reactions were visualized by color precipitation reaction.

Data were analyzed using SPSS for Windows (SPSS Inc., Chicago, IL). Univariate analyses used χ^2 and Fisher exact tests for comparison of categorical data, and Mann-Whitney and Student t tests for continuous variables. In the presence of zero values, Haldane's modification of Woolf's formula was used to calculate the odds ratio (OR) and 95% confidence interval (CI).[13]

Results

Among 47 women with tubal factor infertility and 46 who had tubal ligation, 25 (53%) and 12 (26%), respectively, were antibody positive for *C trachomatis* (OR 3.2; 95% CI 1.3, 7.7). No IgM antibodies were detected. Table 1 presents selected demographic, sexual, and STD data among chlamydia-seronegative and seropositive cases and controls. Overall, cases with tubal infertility were younger (28.1 versus 33.9 years; $P < .001$), had a greater median lifetime number of sexual partners (3 versus 2; $P < .001$), and more commonly had histories of STDs (45 versus 15%; OR 4.5; 95% CI 1.7, 12.1). Although only 15% of infertile women recalled histories of pelvic inflammatory disease (PID), no fertile control reported PID (OR 16; 95% CI 5.5, 47). Thirty-eight infertile women (81%) received antibiotics for undefined abdominal pain compared with seven fertile controls (15%) (OR 22.3; 95% CI 7.7, 64.4). The median number of pregnancies was greater among fertile women. Forty percent of infertile cases had never conceived, whereas 42% had secondary infertility with single pregnancies and 18% had multiple pregnancies. Of the 41 pregnancies reported among infertile cases, 14 (34%) ended in spontaneous abortions compared with 19 of 265 pregnancies (7%) among fertile controls (OR 6.7; 95% CI 2.8, 16).

Chlamydia trachomatis-seropositive and seronegative women with tubal factor infertility were not different in mean age (27.8 versus 28.5 years, respectively; $P = .51$).

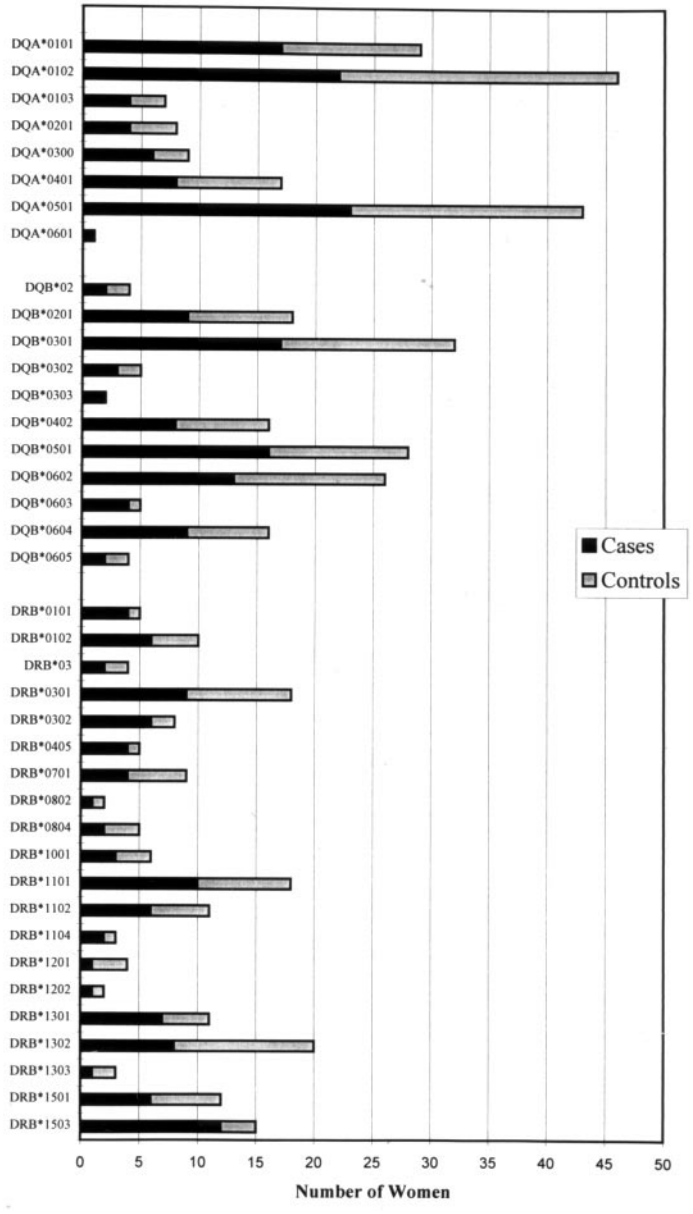

Figure 1. Frequency of human leukocyte antigen class II alleles among infertile women and fertile controls.

A history of PID was more common among seropositive women (OR 2.5; 95% CI 0.4, 14.4), which was consistent with their greater lifetime number of sexual partners than among seronegative women (Table 1). Severity of pelvic scarring, measured by adnexal adhesion classification system, did not differ significantly by serostatus. Twenty-two percent of women conceived after tubal surgery, and the pregnancy rate (OR 0.7; 95% CI 0.2, 2.6) and median number of days from surgery to conception did not differ significantly between *C trachomatis*–seropositive and seronegative infertile women (Table 1).

Two women with tubal infertility had *C trachomatis* detected, but *N gonorrhoeae* was not isolated from any patient or control (Table 2). Although *C trachomatis* antibody was more common among infertile women than fertile controls, the median *C trachomatis* antibody titer among seropositive women was similar between groups ($P = .51$), as was the distribution of antibody to different *C trachomatis* serogroups ($P = .80$). Although not statistically significant, chlamydia heat shock protein-60 antibody was more common among chlamydia-seropositive women with tubal infertility than chlamydia-seropositive controls (OR 2.4; 95% CI 0.4, 14.5). Seven *C trachomatis*–seronegative women (11%) also had chlamydia heat shock protein-60 antibody.

Overall, 20 different HLA DRB1, eight different

Table 3. Selected Human Leukocyte Antigen Class II Alleles in Patients and Controls by Antibody Serostatus

Allele	Tubal infertility		Tubal ligation	
	Microimmunofluorescence positive (n = 25)	Microimmunofluorescence negative (n = 22)	Microimmunofluorescence positive (n = 12)	Microimmunofluorescence negative (n = 34)
DQA*0101	13 (52%)	4 (18%)	3 (25%)	9 (27%)
	$P < .02$, OR 4.9	(95% CI 1.3, 18.6)	$P = .92$, OR 0.9	(95% CI 0.2, 4.2)
DQA*0102	7 (28%)	15 (68%)	5 (42%)	19 (56%)
	$P < .01$, OR 0.2	(95% CI 0.005, 0.6)	$P = .4$, OR 0.6	(95% CI 0.2, 2.1)
DQB*0501	13 (52%)	3 (14%)	9 (27%)	4 (33%)
	$P < .01$, OR 6.8	(95% CI 1.6, 29.2)	$P = .65$, OR 1.4	(95% CI 0.3, 5.8)

OR = odds ratio; CI = confidence interval.

DQA1, and 11 different DQB1 alleles were identified among the 93 genotyped women. Figure 1 depicts the aggregated frequency of HLA DR and DQ loci in women with tubal infertility and fertile controls. Among women with tubal infertility, DQA*0101 and DQB*0501 were positively correlated, and DQA*0102 was negatively correlated with *C trachomatis* seropositivity (Table 3). Thus, 52% of chlamydia-seropositive women with tubal infertility had DQA*0101 compared with 18% of seronegative women (OR 4.9; 95% CI 1.3, 18.6). Fifty-two percent of chlamydia antibody–positive infertile women had DQB*0501 compared with 14% of antibody-negative women (OR 6.8; 95% CI 1.6, 29.2). Twenty-eight percent of chlamydia antibody–positive infertile women had DQA*0102 compared with 68% of seronegative women (OR 0.2; 95% CI 0.005, 0.6). None of the HLA DQ alleles was significantly associated with *C trachomatis* serostatus among controls. No DR allele was significantly associated with *C trachomatis* serostatus among patients or controls (data not shown).

Discussion

This study showed that *C trachomatis* is a significant cause of tubal infertility in Nairobi, Kenya. Based on the prevalence of *C trachomatis* antibodies among infertile women and fertile controls, we estimated that the etiologic fraction of tubal infertility attributable to *C trachomatis* infection in Nairobi was approximately 36%.[14] Whether chlamydia antibody titer diminishes over time is not known. Therefore, we do not know whether the lower prevalence of chlamydia antibodies measured in controls might in part indicate greater mean age. Women with tubal infertility differed from women who had tubal ligation in risk for sexually transmitted infection, in that they reported more lifetime sexual partners and more often reported histories of prior STDs. That was especially true among women with tubal infertility associated with *C trachomatis* antibody who reported more lifetime sexual partners than women with tubal infertility not associated with seropositivity. A history of prior PID was more commonly reported by women with tubal infertility than by fertile controls, but was noticeably uncommon overall. Eighty percent of women with tubal infertility reported receiving antibiotics for a lower abdominal pain syndrome, suggesting that they might have had atypical rather than silent salpingitis before tubal infertility.[1]

More frequent histories of spontaneous abortions reported by infertile women were also consistent with atypical upper genital tract infection. Other investigators found that *C trachomatis* seropositivity and history of PID were associated with an increased risk of early pregnancy loss.[15,16] Thus, it is possible that clinically significant endometritis preceded tubal damage in that group of women, and could explain the increased rate of spontaneous abortions among infertile women. Genetic factors including HLA phenotype also might predispose women to reproductive failure unrelated to *C trachomatis* infection.[17]

Chlamydia trachomatis antibodies were important risk factors for tubal infertility in this study. Detection of active infection using sensitive nucleic acid detection amplification technique showed that most women did not have detectable organisms. Past treatment with antimicrobials might have contributed to low prevalence of microbiologically detectable infection.

In prior studies in Nairobi, *C trachomatis* PID was associated with repeated *C trachomatis* infection, absence of steroid contraceptive use, antibody to chlamydia heat shock protein-60, HLA-A31, and CD4 T cell count under 400 cells/μL among HIV-1–infected women.[6,7,18] Human class I correlation of disease response to *C trachomatis* infection was recently documented in macaque fallopian tube infection models, and in humans with ocular trachomatous scarring in The Gambia.[8,19] Our data suggest that susceptibility to *C trachomatis*–induced tubal fibrosis might be HLA class II–associated. The association of DQA*0101, DQA*0102, and DQB*0501 with *C trachomatis* microimmunofluorescence antibody was restricted to women with tubal factor infertility, suggesting that those HLA class II

alleles might be necessary, but not sufficient risk factors for *C trachomatis* tubal scarring. DQA*0101 and DQA*0102 had opposite associations with *C trachomatis* tubal infertility (OR 4.9 versus 0.2, respectively). Those alleles are similar except for a change at codon 34 from GAG to CAG, causing a glutamic acid to glutamine substitution,[20] which might influence their peptide binding repertoire. We hypothesize that among women with *C trachomatis* tubal infections, those with the DQ alleles might present different chlamydia or host-derived peptides that evoke damaging, protective, or regulatory immune responses by CD4 T lymphocytes. Alternatively, the DQ alleles might be in linkage disequilibrium with an unstudied gene that caused increased risk of *C trachomatis* infection and disease.

Our findings suggest possible major histocompatibility complex class II restriction of susceptibility to *C trachomatis* tubal infertility. Further epidemiologic, animal, and in vitro studies will be required to expand our results and provide additional insight into the immunopathologic mechanisms associated with *C trachomatis* tubal disease.

References

1. Westrom L, Eschenbach D. Pelvic inflammatory disease. In: Holmes KK, Sparling PF, Mardh P-A, Lemon SM, Stamm WE, Piot P, et al, eds. Sexually transmitted diseases. 3rd ed. New York: McGraw-Hill, 1999:783–809.
2. De Muylder X, Laga M, Tennstedt C, Van Dyck E, Aelbers GN, Piot P. The role of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in pelvic inflammatory disease and its sequelae in Zimbabwe. J Infect Dis 1990;162:501–5.
3. Ville Y, Leruez M, Glowaczower E, Robertson JN, Ward ME. The role of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in the etiology of ectopic pregnancy in Gabon. Br J Obstet Gynaecol 1991;98:1260–6.
4. Patton DL, Kuo CC, Wang SP, Brenner RM, Sternfeld MD, Morse SA, et al. Chlamydial infection of subcutaneous fimbrial transplants in cynomolgus and rhesus monkeys. J Infect Dis 1987;155:229–35.
5. Holland MJ, Bailey RL, Conway DJ, Culley F, Miranpuri G, Byrne GI, et al. T helper type-1 (Th1)/Th2 profiles of peripheral blood mononuclear cells (PBMC): Responses to antigens of *Chlamydia trachomatis* in subjects with severe trachomatous scarring. Clin Exp Immunol 1996;105:429–35.
6. Kimani J, Maclean IW, Bwayo JJ, MacDonald K, Oyugi J, Maitha GM, et al. Risk factors for *Chlamydia trachomatis* pelvic inflammatory disease among sex workers in Nairobi, Kenya. J Infect Dis 1996;173:1437–44.
7. Toye B, Laferriere C, Claman P, Jessamine P, Peeling R. Association between antibody to the chlamydial heat shock protein and tubal infertility. J Infect Dis 1993;168:1236–40.
8. Lichtenwalner AB, Patton DL, Cosgrove Sweeney YT, Gaur LK, Stamm WE. Evidence of genetic susceptibility to *Chlamydia trachomatis*-induced pelvic inflammatory disease in the pig-tailed macaque. Infect Immun 1997;65:2250–3.
9. Washington AE, Aral SO, Wolner-Hanssen P, Grimes DA, Holmes KK. Assessing risk for pelvic inflammatory disease and its sequelae. JAMA 1991;266:2581–6.
10. The American Fertility Society classifications of adnexal adhesions, distal tubal occlusion, tubal occlusion secondary to tubal ligation, tubal pregnancies, mullerian anomalies and intrauterine adhesions. Fertil Steril 1988;49:944–55.
11. Wang S, Grayston JT. Human serology in *Chlamydia trachomatis* infection with microimmunofluorescence. J Infect Dis 1974;130:388–97.
12. Bugawan TL, Erlich HA. Rapid typing of HLA-DQB1 DNA polymorphism using nonradioactive oligonucleotide probes and amplified DNA. Immunogenetics 1991;33:163–70.
13. Woolf B. On estimating the relation between blood group and disease. Ann Hum Genet 1955;19:241–3.
14. Denman DW III, Schlesselman JJ. Interval estimation of the attributable risk for multiple exposure levels in case-control studies. Biometrics 1983;39:185–92.
15. Quinn PA, Petric M, Barkin M, Butany J, Derzko C, Gysler M, et al. Prevalence of antibody to *Chlamydia trachomatis* in spontaneous abortion and infertility. Am J Obstet Gynecol 1987;156:291–6.
16. Heisterberg L. Factors influencing spontaneous abortion, dyspareunia, dysmenorrhea, and pelvic pain. Obstet Gynecol 1993;81:594–7.
17. Hataya I, Takakuwa K, Tanaka K. Human leukocyted antigen class II genotype in patients with recurrent fetal miscarriage who are positive for anticardiolipin antibody. Fertil Steril 1998;70:919–23.
18. Westrom L, Joesoef R, Reynolds G, Hagdu A, Thompson SE. Pelvic inflammatory disease and infertility: A cohort of 1,844 women with laparoscopically verified disease and 657 control women with normal laparoscopic findings. Sex Transm Dis 1992;19:185–92.
19. Conway DJ, Holland MJ, Campbell AE, Bailey RL, Krausa P, Peeling RW, et al. HLA Class I and II polymorphisms and trachomatous scarring in a *Chlamydia trachomatis*–endemic population. J Infect Dis 1996;174:643–6.
20. Bodmer JG, Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Charron D, et al. Nomenclature for factors of the HLA system. Tissue Antigen 1997;49:297–322.

Address reprint requests to:
Craig R. Cohen, MD, MPH
Department of Obstetrics and Gynecology
University of Washington at Seattle
Box 356460
Seattle, WA 98104-6460
E-mail: crcohen@u.washington.edu

Received May 21, 1999.
Received in revised form July 27, 1999.
Accepted August 19, 1999.