Cross-Reactivity between *Chlamydia trachomatis* Heat Shock Protein 10 and Early Pregnancy Factor

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*Chlamydia trachomatis* heat shock protein 10 (Chsp10) is associated with chronic genital tract infection with *C. trachomatis*. Chsp10 is homologous to human chaperonin 10 (Cpn10) and early pregnancy factor (EPF), a form of human Cpn10 that is specifically secreted at the start of pregnancy. We investigated cross-reactions between serum anti-Chsp10 antibodies and anti-EPF antibodies in pregnant and nonpregnant patients. Pregnancy was found to be associated with the presence of anti-EPF antibodies, which are specifically induced in pregnant women with a history of *C. trachomatis* infection, and with the presence of serum anti-Chsp10 antibodies. We also found that infertility was associated with the presence of anti-Chsp10 and anti-EPF antibodies. The HLA class II haplotype DR8 DQ4 was associated with the presence of anti-Chsp10 antibodies but not of anti-EPF antibodies.

Heat shock proteins (Hsps) are a highly conserved group of polypeptides that are produced under a variety of stress conditions to preserve cellular functions. The major Hsps are molecular chaperones that direct the folding and assembly of polypeptides in the cell (11). Early pregnancy factor (EPF) has been identified as a homologue of chaperonin 10 (Cpn10). It belongs to the heat shock protein family, but unlike other members of this family, EPF is detected outside the cell. EPF has immunosuppressant (17) and growth factor properties (19), rendering it essential for the growth and survival of the embryo during the pre- and postimplantation periods (2, 3). The passive immunization of pregnant mice with anti-EPF antibodies leads to the retardation of embryonic development and/or inhibition of implantation.

The partial amino acid sequences obtained for platelet-derived EPF are 100% identical to the corresponding sequences of human mitochondrial Cpn10 (8, 14) and 33.3% identical to those of the *Chlamydia trachomatis* chlamydial Hsp 10 (Chsp10) (12) (Fig. 1). The 10-kDa Hsp of *C. trachomatis* has been shown to be associated with chronic infection and sequelae, such as ectopic pregnancy (4). Hsp synthesis is induced in microbial pathogens during infection (6). Microbial Hsps are highly immunogenic and display a high level of conservation through evolution; in addition, immune cells have been shown to recognize both microbial and self-Hsp (22). Therefore, cross-reactions may occur between *C. trachomatis* Chsp10 and human extracellular EPF. Such cross-reactions might interfere with EPF function at the start of pregnancy, thereby playing a role in *C. trachomatis*-associated female infertility.

We studied a population of 716 women in the first trimester of pregnancy. We used a *C. trachomatis*-specific molecular biology detection technique and investigated *C. trachomatis* species-specific and Chsp10-specific serum antibody responses. We tested 230 of the patients for immunoglobulin G (IgG) antibodies specifically recognizing two EPF-derived synthetic peptides. As EPF is essential for embryo implantation and survival, we also investigated a population of 210 women consulting for infertility. These women were tested with a *C. trachomatis*-specific molecular biology detection technique and serological assays for *C. trachomatis* species-specific, Chsp10-specific, and EPF-derived peptide-specific antibody responses. A third population of nonpregnant patients attending a sexually transmitted disease (STD) center was studied to enable us to investigate a possible association between pregnancy and anti-EPF antibody detection.

An association has already been shown between production of anti-*C. trachomatis* Chsp60 antibodies and HLA DQ4 alleles in *C. trachomatis*-infected women (9). We investigated associations between production of anti-Chsp10 antibodies and HLA DRB1 DQBI haplotypes in 81 *C. trachomatis* infected women.

MATERIALS AND METHODS

**Study population.** (i) Pregnant population. A total of 716 women (median age, 24 years [range, 16 to 38 years]) attending the Centre de Gynécologie Obstétrique, Amiens, France, during the first trimester of pregnancy were enrolled for screening for *C. trachomatis* infection. Urine samples were tested by the direct detection transcription-mediated amplification assay (AmpCT; GenProbe) for the presence of *C. trachomatis* rRNA. Serum samples, taken on the same day as the urine samples, were tested by enzyme-linked immunosorbent assay (ELISA) for the presence of anti-Chsp10 IgG antibodies. All patients testing positive for anti-Chsp10 antibodies (n = 82) and 129 randomly selected patients were screened for *C. trachomatis*-specific IgG antibodies and for EPF-specific IgG antibodies. All AmpCT-positive patients (n = 26) were also screened for EPF-
FIG. 1. Deduced amino acid sequences of the 10-kDa Hsp from *Chlamydia trachomatis* (GenBank accession number CTU02555) and human HSP (GenBank accession number Q09UDH1). The sequences corresponding to pEPF1 and pEPF2 are underlined.

specific IgG antibodies. The rates of previous pregnancies and previous histories of STDs among the patients were 34.9 and 4.1%, respectively.

(ii) STD population. A total of 1,103 male and female patients attending the Center for Sexually Transmitted Diseases, Amiens, France, were screened for *C. trachomatis* infections by the AmpCT assay and for the presence of anti-Chsp10 IgG antibodies. Serum samples were tested for *C. trachomatis*-specific antibodies. Levels of EPF-specific antibodies were assessed in 87 patients with a clearly defined *C. trachomatis* status. The rates of previous pregnancies and previous histories of STDs were 55.3 and 3.5%, respectively. None of these patients had detectable β-human choriogonadotrophin in serum (FirstSign; ServiBio, Paris, France).

(iii) Infertile population. A population of 210 women (median age, 26 years [range, 22 to 40 years]) consulting for infertility at the Alfredo Da Costa Maternity Unit and Santa Maria Hospital, Lisbon, Portugal, was included in the study. All 210 patients were screened for tubal diseases by laparoscopy. The contents of the fallopian tubes and cul-de-sac fluids were collected from 176 patients with a spinal needle for laboratory diagnosis of *C. trachomatis* infection. It was not possible to obtain tubal specimens from the remaining 34 patients due to surgical difficulties. All tubal samples were tested with the Amplicor PCR detection assay for the presence of *C. trachomatis* DNA. All serum samples were tested for the presence of specific anti-Chsp10, anti-*C. trachomatis*, and anti-EPF IgG antibodies. The rates of previous pregnancies and previous histories of STDs were 0 and 4.8%, respectively.

Informed consent was obtained from all patients. Azithromycin treatment was prescribed to all acutely infected patients.

**AmpCT assay.** Urine samples were centrifuged and processed for *C. trachomatis*-specific RNA detection as recommended by the manufacturer (Bio-Mérieux-GenProbe). The AmpCT assay is based on the amplification of an RNA target sequence via DNA intermediates. The amplified RNA sequence is specifically detected with a chemiluminescent single-stranded DNA probe complementary to the ampiclon. Labeled RNA-DNA hybrids were quantified in a luminometer.

**PCR-Amplicor assay.** The amplification by PCR of *C. trachomatis* DNA and detection of the ampiclon were carried out according to the manufacturer’s instructions but with slight modifications from the previously described protocol (7). Briefly, tubal aspirates were centrifuged, and the cell pellet was added to 1 ml of 2-SP transport medium. We then added 100 µl of the sample in 2-SP medium to 100 µl of CT or NG lysing buffer (Amplicor; Roche), vortexed the mixture for 1 min, and incubated the mixture at room temperature for 10 min. We added 200 µl of CT or NG specimen diluent (Amplicor) to each tube, vortexed the mixture, and incubated the mixture at room temperature for 15 min. The samples were subjected to an initial cycle of 50°C for 2 min and 95°C for 5 min and then to 35 cycles of denaturation at 91°C, annealing at 62°C, and extension at 72°C in a Perkin-Elmer 2400 thermocycler. At the end of the amplification process, the samples were maintained at 72°C while denaturation solution was added (Amplicor). The denatured amplicons were detected by oligonucleotide capture hybridization in a microtiter plate with colorimetric detection, based on an avidin horseradish peroxidase system. Absorbance was read at an automated microwell reader at 405 nm. The Amplicor internal control detection kit was used to identify specimens containing substances that inhibited the PCR. The internal control test was performed according to the manufacturer’s instructions. Specimens displaying PCR inhibition were subjected to a second round of amplification and detection, and none remained inhibited.

**Chsp10 ELISA.** Recombinant Chsp10 was produced as a 6-His-tagged molecule and purified by nickel-chelate affinity chromatography (12). Microtiter plates were coated with 3 µg of recombinant Chsp10/ml and saturated with 5% skim milk powder in phosphate-buffered saline (PBS). Aliquots of sera diluted 1:80 to 1:2,000 were incubated at 37°C for 20 min. The plates were incubated at 37°C for 40 min, washed with PBS, and then to 35 cycles of denaturation at 91°C for 2 min and 95°C for 2 min and then to 35 cycles of denaturation at 91°C, annealing at 62°C, and extension at 72°C in a Perkin-Elmer 2400 thermocycler. At the end of the amplification process, the samples were maintained at 72°C while denaturation solution was added (Amplicor). The denatured amplicons were detected by oligonucleotide capture hybridization in a microtiter plate with colorimetric detection, based on an avidin horseradish peroxidase system. Absorbance was read at an automated microwell reader at 405 nm. The Amplicor internal control detection kit was used to identify specimens containing substances that inhibited the PCR. The internal control test was performed according to the manufacturer’s instructions. Specimens displaying PCR inhibition were subjected to a second round of amplification and detection, and none remained inhibited.

**EPF peptide ELISA.** Two N-biotinylated peptides, pEPF1 and pEPF2, were synthesized by Neosystem, Strasbourg, France. The first, pEPF1, was a 27-mer corresponding to the central region (amino acids 27 to 53) of EPF (biotin-KG1MLPEKSGKVLGATVYAVGYSK). The second, pEPF2, was a 24-mer corresponding to the C-terminal region (amino acids 73 to 96) of EPF (biotin-PYEGGTKVLKDSDKFLRDGDILKGVYD). The wells were washed three times, and an alkaline phosphatase substrate, para-nitrophenylphosphate, was added. The plates were incubated at room temperature for 60 min, the reaction was stopped by the addition of 3 M NaOH, and absorbance was read at 405 nm. 

Samples were classified as positive if the absorbance value obtained was at least 2 standard deviations above the mean obtained with a panel of 30 microimmunofluorescence-negative samples. Thus, the threshold for positive values was an absorbance value at 405 nm of ≥0.12. Index values were calculated as A405/0.12.

**Serochlamydia peptide C. trachomatis ELISA.** This commercial ELISA technique, based on a major outer membrane protein-derived synthetic peptide that is specific to *C. trachomatis*, was performed according to the manufacturer’s instructions (ServiBio). Serum samples were classified as IgG-positive if the A405 value obtained was higher than the negative control A405 + 0.21.

**EPF peptide ELISA.** Two N-biotinylated peptides, pEPF1 and pEPF2, were synthesized by Neosystem, Strasbourg, France. The first, pEPF1, was a 27-mer corresponding to the central region (amino acids 27 to 53) of EPF (biotin-KG1MLPEKSGKVLGATVYAVGYSK). The second, pEPF2, was a 24-mer corresponding to the C-terminal region (amino acids 73 to 96) of EPF (biotin-PYEGGTKVLKDSDKFLRDGDILKGVYD). The wells were washed three times, and an alkaline phosphatase substrate, para-nitrophenylphosphate, was added. The plates were incubated at room temperature for 15 min. The reaction was stopped by adding 3 M NaOH, and absorbance was read at 405 nm. For each peptide, a sample was classified as positive if its absorbance value was at least double the mean value of the two negative controls (two anti-C. trachomatis-negative and anti-Chsp10-negative sera).

**Inhibition ELISA.** Samples of sera from patients in which anti-Chsp10 and anti-pEPF1 or two IgG antibodies were detected were incubated overnight at 4°C in test tubes with 0.76, 7.6, and 76 µg of pEPF1 or pEPF2/ml at a dilution of 1/80. These concentrations correspond to EPF peptide/Chsp10 protein molar ratios of 1, 10, and 102, respectively. We dispersed 50 µl of the mixture from each tube on the precoated Chsp10 plates. A sample without inhibitor was used to estimate 100% binding. Inhibition coefficients (I.C.) were calculated from the final absorbance values of the test samples by using the formula I.C.(%) = (A0 − A1)/A0 × 100%, in which A0 is the absorption of serum solution without inhibitor and A1 is the absorption of serum solution with peptide inhibitor at a concentration of X. Inhibition curves were constructed for several serum samples (data not shown). Inhibition was considered significant if the I.C. was 50% or more.

**DNA extraction.** Whole-blood samples from 88 patients with detectable serum anti- *C. trachomatis* antibodies were used for DNA extraction. Polymorphonuclear cells were isolated on a Ficoll-Paque gradient, and 106 cells were used for DNA extraction with the High Pure PCR template preparation kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the protocol for the isolation of nucleic acids from cultured cells.

**Partial HLA molecular typing.** Class II locus DRB1 and DQB1 typing was performed with specific sequencing primers according to the Olerup SSP HLA DR DQ low-resolution technique (18). Amplicons were visualized after electrophoresis by ethidium bromide staining of the agarose gel.

**Statistical analysis.** The significance of differences between groups of data was assessed by the χ2 test with Yates’s correction for small numbers and by Student’s t-test. In each group, the distribution of major histocompatibility complex (MHC) genotypes was checked for deviations from Hardy-Weinberg equilibrium by using an exact test (10), and associations between HLA haplotypes and anti-Chsp10 or anti-EPF antibody responses were tested by χ2 analysis. The 95% confidence intervals were calculated.

**RESULTS**

**Humoral immune response to Chsp10.** The prevalence of acute or past *C. trachomatis* infection and of serum anti-Chsp10 and anti-EPF antibodies is summarized in Table 1. The AmpCT direct detection assay gave positive results for
26 patients in the population of pregnant women. Therefore, 3.6% (confidence interval, 2.2 to 5%) of the population studied had acute *C. trachomatis* infection of the genital tract. Anti-Chsp10 antibodies were detected in 26.9% (7 of 26) of the patients with acute *C. trachomatis* infection. Similarly, one-third (30.3% [10 of 33]) of STD patients with acute *C. trachomatis* infection tested positive for antibodies against Chsp10. None of the infertile patients gave a positive result in the Amplicor detection assay. The total seroprevalence of anti-Chsp10 antibodies was 11.4% (82 of 716) in the population of pregnant women, 14.7% (162 of 1,103) in the STD patient population, and 59% (124 of 210) in the infertile population. The prevalence of anti-Chsp10 antibodies was therefore significantly higher (*P* < 10⁻⁵) in the infertile population than in the other two populations tested.

**Humoral immune response to EPF peptides.** The prevalence of antibodies recognizing pEPF1 was 2.6% (6 of 230) and that of antibodies recognizing pEPF2 was 7% (16 of 230) in the pregnant population. For the infertile population, the prevalences of these antibodies were 2.4 (5 of 210) and 4.3% (9 of 210), respectively, and for the nonpregnant STD patient population, the prevalence of these two types of antibody was 1.1% (1 of 87) (Table 2). The prevalence of antibodies against pEPF2 was significantly higher (*P* < 0.01) in patients who had been infected with *C. trachomatis* (AmpCT-positive and/or Serochlamydia peptide *C. trachomatis*-positive) who also had detectable anti-Chsp10 antibodies. In women falling into this category, we detected anti-pEPF1 and anti-pEPF2 antibodies in 11.5 (3 of 26) and 26.9% (7 of 26), respectively, of the pregnant cases, and anti-pEPF2 antibodies were detected in 23.8% (5 of 21) of the infertile cases (Table 2).

Significantly lower frequencies of anti-pEPF2 antibody detection were observed in patients who had been infected with *C. trachomatis* (AmpCT-positive and/or Serochlamydia peptide *C. trachomatis*-positive) but who did not have detectable anti-Chsp10 antibodies and in women without species-specific evidence of acute or past *C. trachomatis* infection (AmpCT-negative and Serochlamydia peptide *C. trachomatis*-negative) (Table 2). Therefore, the presence of anti-EPF peptides antibodies is specifically associated with *C. trachomatis* infection and the concomitant presence of anti-Chsp10 antibodies.

The frequency of anti-EPF antibodies appeared to be similar for infertile and for pregnant patients. However, comparison between pregnant and nonpregnant STD patients revealed that there was a significant association between pregnancy and the detection of anti-EPF antibodies (*P* < 0.02) (2.8 versus 26.9%) (Table 2). Comparisons of nonpregnant STD patients and infertile patients also showed that the presence of anti-EPF antibodies was significantly (*P* < 0.02) associated with infertility, although less strongly than the presence of anti-Chsp10 antibodies (*P* < 10⁻⁵).

**Cross-reactivity between Chsp10 and EPF peptides.** Inhibition ELISA was used to confirm the cross-reactivity of the pEPF1 and pEPF2 peptides with *C. trachomatis* Chsp10. A total of 18 serum samples with detectable antibodies against both Chsp10 and pEPF1 or -2 were tested by inhibition ELISA. Significant inhibition, indicating specific cross-reactivity, was observed in 17 (94%) serum samples with pEPF2, but no such

### Table 1. Prevalence of acute or past *C. trachomatis* infections and of serum anti-Chsp10 and anti-EPF antibodies in pregnant, nonpregnant, and infertile patients

| Patient group | AmpCT and/or Serochlamydia peptide *C. trachomatis* | anti-Chsp10 | anti-pEPF1 | anti-pEPF2 |
|---------------|-----------------------------------------------------|-------------|------------|------------|
| Pregnant (n = 716) | 8.5 (11/129) | 11.4 (82/716) | 2.6 (6/230) | 7 (16/230) |
| Infertile (n = 210) | 16.7 (35/210) | 59 (124/210) | 2.4 (5/210) | 4.3 (9/210) |
| Nonpregnant (n = 1,103) | 7.7 (85/1103) | 14.7 (162/1103) | 1.1 (1/87) | 1.1 (1/87) |

* Values are percent (no. positive/total no. tested) patients positive by the indicated test or for the indicated protein or peptide. *P* values: AmpCT- and/or Serochlamydia pep *C. trachomatis*-positive, *P* < 10⁻⁵; anti-Chsp10-positive, *P* < 10⁻⁵; anti-pEPF1-positive, *P* > 0.5; anti-pEPF2-positive, *P* > 0.2.

### Table 2. Prevalence of anti-EPF antibodies according to *C. trachomatis* infection status and anti-Chsp10 antibody status in pregnant, nonpregnant STD, and infertile patients

| Population group | EPF-derived synthetic peptide | Prevalence among patients with the indicated test result and Chsp status* | *P* |
|------------------|-----------------------------|--------------------------------------------------|-----|
|                  |                             | Chsp10-positive | Chsp10-negative | Chsp10-positive | Chsp10-negative |
| Pregnant (n = 230) | pEPF1 | 11.5 (3/26) | 2.3 (1/43) | 0 (0/24) | 1.4 (2/137) | >0.3 |
|                  | pEPF2 | 26.9 (7/26) | 4.6 (2/43) | 8.3 (2/24) | 3.6 (5/137) | <0.01 |
| Nonpregnant STD (n = 87) | pEPF1 | 2.8 (1/35) | 0 (0/38) | 0 (0/14) | NDb | ND |
|                  | pEPF2 | 2.8 (1/35) | 0 (0/38) | 0 (0/14) | ND | ND |
| Infertile (n = 210) | pEPF1 | 4.8 (1/21) | 5.9 (1/17) | 2 (2/103) | 1.4 (1/69) | ND |
|                  | pEPF2 | 23.8 (5/21) | 11.8 (2/17) | 1 (1/103) | 1.4 (1/69) | <0.01 |

* Values are percent patients positive for anti-EPF antibodies (no. positive/total no. tested) for patients who are *C. trachomatis*-positive by AmpCT and/or Serochlamydia peptide *C. trachomatis* tests (Positive) or patients who are negative by both tests (Negative).

b ND, not determined.
inhibition was observed in any samples with the pEPF1 peptide. The mean inhibition coefficient with pEPF2 was 76% ± 5.9%.

**Association of selected HLA class II haplotypes with Chsp10 antibody.** Overall, 12 different DRB1 alleles and 7 different DQB1 alleles were identified (data not shown). Haplotypes that were assessed in >10% of the patients studied were evaluated for their statistical association with Chsp10 antibody. When the proportions of sera with detectable anti-Chsp10 antibodies among haplotype-positive and -negative women were compared, a significant association was observed between the DR8 DQ4 haplotype and the concomitant presence of anti-Chsp10 antibodies ($P < 0.05$). However, none of the DR8 DQ4 patients had EPF cross-reactive antibodies detected. The DR7 DQ2 haplotype was associated with absence of anti-Chsp10 antibodies ($P < 0.05$). However, anti-Chsp10 antibodies found in DR7 DQ2 or DR17 DQ2 patients represented 27.5% of EPF cross-reactive sera (Table 3).

### DISCUSSION

Hsps are extremely potent immunogens despite their high degree of evolutionary conservation. The immunological recognition of Hsps is associated with several inflammatory conditions (1). We therefore focused our study on patients with a documented acute or past *Chlamydia trachomatis* infection and with *Chlamydia trachomatis*-associated anti-Chsp10 antibodies.

It has been suggested that pelvic inflammatory disease (PID) caused by *Chlamydia trachomatis* and its reproductive system sequelae, such as tubal infertility and ectopic pregnancy, may be mediated by the immune system (5). An antibody against the 60-kDa Hsp of *Chlamydia trachomatis* (Chsp60) in women with *Chlamydia trachomatis*-associated ectopic pregnancy was found to cross-react with peptide epitopes from human Hsp60 (23). Serum antibodies against Chsp10 are commonly detected in patients with chronic *Chlamydia trachomatis* infection and in patients with ectopic pregnancies (4). The very strong association observed in this study between female infertility and the presence of antibodies against Chsp10 is not surprising; a strong association has also been found between serum antibody responses to the 60-kDa chlamydial Chsp60 and PID and between such antibody responses and PID-linked tubal infertility (5).

Antibodies against Chsp10 may act as a marker for autoimmune responses to self-Cpn10/EPF initiated by molecular mimicry. The Cpn10/EPF protein plays a dual role, acting both as a molecular chaperone and as an extracellular hormone necessary for embryo implantation (2, 3). Cross-reactions with EPF may neutralize the activity of this protein, thereby causing secondary infertility due to embryo implantation failure.

The amino acid sequence of *Chlamydia trachomatis* Chsp10 is 48.1 and 12.5% identical to the sequences of pEPF1 and pEPF2, respectively (Fig. 1). Contrary to expectations, inhibition ELISA results indicated that the antibody against pEPF2 cross-reacted with Chsp10, whereas the antibody against pEPF1 did not. Antigenic cross-reactivity may depend on epitope-paratope immunochemistry (20), and as has already been shown for *Chlamydia trachomatis* Chsp60, differences in amino acid sequence between chlamydial and human proteins do not accurately predict which epitopes will be specific or cross-reactive (23).

In vitro studies are required to determine whether the antibodies that cross-react with Chsp10 and EPF neutralize EPF. It was recently shown that all peptides spanning residues 1 to 77 of Cpn10 produced EPF-neutralizing antibodies in a lymphocyte EPF bioassay. One of our peptides, pEPF1, corresponds to this region. In contrast, antibodies against residues 77 to 101 have been shown to be nonneutralizing (21). The second of our peptides, pEPF2, corresponds to this C-terminal region of EPF. Therefore, the cross-reactive anti-EPF antibodies identified in this study are probably nonneutralizing.

Our data also suggest that antibodies against EPF peptide epitopes are not deleterious early in pregnancy, as such antibodies were found in a large proportion of women in the first trimester of pregnancy. Interestingly, anti-EPF antibodies in pregnant women were detected mostly in women with a history of *Chlamydia trachomatis* infection, who also presented anti-Chsp10 antibodies. This finding may be accounted for by the stimulation and expansion of populations of self-EPF cross-specific T cells; these T cells are primed by *Chlamydia trachomatis* Cpn10 and reactivated by EPF secretion and display, which are naturally upregulated during pregnancy.

Our results suggest that there is cross-reactivity between *Chlamydia trachomatis* Cpn10 and certain EPF epitopes. This cross-reactivity seems to be associated with either pregnancy or infertility. In pregnant women, cross-reactivity and the overcomin of tolerance may be accounted for by the pregnancy-induced upregulation of endogenous EPF (15). In infertile women, it may be accounted for by an underlying inflammation (PID)-induced upregulation of endogenous Cpn10 production and the recruitment of memory T cells to the site of upregulated Cpn10 antigen presentation (13, 22).

Studies of the MHC profiles of patients with detectable

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**Table 3. Association of selected HLA DRB1/DQB1 haplotypes with *Chlamydia trachomatis* Chsp10 antibody.**

| Haplotype | % Haplotype-positive (no. positive/total no. tested) | % Haplotype-negative (no. negative/total no. tested) | $p$ | % Haplotype-positive among EPF cross-reactive sera (no. positive/total no. tested) |
|-----------|-----------------------------------------------|-----------------------------------------------|---|-----------------------------------------------|
| DR7 DQ2   | 20 (3/15)                                     | 56 (37/66)                                    | <0.05 | 10.3 (3/15)                                  |
| DR17 DQ2  | 55.5 (10/18)                                  | 47.6 (30/63)                                  | >0.1  | 17.2 (5/15)                                  |
| DR8 DQ4   | 77.8 (7/9)                                    | 45.8 (33/72)                                  | <0.05 | 0 (0/15)                                     |
| DR15 DQ6  | 54.5 (6/11)                                   | 48.0 (34/70)                                  | >0.1  | 10.3 (3/15)                                  |
| DR13 DQ6  | 40 (6/15)                                     | 51.5 (34/66)                                  | >0.1  | 17.2 (5/15)                                  |
| DR1 DQ5   | 54.5 (6/11)                                   | 48.0 (34/70)                                  | >0.1  | 10.3 (3/15)                                  |

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*a* No. positive and no. negative in parentheses are the numbers of serum samples with a Chsp10 optical density at 405 nm of >0.12.

*b* This column indicates the frequency of detection of the selected haplotypes among 15 EPF cross-reactive serum samples.
anti-Chsp10 and anti-EPF antibodies might provide insight into the possible genetic basis of cross-reactivity. The HLA-B8, DR3 (DR17 or DR18) haplotype is associated with a wide variety of diseases with autoimmune features (16). Furthermore, the presence of antibodies against Chsp60 has been shown to be associated with the DQ alleles corresponding to the DQ4 phenotype (9). Our results suggest an association between the presence of antibodies against Chsp10 and the DR8 DQ4 haplotype. However, none of the EPF cross-reactive sera studied corresponded to this haplotype. The majority of EPF cross-reactive sera corresponded to the DR7 DQ2, DR17 DQ2, DR13 DQ6, and DR15 DQ6 haplotypes. Therefore, it seems that if the Chsp10 antibody response operates through the HLA class II genes, EPF cross-reactivity does not necessarily operate through the same susceptibility genes.

This study provides evidence that antibodies against C. trachomatis Chsp10 may cross-react with human EPF peptide epitopes and that these antibodies may act as a marker of female infertility. A relationship between MHC susceptibility genes and the presence of antibodies against Chsp10 protein is also shown. It is unclear whether these antibodies neutralize EPF, and further studies are required to determine whether this is the case.

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