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Correlation of Host Immune Response with Quantitative Recovery of *Chlamydia trachomatis* from the Human Endocervix

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We studied 95 women with uncomplicated *Chlamydia trachomatis* cervical infection. Quantitative isolation of *C. trachomatis* was performed in HeLa 229 cells, and the results were correlated with serum immunoglobulin M and immunoglobulin G antibody to the organism. We found that quantitative cultures for *C. trachomatis* can provide a meaningful measurement by which to evaluate the effect of the acquired immune response. In particular, secretory immunoglobulin A antibody to *C. trachomatis* in cervical secretion demonstrated a striking and inverse correlation with recovery of the organism from the cervix. It is suggested that this component of the immune response may regulate shedding of the organism.

Infections with trachoma biovars of *Chlamydia trachomatis* are characteristically persistent and recurrent. Vigorous immune responses during infection occur, but their role in regulating the infection is obscure. Data from experimental infections suggest that the immune response to *C. trachomatis* may provide some degree of protection to reinfection and may also contribute to some of the manifestations of chronic infection (9).

To investigate correlations of the immune response with shedding of *C. trachomatis*, we studied 95 women with uncomplicated cervical infection. Serum and local endocervical secretions were assayed for antibody to *C. trachomatis*. Peripheral blood mononuclear cells were tested for lymphocyte transformation to *C. trachomatis*. These results were correlated with quantitative cultures of *C. trachomatis*.

Ninety-five women found to have *C. trachomatis* infection by screening during attendance at a Sexually Transmitted Disease Clinic and who remained culture positive when studied 1 to 2 weeks later constituted the study population. None of these women had clinically apparent complications of genital infection with *C. trachomatis*, and none had concurrent gonococcal or genital herpes simplex virus infection at the time of enrollment. Endocervical cultures for *C. trachomatis* were collected with a type III calcium alginate swab on a plastic shaft (Inolex Corp., Park Forest South, Ill.) in 1 ml of SPG (sucrose, 75 g; KH_2PO_4, 0.52 g; Na_2HPO_4, 1.22 g; glutamic acid, 0.72 g; water to 1 liter; pH 7.4 to 7.6) transport media and frozen immediately in dry ice before storage in a −70°C freezer. Screening cultures were performed in cycloheximide-treated McCoy cells in microtiter plates (22). Quantitative cultures were performed in HeLa 229 cells pretreated with diethylaminoethyl dextran (30 μg/ml) (14) with additions of cycloheximide (0.5 μg/ml) in the culture medium for incubation after inoculations. Three culture vials were inoculated with 0.1 ml each of specimen. One vial was used for inclusion detection by Giemsa stain after 3 days of incubation. The remaining two vials were harvested for passage to three new culture vials. Quantitation was performed by the method of Furness et al. (8) with modification (6). Inclusions were counted in each of 30 fields at ×400 magnification with the aid of a micrometer and expressed as inclusion-forming units (IFU) per ml of transport medium according to the following formula: average number of inclusions per 30 fields × 654 = IFU per milliliter. If no inclusions were seen in 30 fields, the entire coverslip was scanned to assess the titer. If no inclusions were seen on scanning the entire coverslip but inclusions did appear when the specimen was blindly passed, the specimen was defined as having less than 10 IFU/ml.

Serological studies were performed with plasma tested at three fourfold dilutions (1:8, 1:32, and 1:128) in the simplified microimmunofluorescence test (21) against a set of *C. trachomatis* elementary body antigens. Each specimen was tested for antibody of immunoglobulin M

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TABLE 1. Percentage of women with antibody to *C. trachomatis* in serum or local endocervical secretions in terms of quantitative recovery of *C. trachomatis* from the cervix

IFU of *C. trachomatis* per ml of transport medium	No. of women	% Of women with:[a]			
		Serum antibody		Local antibody	
		IgM ≥ 1:8	IgG ≥ 1:128	IgMAG[b]	sIgA
$<10^1$	16	6	25	100	63
10^2	27	15	37	88	42
10^3	14	7	43	92	46
10^4	24	29	21	67	25
$>10^4$	14	36	14	71	21

[a] P values were determined by a chi-square test of linear trend. IgM, $P = 0.02$; IgG, $P = 0.24$; IgMAG, $P < 0.01$; sIgA, $P < 0.01$.

[b] IgMAG, Fluorescein-labeled goat anti-human combined immunoglobulins.

(IgM) and IgG specificity by using fluorescein-labeled goat anti-human IgM or IgG conjugate (Hyland Laboratories, Inc., Costa Mesa, Calif.). Local cervical secretions were collected by allowing premeasured (5 by 20 mm) filter paper strips to become saturated with endocervical secretions. Each strip was eluted into 0.2 ml of phosphate-buffered saline. Secretions were collected atraumatically before culturing the cervix to avoid contamination with blood. Specimens were stored at −20°C and tested in the microimmunofluorescence assay without further dilutions. Fluorescein-labeled goat anti-human immunoglobulins (Hyland Laboratories) and rabbit anti-serum secretory component (Bio-Rad Laboratories, Richmond, Calif.) were used to detect antibody to *C. trachomatis*. Rabbit anti-human secretory component conjugate was tested and found not to detect antibody to *C. trachomatis* in plasma and was considered specific for secretory IgA (sIgA).

Peripheral blood lymphocytes collected by venipuncture were tested in a lymphocyte transformation (LT) assay using Renografin-purified serotype L_2 elemenary bodies of *C. trachomatis* as previously described (5). Results are expressed as the stimulation index (SI): SI = mean counts per minute of antigen stimulated culture/mean counts per minute of nonstimulated control culture.

The number of *C. trachomatis* IFU per milliliter was transformed to the base 10 logarithm, and results were expressed as $<10^1$, 10^2, 10^3, 10^4, or $>10^4$, respectively, if <10, 10 to 99, 100 to 999, 1,000 to 9,999, or ≥10,000 inclusions per ml of transport medium were detected. The significance of the correlation of antibody prevalence with quantitative culture results was analyzed by a chi-square test for linear trend. The LT data were analyzed with the one-tailed Student's t test and with analysis of variance.

Table 1 shows the proportion of women who, with each level of quantitative isolation, had the indicated serological finding. As the number of organisms increased, the proportion with IgM antibody to *C. trachomatis* increased ($P < 0.02$). Serum IgM was present in 32% of women with $\geq 10^4$ IFU/ml as compared with 11% of women with $\leq 10^3$ IFU/ml ($P < 0.05$). Since the presence of serum IgM antibody to *C. trachomatis* suggests recent infection, these results suggest that women with the highest inclusion counts are more likely to have been recently infected.

All but four women had serum IgG antibody at a titer of <1:8 detected in the microimmunofluorescence assay. Two of these four women had $>10^4$ IFU/ml, one had 10^2 IFU/ml and the other had 10^3 IFU/ml of recovered *C. trachomatis*. Serum IgG antibody at a titer of ≥1:128 also did not show a significant correlation with quantitative isolation of *C. trachomatis* ($P = 0.24$). Thirty-five percent of women with $<10^3$ IFU/ml had serum IgG ≥1:128 compared with 18% of women with $\geq 10^4$ IFU/ml ($0.05 < P < 0.1$).

Local antibody detected in cervical secretions showed a stronger correlation with quantitative isolation of *C. trachomatis* from the cervix, and in contrast with serum antibody, the presence of local antibody was inversely correlated. Women with the lowest numbers of organisms recovered had the highest prevalence of antibody in cervical secretions ($P < 0.01$). This relationship was most striking with sIgA where 63% of women with $<10^1$ IFU/ml had sIgA to *C. trachomatis* in cervical secretions and only 21% of those with $>10^4$ IFU/ml had such antibody ($P < 0.01$). Furthermore, 50% of women with $\leq 10^3$ IFU/ml isolated had sIgA to the organism in cervical secretions compared with 24% of women with $\geq 10^4$ IFU/ml ($P < 0.01$).

LT assays and quantitative cultures were done in 69 of the 95 women. Fig. 1 shows the mean LTSI observed in women in terms of the level of quantitative culture result. Analysis of variance showed that the relationship of LTSI to results of quantitative cultures overall was only

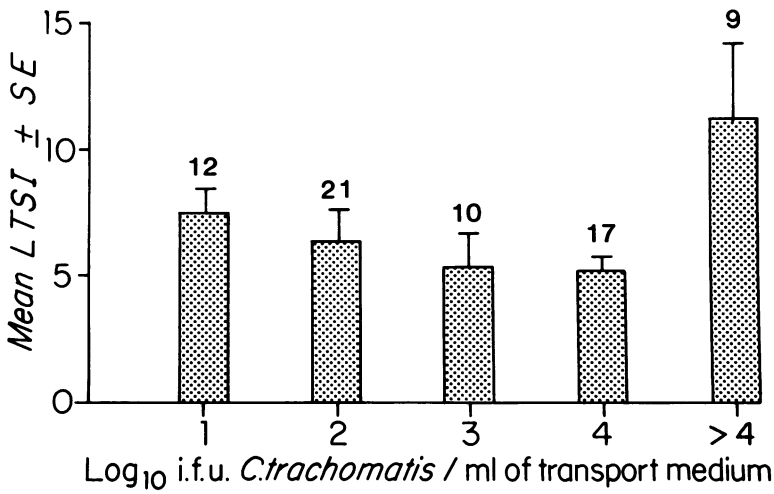

FIG. 1. Relationship of the mean LTSI with *C. trachomatis* antigen to quantitative recovery of *C. trachomatis* from the cervix. The correlation was only of borderline statistical significance ($P = 0.08$, analysis of variance). Women with $>10^4$ IFU/ml had significantly higher mean LTSI than did women with 10^2, 10^3, or 10^4 IFU/ml. The brackets depict the standard error of the mean. The numbers above the bar graph represent the number of women tested.

of borderline statistical significance ($P = 0.08$). Women with $>10^4$ IFU/ml had the highest mean LTSI, and this was significantly greater than the mean LTSI for each of the other groups individually, except for those with $<10^1$ IFU/ml.

The data presented in this report show that quantitative cultures for *C. trachomatis* provide one interesting measure by which to evaluate the effect of the host immune response. The quantitative recovery of *C. trachomatis* was most strongly correlated, in an inverse fashion, with the presence of sIgA antibody to this organism. These results suggest that this response may directly regulate shedding of the organism. The presence of serum IgM antibody to *C. trachomatis*, as expected, correlated directly with quantitative isolation, probably because the organism multiplies to higher titer during acute, recently acquired infection than during later stages of the infection.

Results obtained with the LT assay were more complex. The mean LTSI tended to increase with decreasing titers of organisms, up to 10^4 IFU/ml, suggesting that the LT response was associated with regulation of shedding. However, women who had $>10^4$ IFU/ml had significantly higher mean LTSI than other women, suggesting that the LT response may reflect the antigenic mass of infection, being higher during the acute phase when larger numbers of organisms are present.

The mechanism by which sIgA could regulate shedding of *C. trachomatis* is unexplored. Neutralization of infectivity of *C. trachomatis* by serum antibody in tissue cell culture has been reported by several groups (3, 4, 11). In particular, Howard (11) was able to demonstrate that neutralization with hyperimmune serum was complement dependent. Eye secretions from children with active trachoma have been shown to neutralize *C. trachomatis* infectivity in the owl monkey conjunctivitis model (1, 16). Whether neutralization of chlamydial infectivity represents agglutination, bacteriolysis, or inhibition of attachment has not been defined. The comparative effectiveness and complement dependence of sIgA versus serum IgG or IgM in neutralizing chlamydial infectivity in vitro has not been studied.

sIgA could interfere with the recovery of *C. trachomatis* by mechanisms other than direct neutralization. Fubara and Freter (7) reported that the antibacterial activity of sIgA to *Vibrio cholera* was entirely dependent upon the intact metabolic function of mucosa. These results suggest that cells found within the mucosa were necessary for the antibacterial function of sIgA. Lowell et al. (15) have proposed that monocytes may be the cell type involved in this phenomenon, since they observed that monocytes were especially effective in reducing bacterial viability in the presence of low concentrations of purified serum IgA and in the absence of complement. This mechanism may be operative in the cervix where low concentrations of complement are found. sIgA may also enhance the antimicrobial activity of the peroxidase system against *C. trachomatis*. Yong et al. (23) have demonstrated the striking susceptibility of *C. trachomatis* to the peroxidase-halide-H_2O_2 sys-

tem. Peroxidase present in cervical mucus (18), together with H_2O_2 generated by lactobacilli or streptococci present in the vagina, provide the ingredients for a powerful antimicrobial system (13). Tenovuo et al. have recently reported that IgA preferentially enhances the activity of this system against *Streptococcus mutans* (19).

The regulation of *C. trachomatis* shedding by sIgA could also explain the interaction observed between *Neisseria gonorrhoeae* and *C. trachomatis*. It has been proposed that *N. gonorrhoeae* can reactivate latent *C. trachomatis* infection, since women with gonorrhea have a high frequency of coinfection with *C. trachomatis* (10, 17). This association might derive in part from destruction of sIgA by extracellular IgA1 protease secreted by *N. gonorrhoeae* (2). Similarly, *Haemophilus aegyptius* ocular infection and increased severity of trachoma has also been observed (20). *H. aegyptius* produces IgA1 protease (12).

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