JCI The Journal of Clinical Investigation

Depression of the lymphocyte transformation response to microbial antigens and to phytohemagglutinin during pregnancy.

R C Brunham, … , D A Eschenbach, K K Holmes

J Clin Invest. 1983;72(5):1629-1638. https://doi.org/10.1172/JCI111122.

Research Article

Lymphocyte transformation (LT) responses to Chlamydia trachomatis, to four other microbial antigens, and to phytohemagglutinin (PHA) were studied in 201 women during pregnancy and/or 3-18 wk postpartum. The LT responses to all stimulants tested were significantly depressed during pregnancy when compared with postpartum LT responses. This difference occurred whether LT assays were performed in autologous or pooled heterologous plasma collected from nonpregnant donors. Among women studied in the third trimester and again postpartum, the autologous LT stimulation index (LTSI) rose from 1.7 to 3.4 (P less than 0.001) with C. trachomatis elementary body antigen, from 3.7 to 7.9 (P less than 0.001) with Candida albicans cell wall extract, from 4.5 to 7.8 (P = 0.008) with streptokinase-streptodornase, from 1.7 to 3.0 (P = 0.007) with fluid tetanus toxoid, from 1.7 to 2.8 (P = 0.046) with mumps virus skin test antigen, from 35.5 to 87.0 (P less than 0.001) with PHA (2 micrograms/ml), and from 107.2 to 181.9 (P = 0.007) with PHA (10 micrograms/ml). LT responses to C. trachomatis were compared in 52 pregnant women and 58 nonpregnant women; all the women had C. trachomatis isolated at the time of LT assay. Using either plasma supplement, the mean LTSI with C. trachomatis antigen was significantly higher in nonpregnant women than in pregnant women, regardless of trimester (P less than […]

Depression of the Lymphocyte Transformation Response to Microbial Antigens and to Phytohemagglutinin during Pregnancy

ROBERT C. BRUNHAM, DAVID H. MARTIN, TODD W. HUBBARD, CHO-CHOU KUO, CATHY W. CRITCHLOW, LINDA D. CLES, DAVID A. ESCHENBACH, and KING K. HOLMES, *Departments of Medicine, Obstetrics and Gynecology, and Pathobiology, University of Washington, and the Seattle Public Health Hospital, Seattle, Washington 98114*

ABSTRACT Lymphocyte transformation (LT) responses to *Chlamydia trachomatis*, to four other microbial antigens, and to phytohemagglutinin (PHA) were studied in 201 women during pregnancy and/or 3–18 wk postpartum. The LT responses to all stimulants tested were significantly depressed during pregnancy when compared with postpartum LT responses. This difference occurred whether LT assays were performed in autologous or pooled heterologous plasma collected from nonpregnant donors. Among women studied in the third trimester and again postpartum, the autologous LT stimulation index (LTSI) rose from 1.7 to 3.4 ($P < 0.001$) with *C. trachomatis* elementary body antigen, from 3.7 to 7.9 ($P < 0.001$) with *Candida albicans* cell wall extract, from 4.5 to 7.8 ($P = 0.008$) with streptokinase-streptodornase, from 1.7 to 3.0 ($P = 0.007$) with fluid tetanus toxoid, from 1.7 to 2.8 ($P = 0.046$) with mumps virus skin test antigen, from 35.5 to 87.0 ($P < 0.001$) with PHA (2 μg/ml), and from 107.2 to 181.9 ($P = 0.007$) with PHA (10 μg/ml). LT responses to *C. trachomatis* were compared in 52 pregnant women and 58 nonpregnant women; all the women had *C. trachomatis* isolated at the time of LT assay. Using either plasma supplement, the mean LTSI with *C. trachomatis* antigen was significantly higher in nonpregnant women than in pregnant women, regardless of trimester ($P < 0.001$). Among 12 women who were serially tested and remained culture positive for *C. trachomatis* throughout pregnancy and the postpartum period, the mean autologous LTSI rose from 1.9 in the third trimester to 7.8 postpartum ($P = 0.0004$). These data are the first to show that the immune response to an ongoing bacterial infection is depressed during pregnancy and to definitively document the depressed LT responses during human pregnancy.

INTRODUCTION

While humoral immune responses are generally found to be intact during human pregnancy (1, 2), a large body of evidence suggests that cellular immune responses are impaired. There has been great difficulty in consistently demonstrating depressed immune response in vitro. For instance, it has been reported that the in vitro lymphocyte transformation (LT)[1] response to phytohemagglutinin (PHA) is depressed (3–8), unchanged (9–13), or even increased (14) during pregnancy. Studies in animals have not resolved these discrepancies as some investigators report immunodepression during pregnancy and others do not (15–18).

Several reasons may exist to explain these disparate results. Different in vitro assay systems have been used with different culture conditions, different plasma or serum supplements, and different concentrations of stimulants. Small numbers of patients have been stud-

Dr. Brunham was a recipient of a fellowship from the Medical Research Council of Canada. Address all reprint requests to Dr. Brunham, Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada R3E OW3.

Received for publication 18 May 1982 and in revised form 11 July 1983.

[1] *Abbreviations used in this paper:* C, control; E, experimental; LT, lymphocyte transformation; LTSI, lymphocyte transformation stimulation index; PHA, phytohemagglutinin; SI, stimulation index; SK-SD, streptokinase-streptodornase.

ied and often on a single occasion. The LT response data have been expressed in various ways and statistical methods used for analyses have varied from study to study.

We have reexamined the issue of maternal immunoresponsiveness by measuring the in vitro LT response to a variety of microbial antigens and to PHA in large numbers of women during pregnancy and the postpartum period, by prospectively analyzing trends in the LT response throughout pregnancy and the puerperium, and by comparing pregnant women with nonpregnant women. We also identified a group of women with *Chlamydia trachomatis* infection of the cervix and compared cellular immune responses with chlamydial antigens throughout gestation and the puerperium, and between pregnant and nonpregnant women with a current chlamydial infection. We chose to study infection with *C. trachomatis* because it is common during pregnancy and because the cellular immune response may be important in host defense against this obligately intracellular pathogen.

We observed that during pregnancy the LT response to PHA and to all microbial antigens was significantly depressed when compared with the postpartum period or when compared with that in nonpregnant women, and that this depression was most marked in the third trimester. LT responsiveness was depressed not only to recall microbial antigens, but also to chlamydial antigens among women with current infection with this agent. Depressed LT responses were observed in both autologous and heterologous (nonpregnant) plasma; this suggests that intrinsic cellular changes in lymphocyte regulation are present during human pregnancy. Depression of the cellular immune response during pregnancy, reflected by depressed LT responsiveness, may help account for the increased susceptibility of the pregnant host to certain infections and to reactivation of previously latent infections.

METHODS

Patient population

201 women were studied during pregnancy or postpartum; 70 of them were seen sequentially—initially in the first or second trimester, subsequently late in the third trimester, and then again at 3–18 wk postpartum. 117 women were studied on at least two occasions and one of the two was during pregnancy. All women were studied at the Prenatal Clinic, University Hospital, Seattle, WA. All women were cultured on at least one occasion for *C. trachomatis*. This group was selected to include 78 women with a positive cervical culture for *C. trachomatis* during pregnancy.

58 additional nonpregnant women whose sex partners lacked a history of urethritis and who were detected to have *C. trachomatis* cervical infection by routine screening cultures were studied at the Sexually Transmitted Disease Clinic, Harborview Medical Center, Seattle, WA.

Microbiologic methods

Cultures of *C. trachomatis* were obtained with cotton-tipped or calcium alginate-tipped swabs (type III, Inolex Corp., Park Forest South, IL) from the endocervical canal and were stored in transport media. Specimens were either held at refrigerator temperature if they were to be processed within 24 h or were frozen at −70°C if they were to be tested later. *C. trachomatis* was isolated by three different methods in two cooperating laboratories over the course of the study: initially, in iododeoxyuridine-treated McCoy cells in macroculture (19); later, in cycloheximide-treated McCoy cells in a microtitre test (20); and in some instances, in DEAE dextran-pretreated HeLa 229 cells (21).

Collection and handling of blood

Blood was collected by venipuncture of a peripheral arm vein into preservative-free, heparinized (0.2 ml/10 ml) sterile tubes. Blood was handled at room temperature and transported to the laboratory usually within 4–6 h of collection. Plasma was separated from cells by centrifugation at 100 *g* for 10 min. Cells were diluted in Waymouth's medium (Gibco Laboratories, Santa Clara, CA) and processed for separation of the mononuclear cell fraction by density-gradient centrifugation. Plasma samples were frozen at −20°C until tested in the microimmunofluorescence antibody assay.

Serologic test

All plasma samples were tested for *C. trachomatis* antibody by the microimmunofluorescence technique (22). Each specimen was screened for antibody with the use of the fluorescein-labeled goat anti-human immunoglobulins (IgM, IgG, and IgA combined, Hyland Laboratories, Costa Mesa, CA) at three fourfold dilutions against each of the 15 serovars of *C. trachomatis* elementary bodies. Seropositivity was defined as serum antibody present at a titer $\geq$1:8.

Lymphocyte proliferation assay

Mononuclear cells from peripheral blood were isolated by Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ) centrifugation according to procedures described by Boyum (23). After washing, the cells were suspended in Waymouth's MB 725/L medium with L-glutamine and counted in a hemocytometer chamber after staining with Turk's solution. Viability, as determined by trypan blue exclusion, was >95%. Cells were adjusted to a density of 1×10^6/ml in medium supplemented with 10% autologous plasma or with 10% heterologous plasma, which had been collected and pooled from six nonpregnant donors, and with 100 U penicillin G/ml and 100 μg streptomycin/ml. 0.2 ml of this suspension was added to wells of a microtiter plate (Microtest II, No. 040, Falcon Labware, Oxnard, CA). Stimulants were added in a volume of 25 μl to each of four replicate wells. Cultures were incubated for 3 d with PHA stimulation and 7 d with microbial antigen stimulation at 37°C in 5% CO_2 and 95% humidified air. At 18 h before termination of the cultures, 1 μCi of [^{3}H]thymidine (sp act 6.7 Ci/mmol; New England Nuclear, Boston, MA) was added to each well. Cultures were harvested on filter strips (Microbiological Associates, Walkersville, MD). Filter discs were removed from the strips and allowed to dry before being placed in scintillation vials (Packard Instrument Co., Downers Grove, IL) with 5 ml of Omnifluor scintillation

fluid (New England Nuclear, Gardena, CA). The vials were dark and cold-adapted for at least 1 h before measurement in a scintillation counter (Packard Instrument Co., Downers Grove IL).

Stimulants used in the LT assay

LT assays were performed with *C. trachomatis* and *Candida albicans* antigens in all cases. LT assays that were performed with PHA, tetanus toxoid, mumps, and streptokinase-streptodornase (SK-SD) were added later in the study. LT assays were done in autologous plasma in all cases and when cell numbers permitted, in heterologous plasma.

PHA. PHA (PHA-P, Difco Laboratories Inc., Detroit, MI) was obtained as a lyophylized powder and reconstituted in sterile phosphate-buffered saline (PBS). Protein concentration was determined by Lowry's method (24), and serial dilutions were prepared. Preliminary experiments indicated that 10 μg/ml as final concentration in the well produced the optimal stimulation. PHA was then used at 10 and 2 μg/ml for the LT assay.

Microbial antigens

C. trachomatis. The method of preparation and purification of chlamydial antigens used in this assay has been described (25). Briefly, *C. trachomatis* serotype L2/434/Bu was grown in HeLa 229 cell culture in 32-oz prescription bottles for 3 d. Elementary bodies were separated from host cell material by Renografin (methyl-glucamine diatrizoate, 76% for injection, Squibb Corp., New York) linear-gradient centrifugation. Purified antigen was inactivated with ultraviolet light, diluted in Waymouth's MB 725/L medium, and used at 6.7 μg protein/ml final concentration in the well. This concentration of purified antigen produces optimal stimulation (25). Chlamydial antigen was stored at −65°C in aliquots and thawed immediately before use.

C. albicans. This was obtained from Hollister-Stier Laboratories, Spokane, WA (Allergenic Extract) as a sterile extract of dried cell walls which was diluted 1:10 (wt/vol) in 50% glycerine and contained 0.4% phenol. This preparation was extensively dialyzed against PBS under sterile conditions to remove glycerine and phenol. After dialysis, bacterial cultures were done to document lack of bacterial contamination. Preliminary experiments determined that 13.0 μg/ml final concentration in the well provided optimal stimulation. Samples were aliquoted and frozen at −65°C and thawed immediately before use.

SK-SD. SK (100,000 U)-SD (25,000 U) was obtained from Lederle Laboratories, Pearl River, NY (Varidase) as a lyophilized powder that contained 2.0 mg thimerosol. The preparation was reconstituted with PBS, extensively dialysed against PBS, filter sterilized (0.22 μm Millipore filter, Millipore Corp., Bedford, MA), and was used at 12.5 μg/ml final concentration in the well as the optimal concentration.

Tetanus toxoid. Fluid tetanus toxoid was obtained from Massachusetts Public Health Biologic Laboratories (Boston, MA) and used at 1:300 dilution in PBS. The final concentration in the well was 0.46 Lf U/ml.

Mumps. Mumps skin test antigen (Eli Lilly and Co., Indianapolis, IN) that contained thimerosol was extensively dialyzed against PBS and used at 1.6 μg/ml final concentration in the well as determined by preliminary experiments to provide optimal stimulation.

Expression and analysis of LT data

All statistical tests were performed using 1977-BioMedical Data Program statistical packages. Assay results in autologous and heterologous plasma were analyzed separately for three gestational periods (0–27 wk, 28 wk to delivery, and 3–18 wk postpartum). Further grouping that was based on results of culture and serology for *C. trachomatis* were occasionally used. The *t* test was used for comparisons of the LT response in two gestational periods, or in pregnant vs. nonpregnant culture-positive women (26). Comparisons of the LT response in all three gestational periods, or in relation to various combinations of culture and serology results or the source of plasma were performed using the appropriate linear model (analyses of variance).

Adjustments of experimental (E) counts per minute to reflect changes in control (C) counts per minute are generally used to facilitate qualitative and quantitative analysis of LT response data. However, as evidenced by the number of indices for stimulation used in the literature, the controversy concerning which adjustment for E best reflects the biological nature of its dependence on C has yet to be resolved. Preliminary testing by analysis of covariance of log-transformed, stimulated counts per minute (log E) and control counts per minute (log C) from 31 women tested with both plasma types in all three gestational periods indicated significant dependence of (log E) on (log C) ($P < 0.001$). This was true in autologous plasma and in heterologous plasma.

Fig. 1, *b* and *c* show computer-generated, standard normal plots of two common adjustments of E, the E ÷ C = stimulation index (SI), and E − C, respectively, for all tests done in the third trimester using autologous plasma and *C. trachomatis* as the stimulant. The log (E/C), i.e. (log E) − (log C) shown in Fig. 1 *a*, was suggested biologically because of the logarithmic nature of the LT assay and statistically because logarithmic transformations tend to improve heteroscedasticity and normality characteristics. The dashed diagonal line drawn in Fig. 1 *a* shows the expected distribution of a normally distributed population of numbers. The best approximation to this line is given by the log (E/C). Plots for other gestational periods and stimulants were similar. For these reasons, statistical tests were performed on the log (E/C). To facilitate comparison with previously published data sets, the mean log (E/C) (i.e., lymphocyte transformation stimulation index (LTSI)) has been converted back to the geometric mean SI ($10^{\log(E/C)}$).

Nevertheless, changes in LTSI can result from changes in either numerator E or denominator C counts per minute. To evaluate whether the observed changes in LTSI reflected changes in E or C, we analyzed data of lymphocyte cultures that were incubated with and without *C. trachomatis* antigen for 10 *C. trachomatis* isolate-positive women and 18 *C. trachomatis* isolate-negative seropositive women; all of these women were studied sequentially during all three gestational periods (Fig. 2). We chose this subgroup for analysis because it was considered possible that active ongoing infection could cause in vivo stimulation of lymphocytes; this results in elevation of counts per minute of unstimulated in vitro cultures, which thus artificially suppresses the in vitro LTSI. The results from this analysis show that the rise in LTSI postpartum in culture-positive women is not due to a change in unstimulated counts per minute, and that the unstimulated counts per minute in culture-positive women is similar to the unstimulated counts per minute in culture-negative women, as might be expected for a localized infection.

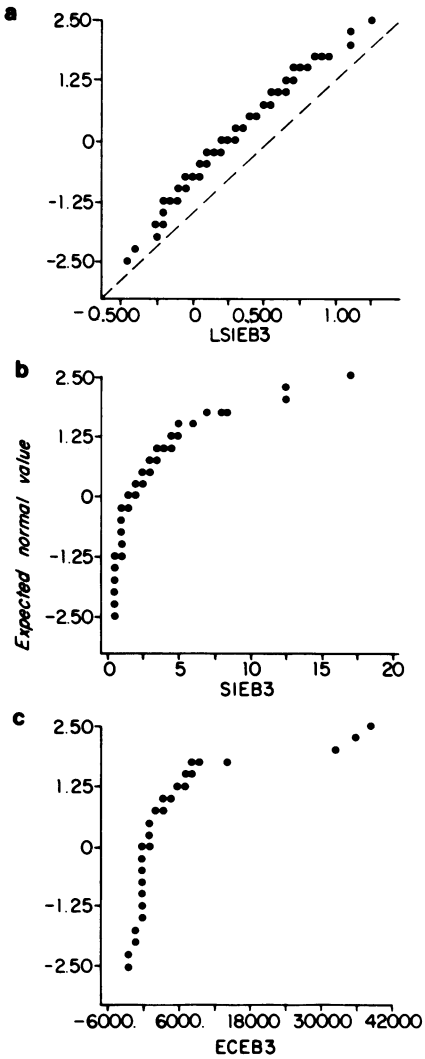

FIGURE 1 Computer-generated normal plot of various indices for results of all LT assays performed with chlamydial antigen during the third trimester. The code for the indices plotted are (*a*) LSI EB3 = log (E/C); (*b*) SI EB3 = (E/C); (*c*) EC EB3 = (E − C). The dashed line in Fig. 1 *a* shows the idealized line expected from a normally distributed population. Similar plots of other time periods and antigens showed analogous results.

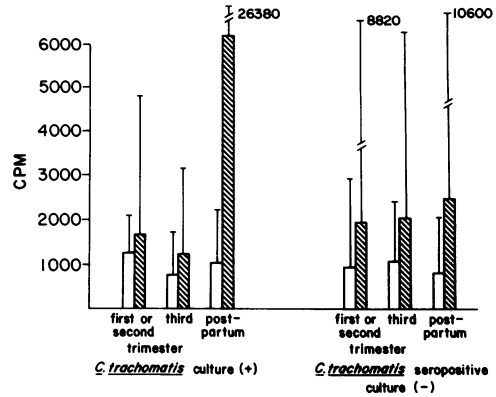

FIGURE 2 Geometric mean counts per minute of in vitro lymphocyte cultures with chlamydial antigen in autologous plasma for 10 *C. trachomatis* culture-positive and 18 *C. trachomatis* seropositive culture-negative women studied sequentially during the first or second trimester, third trimester, and 3–18 wk postpartum. The open bars represent unstimulated counts per minute and the hatched bar stimulated counts per minute. The bracket encloses 1 SD of the mean.

RESULTS

LT response to microbial antigens among women studied during pregnancy and postpartum. Fig. 3 illustrates the mean LTSI that was measured on all 458 assays performed in autologous plasma among the 201 women during the course of pregnancy and 3–18 wk postpartum. Gestation is stratified according to 4-wk intervals from the last menstrual period, and data are shown for those antigens used to test the largest number of women. A clear trend of decreasing mean LTSI was observed through pregnancy for *C. albicans* and SK-SD with a nadir in the third trimester. A similar trend was seen for women infected with *C. trachomatis* during pregnancy; their LT response values fell to levels seen in the uninfected women in the third trimester. The mean LTSI for all of these antigens rose postpartum.

Sequential studies among women followed throughout pregnancy and into the postpartum period. 70 women were followed sequentially through pregnancy with at least one LT assay in the first or second trimester, one assay at 32–36 wk gestation, and one assay at 3–18 wk postpartum. Of these women, 31 were tested for LTSI with *C. trachomatis* antigen in both autologous and pooled heterologous plasma on all occasions, and 26 of the 31 were also tested with *C. albicans* antigen in both autologous and pooled heterologous plasma on all occasions. In either autologous plasma or pooled heterologous plasma, the LT stimulation indices with both microbial antigens were significantly lower during pregnancy than during the postpartum period (Table I). Furthermore, two-way analysis of variance showed that although the LTSI with either antigen was significantly lower in heterologous plasma than in autologous plasma ($P < 0.0001$), the changes that were seen during the course of pregnancy and postpartum were not significantly influenced by plasma source ($P = 0.27$ and $P = 0.39$, respectively).

12 of the serially studied women had persistently positive cultures for *C. trachomatis* at all testing times.

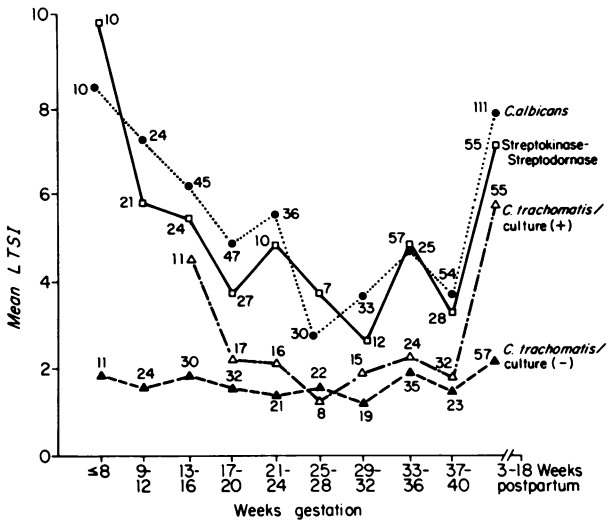

FIGURE 3 All results from the LT assay with *C. trachomatis*, SK-SD and *C. albicans* in the 201 women studied are shown. Results are categorized as to week of gestation. The number adjacent to each point represents the number of women tested at that time with that stimulant.

Among these 12 women, the mean LTSI with *C. trachomatis* antigen in autologous plasma rose significantly from values of 2.5 and 1.9 during pregnancy to 7.8 postpartum ($P = 0.0004$).

Pairwise comparison of lymphocyte proliferation responses in 117 women tested on at least two separate occasions. Although the number of women studied sequentially through pregnancy was not large enough to analyze the repeated measurements of the LTSI to PHA and other antigens by analysis of variance, it was possible to perform pairwise comparison by *t* test for women who were studied in at least two different time periods. As seen in Table II, for all microbial antigens and for both concentrations of PHA, the mean LTSI in autologous plasma was significantly lower in the third trimester of pregnancy than among the same women studied at 3–18 wk postpartum. Similarly, in autologous plasma, the mean LTSI for both concentrations of PHA and for all microbial antigens, except SK-SD and mumps, were significantly lower in women tested during first or second trimester than in the same women studied at 3–18 weeks postpartum. The mean LT responses tended to be lower in the third trimester than in the first or second trimester in the same women, although this was only significant for candida antigen (5.8 vs. 4.2, $P = 0.002$). The LTSI in pooled heterologous plasma also was significantly lower in the third trimester than postpartum for *C. trachomatis*, *C. albicans*, and SK-SD.

As shown in Table III, in both autologous and pooled heterologous plasma, the rise in LT response to *C. trachomatis* from the third trimester to postpartum was greatest for those with a positive culture for *C. trachomatis*. This rise was still significant for those who were culture negative but had serum antibody to *C. trachomatis;* however, there was no significant increase for those who were seronegative. Results were similar when the plasma source was studied more rigorously; a two-way analysis of variance showed that the effect of culture and seropositivity on postpartum LTSI were independent of plasma source (data not shown). Autologous LT response values with chlamydial antigens were lower for all three groups when first or second trimester values were compared with postpartum values; the values reached statistical significance for the culture-positive group (2.49 vs. 4.81, $P = 0.03$) and the seronegative culture-negative group (0.82 vs. 1.77, $P = 0.02$). When first or second trimester values were compared with third trimester values, no significant differences were observed among the culture-positive or the culture-negative seropositive groups. Unexpectedly, LT response values were higher

TABLE I

LTSI for C. trachomatis and C. albicans Antigens, using Autologous and Pooled Heterologous Plasma among Women Followed serially and Tested at least Once in the First or Second Trimester, Once at 32–36 wk Gestation, and Once Postpartum

Stimulant	Plasma	Geometric mean LTSI: First to second trimester	Geometric mean LTSI: Third trimester	Geometric mean LTSI: Postpartum	Effect of stage of pregnancy on LTSI*	Effect of plasma source on LTSI‡	Influence of plasma source on effect of pregnancy stage on LTSI‡
C. trachomatis	Autologous	1.8	1.8	3.8	$P = 0.0002$	$P = <0.0001$	$P = 0.27$
$n = 31$	Heterologous	1.2	1.0	2.0	$P = 0.003$		
C. albicans	Autologous	4.6	3.5	7.4	$P = 0.0005$	$P = <0.0001$	$P = 0.39$
$n = 26$	Heterologous	2.6	2.5	5.2	$P = 0.011$		

* One-way analysis of variance for repeated measures.
‡ Two-way analysis of variance for repeated measures.

TABLE II

Pairwise Comparison of LTSI in Women Studied at least Once during Pregnancy and Again Postpartum

Stimulant	Geometric mean LTSI in autologous plasma		Geometric mean LTSI in heterologous pooled plasma	
	First or second vs. postpartum	Third vs. postpartum	First or second vs. postpartum	Third vs. postpartum
C. trachomatis	$n = 81$ 1.96 vs. 2.9 $P = 0.004$	$n = 87$ 1.7 vs. 3.4 $P < 0.001$	$n = 40$ 1.48 vs. 2.18 $P = 0.05$	$n = 42$ 1.12 vs. 1.95 $P = 0.002$
C. albicans	$n = 77$ 5.7 vs. 7.9 $P = 0.002$	$n = 86$ 3.7 vs. 7.9 $P < 0.001$	$n = 38$ 3.64 vs. 5.45 $P = 0.104$	$n = 37$ 3.07 vs. 5.64 $P = 0.01$
SK-SD	$n = 30$ 6.4 vs. 8.6 $P = 0.21$	$n = 34$ 4.5 vs. 7.8 $P = 0.008$	$n = 19$ 5.38 vs. 7.66 $P = 0.396$	$n = 19$ 3.03 vs. 5.58 $P = 0.044$
Tetanus toxoid	$n = 16$ 2.3 vs. 3.5 $P = 0.02$	$n = 25$ 1.7 vs. 3.0 $P = 0.007$	$n = 11$ 2.68 vs. 3.69 $P = 0.491$	$n = 15$ 2.44 vs. 2.38 $P = 0.912$
Mumps	$n = 16$ 2.2 vs. 2.97 $P = 0.371$	$n = 21$ 1.7 vs. 2.8 $P = 0.046$	Not Done	Not Done
PHA (10 μg/ml)	$n = 22$ 138.0 vs. 190.5 $P = 0.027$	$n = 34$ 107.2 vs. 181.9 $P = 0.007$	$n = 8$ 184.1 vs. 238.8 $P = 0.515$	$n = 9$ 139.6 vs. 144.9 $P = 0.924$
PHA (2 μg/ml)	$n = 22$ 26.9 vs. 95.5 $P = 0.001$	$n = 32$ 35.5 vs. 87.0 $P < 0.001$	$n = 8$ 52.4 vs. 120.2 $P = 0.241$	$n = 9$ 66.8 vs. 70.5 $P = 0.646$

in the third trimester than in the first or second trimester for seronegative women (1.75 vs. 0.86, $P = 0.02$). This aberrant observation is unexplained. As can be seen also from Table III, the LTSI in autologous plasma with chlamydial antigens was actually very similar for these three groups of women during pregnancy, but rose significantly postpartum only for those who were culture positive or seropositive. Results were similar in heterologous

TABLE III

Pairwise Comparison of LTSI with C. trachomatis among Women Studied during the Third Trimester of Pregnancy and Postpartum when Stratified by Culture and Serologic Results

Plasma source	LTSI during third trimester vs. LTSI postpartum		
	C. trachomatis isolation positive	*C. trachomatis* isolation negative but seropositive	*C. trachomatis* isolation negative and seronegative
Autologous plasma	$n = 40$ 1.7 vs. 5.4 $P < 0.001$	$n = 27$ 1.7 vs. 2.6 $P = 0.02$	$n = 7$ 1.7 vs. 1.8 $P = 0.9$
Pooled heterologous plasma	$n = 18$ 1.18 vs. 3.00 $P = 0.007$	$n = 21$ 1.04 vs. 1.45 $P = 0.054$	$n = 4$ 1.49 vs. 1.95 $P = 0.81$

plasma. The rise in postpartum LT response to candida antigen was not related to chlamydia culture or serologic status (data not shown).

LT response to C. trachomatis antigen in women with chlamydial infection: comparison of pregnant or parturient women to nonpregnant women. From the above results, it cannot be determined whether the differences in LT response between pregnant and parturient women are attributable to depressed reactivity during pregnancy or heightened responsiveness postpartum or both. To assess this issue, we compared women at various stages in pregnancy or in the postpartum period with nonpregnant women and examined LT responses to chlamydial and candidal antigens among those with positive *C. trachomatis* cervical cultures at the time of testing. In both pregnant and nonpregnant women, *C. trachomatis* infection was initially detected by routine screening examination. Among women that were currently infected with chlamydia, the mean LTSI with chlamydial antigen in both autologous and heterologous plasma was significantly higher among nonpregnant women than among pregnant women, regardless of gestational age (Table IV). In contrast, the mean LT response to chlamydial antigens in autologous plasma was slightly higher among postpartum women with chlamydial infection than among nonpregnant women with chlamydial infection ($P = 0.09$), while no difference between postpartum and nonpregnant women was seen with pooled heterologous plasma. The mean LT responses to candidal antigens were significantly lower in the third trimester than postpartum ($P < 0.001$), and higher postpartum than in nonpregnant women, although this difference was not significant ($P = 0.2$). Thus, the difference in LTSI between pregnant and parturient women appears to be related to depression during pregnancy.

Although pregnancy plasma has been observed to have potent suppressive activity (27–33), we conclude from these analyses that depressed LT responses are apparent during pregnancy both in autologous and heterologous plasma, and that LT depression is not simply due to immunosuppressive activity of pregnancy plasma.

DISCUSSION

This study convincingly establishes in a large number of women studied sequentially that cellular immune function was depressed during pregnancy. We found that LT responses to a variety of microbial antigens and to PHA were depressed. Depression in the LT assay was evident when pregnant subjects were compared with nonpregnant subjects, or when LT responses of individual subjects were compared at different points

TABLE IV
Unpaired Comparison of LTSI Responses of Pregnant or Parturient Women vs. Nonpregnant Women

Stimulant	Third trimester vs. nonpregnant	First or second trimester vs. nonpregnant	Postpartum vs. nonpregnant
Geometric mean LTSI in autologous plasma			
C. trachomatis	n = 36 vs. 58	n = 43 vs. 58	n = 18 vs. 58
	1.9 vs. 4.8	2.3 vs. 4.8	6.6 vs. 4.8
	$P < 0.001$	$P < 0.001$	$P = 0.09$
C. albicans	n = 36 vs. 58	n = 41 vs. 58	n = 18 vs. 58
	3.7 vs. 7.8	6.5 vs. 7.8	10.5 vs. 7.8
	$P < 0.001$	$P = 0.41$	$P = 0.2$
Geometric mean LTSI in pooled heterologous plasma			
C. trachomatis	n = 26 vs. 57	n = 22 vs. 57	n = 28 vs. 57
	1.4 vs. 4.0	1.4 vs. 4.0	3.2 vs. 4.0
	$P < 0.001$	$P < 0.001$	$P = 0.46$
C. albicans	n = 26 vs. 56	n = 22 vs. 56	n = 28 vs. 56
	3.0 vs. 6.5	4.6 vs. 6.5	6.3 vs. 6.5
	$P < 0.01$	$P = 0.25$	$P = 0.6$

LT assay performed in autologous and heterologous plasma. All women had a positive *C. trachomatis* culture at the time of testing.

in pregnancy or puerperium. All LT responses tended to be lower in the third trimester than earlier in pregnancy, although this difference was significant only for candida antigen. The LT response was depressed not only to recall microbial antigens, such as mumps, SK-SD, and tetanus toxoid, but also to a microorganism (*C. trachomatis*) with which the pregnant subject was currently infected. This is the first study which documents a clear depression of the immune response to an ongoing bacterial infection during pregnancy. LT hyporesponsiveness among pregnant women occurred regardless of the type of plasma supplementation of LT assay.

While the depression of LT responsiveness during pregnancy may serve to promote survival of the fetus as an allograft, such depression may also promote excessive maternal susceptibility to infectious agents. A great deal of clinical literature suggests that the pregnant host responds less well than the nonpregnant host to a wide variety of infectious agents. Of viral infections, hepatitis (34), influenza (35), measles (36), smallpox (37), and poliomyelitis (38) have all been reported to have either higher attack rates or excessive morbidity or mortality for the pregnant host. A recent prospective study of non-A-non-B hepatitis showed that pregnant women had an eightfold excess attack rate when compared with nonpregnant women or with men (34). Furthermore, infected pregnant women had a 10-fold excess of fulminant hepatic failure when compared with infected nonpregnant women or with men. Mycotic infections also are reported to be more common or more severe in pregnancy. Vaginal isolation rates of *C. albicans* are increased during pregnancy and abruptly decrease in the puerperium (39). Coccidiomycosis has been reported to disseminate more frequently in the pregnant host (40). Untreated malarial infections have been shown to worsen during pregnancy (41). Among women followed longitudinally before and during pregnancy, parasitemia occurred 4–12 times more frequently during pregnancy than before pregnancy or in the nonpregnant comparison group. Among bacterial infections, leprosy has been noted to downgrade during pregnancy and upgrade in the puerperium. A recent prospective study of 114 women with various forms of leprosy showed that worsening of the type of leprosy occurred in 37% during pregnancy and usually during the third trimester (42). More than half of the patients whose leprosy worsened in pregnancy experienced erythema nodosum leprosum or other reversal reaction postpartum. In the nonpregnant host, such reversal reactions are associated with markedly increased LT responses to *Mycobacterium leprae* antigens (43).

The mechanism underlying depression of the LT response during pregnancy has not been resolved in this study. Since lymphocytes obtained from pregnant women continued to demonstrate hyporesponsiveness in the LT assay irrespective of the plasma source, plasma immunoregulators do not easily account for this effect. This is not to say that plasma obtained from pregnant women is not immunosuppressive; we (unpublished observations), as well as others (27–33), have noted a suppressive influence of plasma from pregnant women on in vitro LT. It may be that pregnancy plasma acts as an immunoregulator by altering cell circuits. Alpha-fetoprotein, a fetal plasma immunoregulator, has been shown to induce suppressor cells in vitro (44). A cellular basis for impaired LT response during pregnancy is strongly suggested by the recent observation that the number of T cells bearing phenotypic markers for the helper T cell subclass is decreased during pregnancy and that this alteration is most marked in the third trimester; no change in the numbers of suppressor T cells was noted (45). Helper T cells have been shown to be required for in vitro lymphocyte proliferation in response to antigens (46). Deficiency in helper T cells during pregnancy may explain our observation that LT responses are depressed. In this regard, it would be interesting to observe whether lymphocytes obtained from nonpregnant donors are altered in T cell subclasses when incubated in pregnancy plasma.

It is possible that altered kinetics of the in vitro LT response due to active (as opposed to past) infection or to pregnancy could have contributed to the apparent immunosuppression seen in this study. However, we have previously shown that the peak LT response to chlamydial antigen in *C. trachomatis* culture-positive patients occurred at 7 d (25). The kinetics of the response were also assessed in this study in eight culture-positive women studied during the second and third trimester of pregnancy and in three women studied at 4–6 wk postpartum. In seven of eight pregnant women and two of three postpartum women, the in vitro LTSI was higher after 7 d than after 3, 5, and/or 9 d of incubation.

In summary, we have shown that pregnant women have significantly depressed LT responses to a variety of microbial antigens and to PHA when compared with nonpregnant women. Longitudinal studies of individual women have shown that, in the postpartum period, the LT responses are significantly increased. The depressed LT responses during pregnancy may underlie the altered host-parasite relationships observed during pregnancy.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Dr. Jason McLurg, Laura Koutsky, Dr. Julie Adams, and Deborah Hummel.

This work was supported by National Institutes of Health grants AI 19121, AI 14180, and EY 00219.

REFERENCES

1. Maroulis, G. B., R. H. Buckley, and B. J. Younger. 1971. Serum immunoglobulin concentrations during normal pregnancy. *Am. J. Obstet. Gynecol.* 109:971–976.
2. Murray, D. L., D. T. Imagawa, D. M. Okada, and J. W. St. Gene. 1979. Antibody response to monovalent A/New Jersey/8/76. Influenza vaccine in pregnant women. *J. Clin. Microbiol.* 10:184–187.
3. Thong, Y. H., R. W. Steele, M. M. Vincent, S. A. Hensen, and J. A. Bellant. 1973. Impaired in vitro cell-mediated immunity to rubella virus during pregnancy. *N. Engl. J. Med.* 289:604–606.
4. Finn, R., C. A. St. Hill, A. J. Govan, I. G. Ralfo, F. J. Gurney, and V. Denye. 1972. Immunological responses in pregnancy and survival of fetal homograft. *Br. Med. J.* 1:150–152.
5. Tomoda, Y., M. Fuma, T. Mirva, N. Saiki, and N. Ishizuka. 1976. Cell-mediated immunity in pregnant women. *Gynecol. Invest.* 7:280–292.
6. Petrucco, O. M., R. F. Seamark, K. Holmes, L. J. Forbes, and R. C. Symons. 1976. Changes in lymphocyte function during pregnancy. *Br. J. Obstet. Gynaecol.* 83:245–250.
7. Fujisaki, S., N. Mori, T. Sasaki, and M. Maeyama. 1979. Cell-mediated immunity in human pregnancy. Changes in lymphocyte reactivity during pregnancy and postpartum. *Microbiol. Immunol.* 23:899–907.
8. Yamamoto, T., H. Hirata, H. Taniguchi, Y. Kawai, A. Uematusu, and Y. Sugiyama. 1980. Lymphocyte transformation during pregnancy: an analysis using whole-blood culture. *Obstet. Gynecol.* 55:215–219.
9. Hsu, C. C. S. 1974. Peripheral blood lymphocyte responses to phytohemagglutinin and pokeweed mitogen during pregnancy. *Proc. Soc. Exp. Biol. Med.* 146:771–775.
10. Poskitt, P. K. F., E. A. Kurt, B. P. Benoy, R. M. Selvary, A. J. Sbarra, and G. W. Mitchell. 1977. Response to mitogen during pregnancy and the postpartum period. *Obstet. Gynecol.* 50:319–323.
11. Birkeland, S. A., and K. Kristoffersen. 1977. Cellular immunity in pregnancy: blast transformation and rosette formation of maternal T and B lymphocytes. *Clin. Exp. Immunol.* 30:408–412.
12. Covelli, H. D., and R. T. Wilson. 1978. Immunologic and medical considerations in tuberculin-sensitized pregnant patients. *Am. J. Obstet. Gynecol.* 132:256–259.
13. Gehrz, R. C., W. R. Christianson, K. M. Linner, M. M. Conroy, S. A. McCue, and H. H. Balfour. 1981. A longitudinal analysis of lymphocyte proliferative responses to mitogens and antigens during human pregnancy. *Am. J. Obstet. Gynecol.* 140:665–670.
14. Carr, M. C., D. P. Stites, and H. H. Fundenberg. 1973. Cellular immune aspect of the human fetal maternal relationship. II. In vitro response of gravida lymphocytes to phytohemagglutinin. *Cell. Immunol.* 8:448–454.
15. Anderson, D. J. 1978. The responsiveness of various maternal mouse lymphocyte populations to mitogenic stimulation *in vitro*. *Cell. Immunol.* 41:150–156.
16. Harrison, M. R. 1976. Maternal immunocompetence II. Proliferative responses of maternal lymphocytes in vitro and inhibition by serum from pregnant rats. *Scand. J. Immunol.* 5:881–889.
17. Pavia, C. S., and D. P. Stites. 1979. Humoral and cellular regulation of alloimmunity in pregnancy. *J. Immunol.* 123:2194–2200.
18. Suzubik, K., and T. B. Tomasi. 1979. Immune responses during pregnancy. Evidence of suppressor cells for splenic antibody responses. *J. Exp. Med.* 150:889–908.
19. Wentworth, B. B., and E. R. Alexander. 1974. Isolation of *Chlamydia trachomatis* by use of 5-ido-2-deoxyuridine-treated cells. *Appl. Microbiol.* 27:912–917.
20. Yoder, B. L., W. E. Stamm, C. M. Koester, and E. R. Alexander. 1981. Microtest procedure for isolation of *Chlamydia trachomatis*. *J. Clin. Microbiol.* 13:1036–1039.
21. Kuo, C. C., S. P. Wang, B. B. Wentworth, and J. T. Grayson. 1972. Primary isolation of TRIC organisms in HeLa 229 cells treated with DEAE-dextran. *J. Infect. Dis.* 125:665–668.
22. Wand, S. P., and J. T. Grayson. 1974. Human serology in *Chlamydia trachomatis* infection with microimmunofluorescence. *J. Infect. Dis.* 130:388–397.
23. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *J. Clin. Lab. Invest.* 21(Suppl. 97):77–89.
24. Lowry, O. H., N. J. Rosenbrough, A. L. Fan, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.
25. Brunham, R. C., D. H. Martin, C. C. Kuo, S. P. Wangk, C. E. Stevens, T. Hubbard, and K. K. Holmes. 1981. Cellular immune response during uncomplicated genital infection with *Chlamydia trachomatis* in humans. *Infect. Immun.* 34:98–104.
26. Armitage, P. 1973. *In* Statistical Methods in Medical Research. Blackwell Scientific Publications, London. 189–217.
27. Kasakura, S. 1971. A factor in maternal plasma during pregnancy that suppresses the reactivity of mixed leukocyte cultures. *J. Immunol.* 107:1296–1301.
28. St. Hill, C. A., R. Finn, and V. Denye. 1973. Depression of cellular immunity in pregnancy due to a serum factor. *Br. Med. J.* 3:513–514.
29. McIntyre, J. A., and W. P. Faulk. 1979. Maternal blocking factors in human pregnancy are found in plasma not serum. *Lancet.* II:821–823.
30. Noonan, F. P., W. J. Halliday, H. Morton, and G. J. A. Clunie. 1979. Early pregnancy factor is immunosuppressive. *Nature (Lond.).* 278:649–651.
31. Hammarstrom, L., T. Fuchs, and C. I. E. Smith. 1979. The immunodepressive effect of human glucoproteins and their possible role in the nonrejection process during pregnancy. *Acta Obstet. Gynecol. Scand.* 58:477–422.
32. Bissenden, J. G., N. R. Ling, and P. MacKintosh. 1980. Suppression of mixed lymphocyte reactions by pregnancy serum. *Clin. Exp. Immunol.* 39:195–200.
33. Okamura, K., M. Kuramoto, Y. Hamazaki, K. Takahashi, and M. Suzuki. 1980. Immunosuppressive factors in pregnancy serum: a preliminary report. *Tohoku J. Exp. Med.* 130:11–33.
34. Khuroo, M. S., M. R. Teli, S. Skidmore, M. A. Sofi, and M. I. Khuroo. 1981. Incidence and severity of viral hepatitis in pregnancy. *Am. J. Med.* 70:252–255.
35. Greenberg, M., H. Jacobziner, J. Pakter, and B. A. G. Weisl. 1958. Maternal mortality in the epidemic of Asian influenza, New York City, 1957. *Am. J. Obstet. Gynecol.* 76:896–902.
36. Christensen, P. E., H. Schmidt, H. O. Ban, V. Andersen,

B. Hordal, and O. Jensen. 1953. An epidemic of measles from Southern Greenland, 1951. *Acta Med Scand.* 144:430–449.
37. Rao, A. R., I. Prahlad, M. Swaminathan, and A. Lakshmi. 1963. Pregnancy and smallpox. *J. Indian Med. Assoc.* 40:353–363.
38. Siegel, M., and M. Greenberg. 1955. Incidence of poliomyelitis in pregnancy. Its relation to maternal age, parity and gestational period. *N. Engl. J. Med.* 253:841–847.
39. Odds, F. C. 1979. Factors that predispose the host to candidosis. *In* Candida and Candidosis. F. C. Odds, editor. University Park Press, Baltimore. 75.
40. Vaughan, J. E., and H. Ramirex. 1951. Coccidioidomycosis as a complication of pregnancy. *Calif. Med.* 74:121–125.
41. Gilles, H. M., J. B. Lawson, M. Sibilas, A. Voller, and N. Allan. 1969. Malaria, anaemia and pregnancy. *Ann. Trop. Med. Parasitol.* 3:245–263.
42. Duncan, M. E., R. Melsom, J. M. H. Pearson, and D. S. Ridley. 1981. The association of pregnancy and leprosy. *Lepr. Rev.* 52:245–262.
43. Bjune, G., C. St. Barnetson, D. S. Ridley, and G. Dronvall. 1976. Lymphocyte transformation test in leprosy; correlation of the response with inflammation of the lesions. *Clin. Exp. Immunol.* 25:85–94.
44. Murgita, R. A., E. A. Goid, S. Kontiainen, and H. Wogzell. 1977. α-fetoprotein induces suppressor T cells in vitro. *Nature (Lond.).* 267:257–259.
45. Snidama, V., F. Pacini, S.-L. Yang, A. Moawad, M. Reily, and L. J. DeGrott. 1982. Decreased levels of helper T cells: a possible cause of immunodeficiency in pregnancy. *N. Engl. J. Med.* 307:352–356.
46. Reinherz, E. L., P. C. Kung, G. Goldstein, and S. F. Schlossman. 1979. Separation of functional subsets of human T-cells by monoclonal antibody. *Proc. Natl. Acad. Sci. USA.* 76:4061–4065.