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Human Lymphocyte Subpopulations: The Effect of Pregnancy[1] (39463)

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The pregnancy state has always been an immunological enigma in that the fetus can survive in the uterus across a histocompatibility barrier without undergoing transplantation rejection. That this may be related to a modification of immunological status of the mother is suggested by the observations that (i) her lymphocytes respond poorly to mitogenic stimulation, especially in the presence of autologous serum (1), (ii) skin graft survival is prolonged (2), and (iii) the response to tuberculin skin testing is depressed (3). The present investigations were carried out to ascertain if these phenomena might be due to changes in the pool sizes of the various lymphocyte subpopulations.

Methods. Subjects. Blood was drawn from two groups of women after obtaining their informed consent: The first group consisted of females in the third trimester of a normal pregnancy who were attending the UCLA prenatal clinic; the second group consisted of normal nonpregnant female volunteers of child-bearing age who had never taken oral contraceptives or other hormonal supplements or had not taken either within the previous 30 or more days.

Lymphocyte Preparations. Blood was drawn in heparinized tubes between 9 AM and 11 AM in all subjects. Total lymphocyte counts were determined by routine methods from total white blood cell counts and differential blood smears. Lymphocytes were separated from peripheral blood by Ficol-Hypaque sedimentation and then incubated with latex beads in serum-free media at 37° for 30 min to facilitate the later identification of monocytes, as previously described (4).

B-Lymphocytes. Two receptors found primarily on B-lymphocytes were identified, namely, the receptor for aggregated IgG (AggIgG) and the receptor for complement. The receptor for AggIgG was identified as described elsewhere (4). After being incubated with heat-aggregated gamma globulin, lymphocytes were washed and incubated with fluorescein-conjugated goat anti-human gamma globulin antisera. After being washed the cells were examined live under phase contrast and epifluorescent microscopy. Since the fluorescein-conjugated antisera had polyvalent activity, the assay did, in fact, identify lymphocytes with surface immunoglobulin and/or receptors for aggregated IgG.

The method for the detection of the receptor for complement was modified slightly from that of Mendes (5). Zymosan granules were disaggregated by sonication and washed in Hank's balanced salt solution (HBSS). They were sensitized with a 1:5 dilution of fresh or deep frozen human serum in HBSS. After washing and resuspending the complement-coated granules (ZyC) to 10^8/ml., 0.1-ml lymphocyte suspension (5×10^6/ml) was mixed with 0.1 ml ZyC and spun at 50*g* for 5 min. After overnight incubation at 4° the cells were resuspended, several drops of 0.1% crystal violet solution (to identify lymphocytes) were added, and a minimum of 400 cells were examined. A lymphocyte binding two or more granules was considered to have receptors for complement.

T-lymphocytes. T-lymphocytes were identified by their ability to bind sheep red blood cells (SRBC) as described elsewhere (4). Briefly 0.1 cc of a 0.5% SRBC solution supplemented with 20% fetal calf serum (previously absorbed with SRBC) was mixed with 0.1 cc of lymphocyte suspension (5×10^6/ml). After incubation at 37° for 5 min and spinning at 50*g* for 5 min, the mixture was incubated overnight at 4°. Just prior to examination, crystal violet was added and the cells were resuspended on a

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vertical rotator for 2.5 minutes. Lymphocytes which bound three or more SRBC were considered as T-lymphocytes.

Statistical Analyses. All results were calculated and expressed as mean ± standard error. Comparison between groups was made using Student's *t* test.

Results. The recoveries of mononuclear cells from the interface of the Ficol-Hypaque gradient separation of blood were similar in pregnant and nonpregnant subjects (66.2 ± 4.8% vs 64.2 ± 6.2%).

In Table I are shown the percentages and absolute numbers of lymphocytes and lymphocyte subpopulations in pregnant and nonpregnant females. Of particular note is that in pregnant females there was a significant reduction in cells with receptors for AggIgG as compared to their nonpregnant counterparts ($p < 0.001$). Conversely, cells with receptors for complement (the other B cell marker) were equal in numbers in both groups. The percentage of T-lymphocytes was increased significantly in the pregnant group although the absolute numbers were not.

Discussion. In many respects pregnancy is equivalent to an organ transplantation across a histocompatibility barrier. The fact that the fetus is seldom rejected (aborted) may be, in part at least, a reflection of a modification of the mother's immunological mechanisms (6). By identifying and quantitating B- and T-lymphocyte subpopulations, which are thought to regulate humoral and cell-mediated immunity, the present investigation examined the hypothesis that such modification may result from changes in the absolute pool sizes of certain lymphocyte subpopulations. While the absolute numbers of cells with receptors for sheep red blood cells (SRBC) and for complement were similar in both pregnant and nonpregnant females, the absolute numbers of lymphocytes with receptors for aggregated IgG (AggIgG) were significantly reduced in pregnant females. In normal subjects these two lymphocyte subpopulations represent two discrete but partially overlapping subpopulations of the B-series. The present findings suggest that these two lymphocyte subpopulations are affected to different degrees by pregnancy. While lymphocytes with complement receptors appear to be unaffected, those with receptors for AggIgG are diminished. These findings would tend to fortify those of two previously published studies in which surface markers of peripheral blood lymphocytes were examined during pregnancy. While Gergely and his associates examined surface immunoglobulin (SIg) and receptors for SRBC on the lymphocytes of pregnant females, Brain *et al.* examined only for cells with SIg (7, 8). Both

TABLE I. PERCENTS AND ABSOLUTE NUMBERS OF LYMPHOCYTE SUBPOPULATIONS IN PERIPHERAL BLOOD AT 9:00 A.M. IN PREGNANT AND NONPREGNANT FEMALES[a].

	Nonpregnant female controls	Pregnant females
Absolute lymphocyte count (per mm³)	2799 ± 213 (23)[b]	2444 ± 164 (28)
% AggIgG[c]	21.9 ± 1.3 (23)	13.1 ± 0.7[d] (23)
Absolute AggIgG Count (per mm³)	614 ± 68	307 ± 28[d]
% CRL[e]	13.6 ± 1.4 (13)	14.0 ± 0.9 (15)
Absolute CRL Count (per mm³)	357 ± 42	338 ± 39
% T	70.7 ± 1.7 (10)	77.0 ± 0.9[f] (15)
Absolute T lymphocyte Count	1727 ± 191	1900 ± 187

[a] = All values expressed as mean ± standard error.
[b] = Number of subjects tested given in parentheses.
[c] = Lymphocytes with receptors for aggregated IgG.
[d] = ($p < 0.001$).
[e] = Lymphocytes with receptors for complement.
[f] = ($p < 0.01$).

groups found these subpopulations to be in normal numbers.

The findings here are compatible with the hypothesis that the immunological changes which occur during pregnancy may be mediated, at least in part, by one or more of the following alterations in certain lymphocyte subpopulations: (i) A decrease in the total number of certain subpopulations throughout the body; (ii) A redistribution of certain subpopulations from peripheral blood to other organs; (iii) Certain subpopulations may remain in the peripheral blood but their receptors may be modified so that they are no longer detectable. Changes in pool size or in receptors alone, however, may not be directly related to changes in cell function. Therefore, to know the true significance of the findings here, they must be correlated with studies of cell function directly, i.e., antibody-dependent cell-mediated cytotoxicity or *in vitro* immunoglobulin synthesis to evaluate the functional capacity of cells with receptors for AggIgG (9–11).

Since the blood levels of the steroid sex hormones (estrogen, progesterone, and possibly other steroidal hormones) rise in pregnancy, attempts have been made with some success by many investigators to reproduce the immunologic abnormalities of pregnancy by the administration of these hormones to nonpregnant subjects. Whether the changes seen in lymphocyte receptors during pregnancy can be reproduced by the administration of steroidal sex hormones is presently under investigation in our laboratory.

Summary. Peripheral blood lymphocytes from pregnant and nonpregnant females were studied for the presence of the following surface receptors: (i) Receptor for heat-aggregated human IgG (AggIgG); (ii) Receptor(s) for complement components; (iii) Receptor for sheep red blood cells (SRBC).

Absolute numbers of lymphocytes with receptors for complement and those with receptors for SRBC were present in equal numbers in both groups. However, in the pregnant females there was a significantly lower number of lymphocytes with receptors for AggIgG. It is hypothesized that some of the immunological changes which occur during pregnancy may be mediated partly through changes in the total numbers of certain lymphocyte subpopulations.

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