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Peripheral Blood Lymphocyte Responses to Phytohemagglutinin and Pokeweed Mitogen during Pregnancy (38190)

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(Introduced by P. Y. Paterson)

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The human fetus carries paternal antigens early in embryonic life and immunobiologically speaking represents a foreign tissue graft (1). The basic mechanisms responsible for the "extended take" of this allograft have remained an enigma. One of the proposed mechanisms (2) is that pregnant women have diminished immune responses which are insufficient to reject the fetus.

We studied the *in vitro* responses of pregnant women's peripheral blood lymphocytes to pokeweed mitogen (PWM), a stimulant of both thymus-derived T-lymphocytes (T-cells) and bone marrow-derived B-lymphocytes (B-cells) and to phytohemagglutinin (PHA), a predominant T-cell mitogen (3, 4). We have found that pregnant women's lymphocytes exhibit a diminished response to PWM whereas their response to PHA is not altered. In addition, we have confirmed the findings of others that plasma factors occur in association with pregnancy which inhibit lymphocyte responses to both of these mitogens.

Materials and Methods. Eleven pregnant women ranging in age from 21 to 39 years (mean 28 years) from the Northwestern University, McGaw Medical Center Prenatal Clinic formed the study group. With one exception (S. A.), all were over 34 weeks of gestation. All but 2 had normal uneventful courses of pregnancy. Patient O. G. had mild diabetes. N. R. contracted a varicella infection 10 days after conception and had a history of severe asthma. The number of previous pregnancies varied from 0 to 6 with a mean of 1.45.

Thirteen clinically well female laboratory personnel not on any medications formed the control group. Their age varied from 21 to 36 years with a mean of 26 years.

The lymphocyte cultures were performed as reported previously (5). The peripheral blood leukocytes which were separated by sedimentation of erythrocytes were washed 3 times with Roswell Park Memorial Institute (RPMI) medium 1640 containing 200 units/ml of penicillin and 200 μg/ml of streptomycin. After total and differential cell counts, cultures consisting of 0.5×10^6 lymphocytes in 0.5 ml medium with 20% autologous or an allogeneic reference plasma were set up in triplicate or quadruplicate. Allogeneic reference plasma (or reference plasma) is a plasma taken from a clinically well donor and in which lymphocytes from all control and pregnant women were cultured in order to compare the responsiveness of washed lymphocytes. PHA-P (Difco) at final concentrations of 6.25, 12.5 and 25 μg/ml and PWM (GIBCO) at final concentrations of 0.2, 0.4, 0.8 and 1.6 μl of reconstituted vial per ml were added to the culture. The reconstituted PWM contains about 1 mg protein per ml. The cultures were maintained at 37° in a humidified 5% CO_2–95% air atmosphere for 3 days after addition of PHA-P and 6 days after addition of PWM. For monitoring the lymphocyte responses, the cells were labeled with tritiated thy-

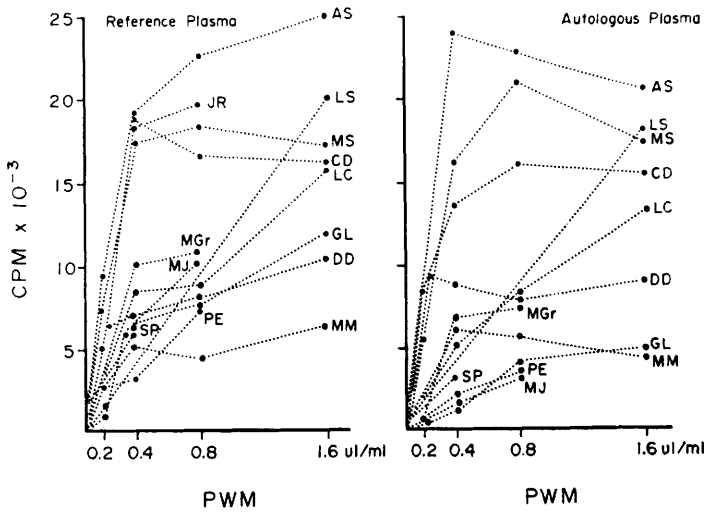

FIG. 1. Tritiated thymidine uptake of lymphocytes from control women stimulated by varying doses of PWM in allogeneic reference plasma and autologous plasma.

midine (0.5 μCi per culture) for 24 hr before harvest. At the time of harvest, cells were washed twice with 2 ml of normal saline and then twice with 2 ml of 5% trichloroacetic acid. The precipitates were dissolved in 0.5 ml Hydroxide of Hyamine 10x(Packard). Ten ml of Bray's solution was added and radioactivity was counted in a Packard scintillation counter (model 3951). The results are expressed in mean counts per min (CPM) of triplicates. In case of quadruplicate cultures, the 4th culture was used for viable cell count using 0.2% trypan blue in normal saline. The results are expressed as the percentages of viable cells after 6 days of culture relative to the original number of lymphocytes put into the culture (i.e., 0.5 $\times$ 10^6 cells) which is defined as 100%.

Results. The results of lymphocyte responses to PWM of control and pregnant women are shown in Figs. 1 and 2. When cultured in the reference plasma, markedly diminished response of lymphocytes from

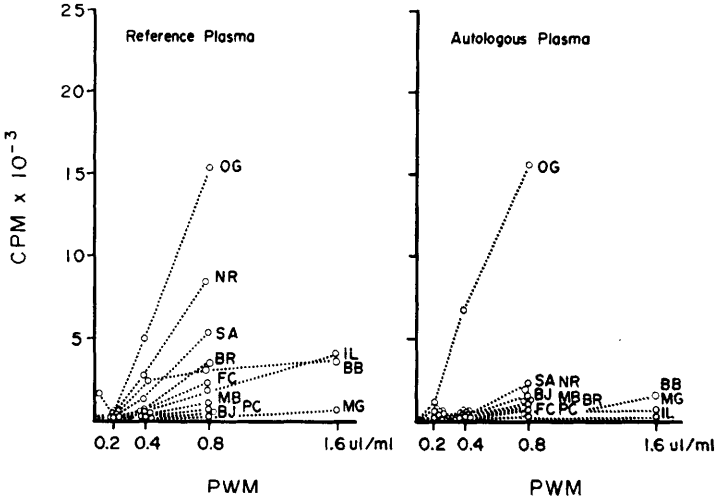

FIG. 2. Tritiated thymidine uptake of lymphocytes from pregnant women stimulated by varying doses of PWM in allogeneic reference plasma and autologous plasma.

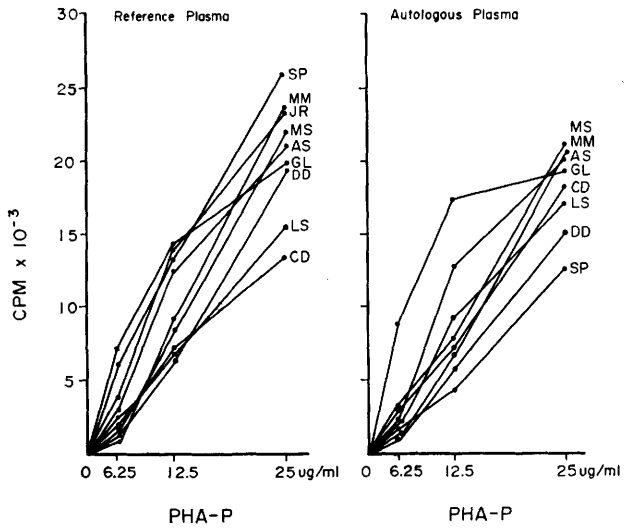

FIG. 3. Tritiated thymidine uptake of lymphocytes from control women stimulated by varying doses of PHA-P in allogeneic reference plasma and autologous plasma.

pregnant women was noted with all 4 dosages of PWM in comparison with that from control women. The depression of lymphocyte response of pregnant women was further enhanced (except in patient O. G.) by maintaining the culture in the autologous plasma indicating the presence of inhibitors in the plasma. With the reference plasma, the difference in lymphocyte responses between 2 groups of individuals at each dosage of PWM was highly significant ($P < 0.001$). The diminished lymphocyte response was not related to the viability of lymphocytes which, after 6 days of culture, ranged from 50% to 101% with a mean ± standard deviations (S.D.) of 67.8 ± 14.8% in the control group and ranged from 35% to 104% with a mean ± S.D. of 72.9 ± 21.3% in the pregnant group. The diminished lymphocyte response also did not appear to be related to a higher number of polymorphonuclear cells

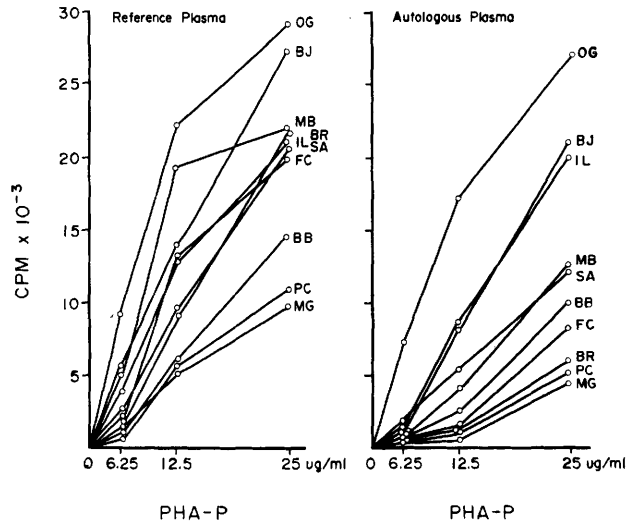

FIG. 4. Tritiated thymidine uptake of lymphocytes from pregnant women stimulated by varying doses of PHA-P in allogeneic reference plasma and autologous plasma.

(polys) contaminating the cultures of the pregnant group (6), because of the wide overlap in the degree of contamination of the polys between the 2 groups. The ratio of polys to lymphocytes in the control group ranged from 0.41 to 4.88 with a mean ± S.D. of 1.98 ± 1.25, while that in the pregnant group ranged from 1.94 to 4.88 with a mean ± S.D. of 3.37 ± 0.84. Examination of individual cases also excludes contamination by the polys as a significant cause of the depressed lymphocyte response to PWM.

The results of lymphocyte responses to PHA-P are shown in Figs. 3 and 4. When cultured in the reference plasma there was no statistical difference between the lymphocyte response of the control and the pregnant groups for each dose of PHA-P ($P > 0.05$). However, all plasma specimens from pregnant women inhibited lymphocyte response to PHA-P in comparison to reference plasma. And if compared with that of the control women, the inhibition of lymphocyte response by plasma from pregnant group is significant at PHA dose of 12.5 μg/ml ($P < 0.05$).

The plasma of a pregnant subject O. G. inhibited lymphocyte response to PHA-P while that to PWM was not affected. An opposite phenomenon was noted with the plasma from a control subject G. L. (Table I). In the control group, 2 other plasma samples (S. P. and J. R.) were found to contain inhibitors of both PHA and PWM stimulated lymphocyte responses.

Discussion. Studies on the lymphocyte response *in vitro* to PHA during pregnancy have been reported with conflicting results. A normal (7, 8), hypoactive (9) or hyperactive (10) response has been reported. In those studies which showed normal or hyperactive response to PHA, a plasma inhibitor depressed the response to PHA. Our results indicate that while both hypo- and hyper-active responses may occur, lymphocyte reactivity to PHA during pregnancy is generally normal. And we confirmed the findings of others that in all plasmas from pregnant women there are inhibitors of lymphocyte response to PHA.

Studies on the lymphocyte response to

TABLE I. Lymphocyte Responses (Expressed as Mean CPM) of a Pregnant (O. G.) and a Normal (G. L.) Individuals Cultured with 20% Reference or Autologous Plasma, Demonstrating Dissociation of Plasma Inhibitory Effects on Lymphocyte Responses to PHA and PWM.

Sources of lymphocytes	Sources of plasma	Doses of PHA (μg/ml)				Doses of PWM (μl/ml)			
		0	6.25	12.5	25.0	0	0.2	0.4	0.8	1.6
O. G.	Reference	313	9,224	22,289	29,345	203	615	5,007	15,340	—
O. G.	Autologous	283	7,104	17,184	26,877	403	1,006	6,725	15,565	—
G. L.	Reference	168	7,218	14,096	20,195	251	2,014	3,263	7,340	11,978
G. L.	Autologous	171	8,798	17,321	19,471	264	477	1,259	4,107	5,142

PWM during pregnancy have not been reported. Our results which demonstrate impaired response of pregnant women's lymphocytes to PWM independent of the plasma lymphocyte inhibitors cannot be explained on the basis of a difference in cell viability and appears unrelated to higher numbers of polys contaminating the cultures of pregnant group. Studies from various sources have established that PHA is a predominant T-cell mitogen whereas PWM stimulates both T- and B-cells (3, 4). Since lymphocytes of pregnant women responded normally to PHA, it seems reasonable to postulate that the depressed B-cell reactivity is the major cause of diminished response of their lymphocytes to PWM. Our results suggest that this intrinsic B-cell hypo-reactivity in conjunction with plasma lymphocyte inhibitors produced marked impairment of lymphocyte response to PWM in pregnant group.

Inhibition of PHA-stimulated lymphocyte response by plasma studied by us is generally associated with inhibition of PWM-stimulated lymphocyte response. Similar findings were also found in plasmas from patients with ataxia telangiectasia (11). Our observation that the plasma which inhibited lymphocyte response to one mitogen may not inhibit the response to the other (see Table I) deserves attention. This indicates that different plasma factors are involved in inhibition of PHA- and PWM-induced lymphocyte responses. It would be of importance to further study and clearly document this phenomenon.

Some plasma from our control group (S. P. and J. R.) were also found to have inhibitors of PHA- and PWM-stimulated lymphocyte responses. It has been shown that patients with relatively minor conditions such as allergic rhinitis may also have inhibitors in their plasmas (5, 12). It is possible that our control individuals whose plasma contained inhibitors had unrecognized minor allergic disorders.

Summary. Peripheral blood lymphocytes from women in late gestation were cultured in an allogeneic reference plasma and autologous plasma. Lymphocytes cultured with autologous plasma exhibited markedly low response to both pokeweed mitogen (PWM, a B- and T-cell stimulant) and phytohemagglutinin (PHA, a predominant T-cell stimulant). When cultured with the reference plasma, lymphocytes from pregnant women showed a markedly impaired response to PWM whereas their responses to PHA was essentially the same as that of age-matched clinically well nonpregnant control women. The findings may suggest B-lymphocyte alterations during pregnancy in addition to plasma lymphocyte inhibitors. Dissociation of plasma inhibitory effects on lymphocyte responses to PHA and PWM was also noted.

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