IMMUNE RESPONSES DURING PREGNANCY

Evidence of Suppressor Cells for Splenic Antibody Response*

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Mammalian pregnancy is a unique immunological paradox because the fetus, which bears paternal histocompatible antigens which are alien to the maternal immune system can survive normally as an allograft in the mother. Several mechanisms have been proposed to explain fetal acceptance including nonspecific suppression of maternal immunity (1). Previous studies have suggested that factors in pregnant sera play a role in the suppression of maternal immunity (2–9), including sex hormones (2, 3), serum-blocking antibody (4), pregnancy-associated proteins (5), α-fetoprotein (6, 7), and as yet undefined factors. However, none of these have been established as a major factor in the suppressed maternal immunity. Moreover, contradictory results have been recently reported by Smith (8) and Hamilton and Hellström (9), who claim that sera from syngeneically or allogeneically pregnant mice do not inhibit the responses of normal lymphocytes to alloantigens. The reasons for these discrepancies are unclear. Other evidence which suggests suppressed maternal immunity during pregnancy includes depressed mixed lymphocyte reactions (10), diminished mitogen responses (6, 11), decreased leukocyte migration (12), and delayed skin graft rejection (13). An increased frequent occurrence of severe viral infection during pregnancy (14) has also been postulated to be related to suppressed maternal immunity. There have, however, been very few reports investigating the antibody response following challenge in pregnant animals. One study (15) reports that the circulating antibody titers to several antigens including sheep erythrocytes (SRBC)[1] were not significantly altered during pregnancy while we have previously reported a decreased splenic plaque-forming-cell (PFC) response following in vivo immunization of pregnant mice (7).

The present study shows that in vitro IgM antibody synthesis against SRBC is decreased in the pregnant spleen cells compared to controls, and moreover this appears to be mediated by nonspecific splenic suppressor cells. At least two populations of pregnant spleen cells were shown to exert a nonspecific suppressor effect. One is a T lymphocyte and the other a nylon wool-adherent cell present in the B-cell-enriched macrophage-depleted fraction. Pregnant spleen cells cultured in vitro secrete soluble supernate factors which decrease the primary IgM antibody response of normal spleen cells. We were unable to demonstrate suppressor cells in the pregnant lymph node

* This work was supported by grant CA-18204, awarded by the National Cancer Institute, Department of Health, Education, and Welfare, and by National Institutes of Health grant HD-09720.

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[1] *Abbreviations used in this paper*: BSS, balanced salt solution; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; LNC, lymph node cell; LPS, lipopolysaccharide; OVA, ovalbumin; PFS, plaque-forming cells; PHA, phytohemagglutinin; PPD, purified protein derivative; SRBC, sheep erythrocytes.

J. Exp. Med. © The Rockefeller University Press · 0022-1007/79/10/0898/11 $1.00
Volume 150 October 1979 898–908

cells of cervical, axillary, and popliteal regions. Finally, mitogen- and antigen-induced lymph node cell proliferative responses including those lymph nodes draining the uterus, were not significantly different in pregnant and control animals.

Materials and Methods

Animals. CBA/J mice were purchased from The Jackson Laboratory, Bar Harbor, Maine, and BALB/c mice from Cumberland View Farm, Clinton, Tenn. Pregnant CBA/J mice were obtained by mating CBA/J female mice to CBA/J male mice (syngeneically pregnant), and to BALB/c male mice (allogeneically pregnant) in our animal facility. The time of pregnancy was determined by examination of vaginal plugs.

In Vivo Antibody Synthesis. Approximately 10- to 13-d pregnant CBA/J mice and age-matched CBA/J female mice were used. 5×10^8 SRBC were injected i.p. 4 d later spleens were removed and the primary IgM antibody responses against SRBC were studied using Jerne's hemolytic plaque assay as modified by Cunningham and Szenberg (16).

To study the antibody responses of lymph node cells, 10×10^7 SRBC were injected into both footpads of control or pregnant mice. 4 d later the popliteal lymph nodes were removed and the PFC assay performed. The assay was done on individual mice, and the mean and SE obtained.

In Vitro Antibody Response. Approximately 15-d pregnant and age-matched control CBA/J female mice were employed for the in vitro antibody responses and the mitogen-induced proliferative responses. 10×10^6 spleen cells were cultured with 10×10^6 SRBC in a Marbrook chamber for 4 d at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Culture media consisted of RPMI-1640 supplemented with 5% heat-inactivated fetal calf serum (FCS) and horse serum, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 2 mM glutamine (all supplements from Grand Island Biological Co., Grand Island, N.Y.). The cultures were performed on lymph node and spleen cells from individual mice or on pools of 4–5 mice. The mean numbers of PFC per culture were calculated from the result of triplicate cultures.

Mitogen-induced Blastogenesis. 2×10^5 spleen cells or lymph node cells were placed into each well of a flat-bottomed microtiter plate (No. 3042, Falcon Plastics, Div. of Becton, Dickinson & Co., Oxnard, Calif.) and were routinely cultured in the presence of an optimal dose (4.0 μg/ml) of phytohemagglutinin (PHA; The Wellcome Research Laboratories, Beckenham, Kent, England), or 50 μg/ml of *Escherichia coli*, lipopolysaccharide (LPS; Difco Laboratories, Detroit, Mich.). However, various doses of PHA (1, 2, 4, and 8 μg/ml) and LPS (10, 20, 50, and 100 μg/ml) have also been employed to be certain that there was no shift in the dose response curve. The culture media consisted of the same components as indicated for the in vitro antibody response except that the serum supplement was 5% heat-inactivated FCS. The cultures were maintained at 37°C for 3 d in a humidified atmosphere of 5% CO_2 and 95% air. 16 h before harvesting, 1 μCi of [^{3}H]methyl thymidine in 20 μl of RPMI-1640 was added to each well. The cells were harvested on a glass fiber paper using a Mash II harvester (Microbiological Associates, Walkersville, Md.) and dissolved in 2 ml of Aquasol (New England Nuclear, Boston, Mass.). The radioactivity was then determined using a Beckman scintillation counter (Beckman Instruments, Fullerton, Calif.). All determinations were performed in triplicate and the data is expressed as the mean cpm ± SE.

Antigen-induced Lymph Node Cell Proliferation. Antigen-induced lymph node cell (LNC) proliferative assay was performed using a modification of the method described by Alkan (17). Briefly, 100 μg of ovalbumin (OVA; Sigma Chemical Co., St. Louis, Mo.) emulsified in H37Ra complete adjuvant (Difco Labs) was injected into ~10- to 12-d pregnant CBA/J mice and age-matched control mice at three different sites: anterior and posterior footpads, and s.c. in the base of the tail. 7 d later draining lymph nodes of these three immunization sites were separately obtained from axillary, popliteal, and para-aortic (uterus-draining) regions, and 4×10^5 sensitized LNCs from each region were cultured in vitro with either 250 μg/ml of OVA or 50 μg/ml of purified protein derivative (PPD; Connaught Laboratories, Toronto, Canada). 4 d later DNA synthesis was measured as described in mitogen-induced blastogenesis.

Cell Preparation. Single cell suspensions of spleen and lymph node cells were prepared by gentle teasing and passing the cells through a screen mesh. After being washed twice in cold

Hank's balanced salt solution (BSS), the cells were resuspended in culture media. In some experiments erythrocytes contained in spleens were lysed using ammonium-chloride-potassium lysing buffer. Nylon-wool-nonadherent T lymphocytes isolated from spleens (subsequently referred to as spleen T cells) were obtained by the method described by Julius et al. (18). Nylon-wool-adherent cells were obtained using a modification of the method described by Handwerger and Schwartz (19). Briefly, after nonadherent T cells were eluted, the nylon wool column was washed with warm culture media. The nylon wool was then compressed with a plunger and flushed with cold culture media. This process was repeated three to four times and the cells resuspended in culture media. Splenic macrophages were obtained as follows: 10×10^7 spleen cells in 10 ml culture media were plated on 100×20-mm plastic Petri dishes (Falcon Plastics) and incubated for 2 h at 37°C. The nonadherent cells were removed, the plates washed with media, and the cells adherent to the dishes were then dislodged by vigorous pipettings using Ca^{++}-free Hank's BSS containing 0.02% EDTA. The cells were washed three times before use. The recovered adherent cell population showed >95% viability by trypan blue exclusion, and contained predominantly macrophages (85–90% as determined by latex ingestion or esterase staining).

Pretreatment with anti-Thy 1.2 serum plus complement was done as follows: $\sim 50 \times 10^6$ cells were incubated with a 1:20 dilution of anti-Thy 1.2 serum (AKR anti-C3H thymocyte, purchased from Bionetics Laboratory Products, Litton Bionetics Inc., Kensington, Md.) for 30 min at room temperature. The cells were washed once and resuspended in a 1:10 dilution of guinea pig complement. After incubation at 37°C for 45 min, the cells were washed three times. PHA- and LPS-induced blastogenesis was used to assess the effectiveness of this treatment. All preparations showed a marked diminution of the PHA response and essentially no change in the LPS response.

Results

In Vivo and In Vitro Responses of Pregnant vs. Age-matched Control Mice. As shown in Table I, a significant decrease of the primary IgM antibody response in vivo against SRBC was observed in the pregnant spleen cells, compared to that of the age-matched controls. However, there was no difference in the in vivo response of lymph node cells between the pregnant and control groups. Table II shows the results of the in vitro primary IgM antibody responses. The PFC response of the pregnant spleen cells were two to three times less than those of controls. Similar decreases were obtained with both syngeneically and allogeneically pregnant spleen cells and also in the cultures stimulated with various doses of SRBC (data not shown). The PFC responses in vitro of the lymph node cells from cervical, axillary, and popliteal regions were essentially identical in pregnant and control animals; this experiment was repeated three times with essentially identical results.

Table III shows the results of the mitogen-induced blastogenesis of spleen cells and lymph node cells including those of draining lymph nodes of the uterus. The data shows that there was no significant difference between the two groups of mice. Similar results were obtained when various concentrations of mitogens as well as different cell densities in the culture were employed (data not shown).

Table IV demonstrates that ovalbumin and PPD-induced lymph node cell proliferation are not significantly altered in pregnant animals.

Is the Low PFC Response of the Pregnant Spleen Cells a Result of Suppressor Cells? The experiment shown in Fig. 1 was performed in an attempt to determine whether or not suppressor cells were involved in the decreased PFC response of the pregnant spleen cells. Various numbers of spleen cells from ~15-d pregnant or age-matched control mice were added to the normal spleen cell culture containing 10×10^6 normal spleen cells/chamber, and the mixture cultured with 10×10^6 SRBC for 4 d. As shown in

TABLE I
Primary In Vivo IgM Response

Cell	PFC/1 × 10^6 cells	
	Control mice	Pregnant mice
	mean ± SE	
Spleen	1,206 ± 151 (9)	635 ± 80 (18)*
Lymph node	146 ± 15 (4)	178 ± 19 (4)‡

Pregnant (syngeneically) CBA/J mice and age-matched control CBA/J female mice were immunized i.p. with 5 × 10^8 SRBC and 4 d later direct IgM PFC assay performed on spleen cells. For direct IgM responses of lymph node cells, 10 × 10^7 SRBC were injected into both footpads, and 4 d later PFC assay performed on popliteal lymph node cells. The assay was performed on individual mice and the mean value obtained. The number in parentheses represents the number of mice studied.

* $P < 0.01$ compared to control mice.

‡ No significant difference observed.

TABLE II
Primary In Vitro IgM Response

Cell	SRBC in culture	PFC/culture	
		Control mice	Pregnant mice
		mean ± SE	
Spleen	+	2,412 ± 238 (4)	948 ± 106 (4)*
	−	151 ± 13	62 ± 11
Lymph node	+	1,210 ± 104 (3)	1,320 ± 134 (3)‡
	−	58 ± 7	64 ± 10

10 × 10^6 cells were cultured with 10 × 10^6 SRBC in a Marbrook chamber and 4 d later direct IgM PFC assay performed. The cultures were done on individual mice and the mean value of PFC response obtained. The number in parentheses represents the number of mice studied.

* $P = 0.01$ compared to control mice.

‡ No significant difference observed.

Fig. 1, the addition of pregnant spleen cells suppressed the PFC responses of the normal spleen cells. Cell viability studies using trypan blue exclusion showed that the pregnant spleen cells were not cytotoxic to the normal spleen cells. The data suggests that suppressor cell activities are evident in the spleen cell population of pregnant mice. No suppressor cells were observed in the lymph node cell population (of cervical, axillary, and popliteal regions).

Suppressor Cell Activity of Subpopulations of Pregnant Spleen Cells. To further characterize the suppressor cells in the pregnant spleen, the cells from pregnant or control mice were passed over a nylon wool column. An aliquot of the nylon-wool-nonadherent cells (T-cell-enriched population) was treated with anti-Thy 1.2 serum plus complement, and resuspended in the volume of culture media required to give a 10 × 10^6/ml cell concentration for the T cells with no anti-Thy 1.2 serum treatment. 1 ml of the cell solution of either anti-Thy 1.2 treated or nontreated nylon-wool-purified T cells were added to the normal spleen cell culture containing 10 × 10^6 cells/chamber. As shown in Table IV, the pregnant spleen T cells before treatment with anti-Thy 1.2 serum are suppressive to the PFC responses of the normal spleen cell cultures and suppression was significantly reduced by the anti-Thy 1.2 serum plus complement

TABLE III

Mitogen-induced Blastogenesis of Lymphocytes from Pregnant and Age-matched Control Mice

Source of cells	Mitogen	[^{3}H]TdR uptake	
		Control mice	Pregnant mice
	μg/ml	*cpm* × 10^{-3} ± *SE*	
Spleen	—	9.2 ± 0.4	12.6 ± 1.8
	PHA 4.0	124.9 ± 5.9	157.2 ± 9.5
	LPS 50	75.8 ± 3.5	80.9 ± 6.2
Lymph nodes cervical and axillary	—	1.6 ± 0.3	2.0 ± 0.3
	PHA 4.0	121.8 ± 9.2	126.8 ± 12.6
	LPS 50	49.6 ± 4.0	42.3 ± 4.0
Lymph nodes draining uterus (para-aortic)	—	2.4 ± 0.6	3.4 ± 0.2
	PHA 4.0	108.2 ± 12.2	116.2 ± 8.4
	LPS 50	39.7 ± 2.2	43.5 ± 1.9

The assay was performed on individual mice and the mean value obtained from four mice of each group.

TABLE IV

Antigen-induced Proliferation of LNC from Various Regions in Pregnant and Age-matched Control CBA/J Mice

Source of sensitized LNC	Antigen	[^{3}H]TdR uptake*	
		Control	Pregnant
	μg/ml		
Axillary LNC	—	4,907 ± 1,102	4,626 ± 320
	OVA 250	51,735 ± 8412	44,057 ± 6,238
	PPD 50	56,724 ± 10,004	67,719 ± 9,442
Popliteal LNC	—	4,500 ± 359	5,354 ± 182
	OVA 250	53,626 ± 2,236	49,720 ± 4,018
	PPD 50	50,378 ± 6,328	60,561 ± 7,124
Para-aortic LNC (uterus draining)	—	4,879 ± 306	4,359 ± 384
	OVA 250	39,702 ± 2,032	32,920 ± 4,012
	PPD 50	50,306 ± 6,340	44,180 ± 6,002

Immunization and the subsequent in vitro culture were performed on individual mice as described in Materials and Methods.

* Values represent the mean ± SE of three mice of each group.

treatment (Table V, exp. B). However, this treatment did not completely eliminate suppression because the PFC responses of the cultures to which anti-Thy 1.2-treated spleen T cells of pregnant mice were added were still less than those of the corresponding control cultures. The nylon-wool-adherent spleen cells of pregnant mice were also suppressive to the normal spleen cell cultures and suppression was not significantly altered by treatment with anti-Thy 1.2 serum plus complement (Table V, exp. C). In other experiments macrophages were depleted by adherence to Petri dishes before cells were applied to a nylon wool column. Nonadherent cells were then applied to the nylon wool column. Note in Table V, exp. D that the pregnant cells nonadherent to Petri dishes are suppressive. The nylon-wool-adherent cells (B-cell-enriched macrophage-depleted fraction) contained 80–85% surface immunoglobulin as determined by fluorescein isothiocyanate (FITC) anti-mouse kappa antibody and <1% macrophages by latex ingestion or esterase staining. The data (Table V, exp. E) shows that

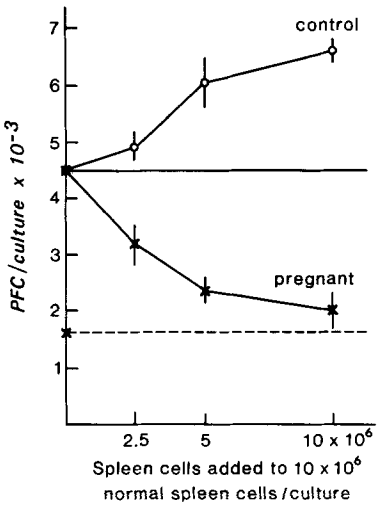

FIG. 1. Pregnant spleen cells suppress the PFC response of normal spleen cells. Various numbers of either pregnant (×) or control (○) spleen cells were added to the culture containing 10×10^6 normal spleen cells, and the mixture cultured with 10×10^6 SRBC and 4 d later direct IgM PFC assay performed. Horizontal lines represent PFC responses of 10×10^6 normal spleen cells (solid line) and 10×10^6 pregnant spleen cells (dotted line).

TABLE V

Suppression of the Normal PFC Responses by Subpopulations of Pregnant Spleen Cells

Experiment group	Cells added to normal cultures	Source of treated cells added to normal spleen cell cultures	
		Control mice*	Pregnant mice*
		PFC/culture ± SE	
A	No treatment	5,934 ± 390	2,484 ± 138 (58)‡
B	Nonadherent to nylon wool No anti-Thy 1.2 treatment	5,336 ± 240	3,098 ± 119 (42)
	Nonadherent to nylon wool§ Anti-Thy 1.2 treatment	5,200 ± 380	4,624 ± 380 (12)
C	Nylon wool adherent No anti-Thy 1.2 treatment	6,854 ± 386	2,186 ± 690 (69)
	Nylon wool adherent§ Anti-Thy 1.2 treatment	6,528 ± 420	2,300 ± 204 (65)
D	Nonadherent to Petri dishes	5,355 ± 362	2,520 ± 198 (54)
E	Nonadherent to Petri dish Adherent to nylon wool (B-cell enriched)	6,486 ± 363	2,012 ± 55 (70)
F	Adherent to Petri dish‖ (macrophage enriched)	6,992 ± 376	7,344 ± 386 (—)

* 10×10^6 treated cells added to cultures containing 10×10^6 normal spleen cells.

‡ Figures in parentheses indicate percent suppression, compared to the correspnding control PFC responses.

$$\text{Percent suppression} = \frac{\text{PFC (control cells added)} - \text{PFC (pregnant cell added)}}{\text{PFC (control cells added)}} \times 100.$$

§ The residual cells were resuspended in a volume equal to that required to give 10×10^6/ml for the cells with no anti-Thy 1.2 treatment, and 1 ml of cell suspension added to the control cultures.

‖ 3×10^6 cells of macrophage population added to the normal cultures.

TABLE VI

Secretion of Soluble Suppressive Factor(s) In Vitro

Group	Cells in culture	Source of supernate*	PFC/culture ± SE	
			Exp. 1	Exp. 2
A	Normal spleen	Fresh medium	3,054 ± 284	4,508 ± 262
B	Normal spleen	Normal cell culture	2,208 ± 207	4,048 ± 248
C	Normal spleen	Pregnant cell culture	973 ± 73 (56)	2,162 ± 207 (47)

Figures in parentheses indicate percentage suppression compared to the control of PFC response (group B).

* Supernates were obtained from 4-d cultures of normal or pregnant spleen cells (10×10^6/ml) and 1 ml of the supernate added to the inner compartment of a Marbrook chamber.

the addition of 10×10^6 cells of the B-cell-enriched macrophage-depleted fraction suppressed the PFC responses of normal spleen cells. In another experiment, splenic macrophages were obtained from pregnant and control mice, as outlined in Materials and Methods, and 3×10^6 cells of the macrophage-enriched population was added to the normal spleen cell cultures. Considering that ~8–10% of pregnant spleen cells are latex-phagocytizing cells, it was calculated from the data on suppression by whole spleen cells that the addition of 3×10^6 cells of the macrophage-enriched population should provide sufficient cells for suppression. However, the results show that there was no suppression by the pregnant macrophage population; the PFC response was about the same as those where the same numbers of cells from the control macrophage population were added (Table V, exp. F).

Suppression of PFC Responses by the Culture Supernate. To determine whether or not pregnant spleen cells produce a soluble suppressive factor(s), 10×10^6 spleen cells from ~15-d pregnant and age-matched control mice were cultured in 1 ml of culture media with or without 10×10^6 SRBC in the Linbro Costar flat-bottom culture plates (Costar, Data Packaging, Cambridge, Mass.). 4 d later the supernatant medium was obtained and centrifuged at 2,000 rpm for 10 min to remove cells. 1 ml of the supernatant medium was added to the inner compartment of a Marbrook chamber containing 1 ml of the cell suspension (10×10^6/ml) of normal spleen cells. As shown in Table VI, the supernate of the control spleen cells showed a slightly decreased PFC response compared to the addition of fresh culture medium. However, the supernate of pregnant spleen cells was strikingly suppressive compared to that of control spleen cells ($P < 0.05$) suggesting the secretion of a soluble suppressive factor(s) from pregnant spleen cells. This experiment was repeated four times with essentially identical results. Antigenic stimulations of pregnant spleen cells with SRBC did not appear to alter the production of the factor(s), because the supernatant medium of the culture with SRBC added suppressed to the same degree as that shown in Table VI.

Discussion

Several recent studies have suggested that suppressor cell activity may be involved in pregnancy-associated decreased immune responses (9, 20–22). For example, Clark and MacDermott (20) have presented evidence for the presence of a suppressor cell(s) for paternal antigens in the mixed lymphocyte cultures of the cells from the draining lymph nodes of the uterus, although the type of the suppressor cell(s) was not defined

in this study. Adoptive transfer of spleen cells from multiparous mice to virgin recipients has also been reported to induce tolerance to the H-Y antigen in male skin graft and this appears to be mediated by suppressor T cells (21). Similar suppressor T cells have also been reported in humans (22). In addition, it has been shown by Hamilton and Hellström (9) that the mixtures of lymphocytes from pregnant and virgin mice are less reactive to alloantigen stimulation than the individual cell population alone, suggesting the presence of nonspecific suppressor cells in pregnant mice. Our study has presented evidence for the involvement of suppressor cell activity in the decreased splenic antibody responses of pregnant mice. The primary IgM antibody synthesis against SRBC in vivo as well as in vitro are markedly decreased in the spleen cells of pregnant mice and this decrease of antibody synthesis is probably mediated via nonspecific suppressor cells. This is evidenced by the observation that the addition of pregnant spleen cells to normal spleen cell cultures causes a significant suppression of PFC responses of normal spleen cells. Whether the diminished primary and secondary IgG and IgA splenic antibody responses of pregnant mice previously reported from this laboratory (7) are mediated by suppressor cells has not been explored.

An interesting observation is that the suppressor cell activity in pregnant mice is evident only in spleen cells and not in lymph node cells. Similar observations of the preferential localization of suppressor cells in spleens rather than lymph nodes has been described in animals under conditions of tumor growth (23, 24), graft vs. host (GVH) reactions (25), and certain bacterial infections, as well as in animals which have been immunized with a large dose of antigen (26, 27). It has been suggested that the microenvironment of the spleen may be favorable to the generation and/or trapping of suppressor cells (25). Gershon et al. (25) have described that under certain experimental conditions immune responses are suppressed by a subpopulation of thymocytes and/or thymus-derived cells. These cells are short-lived T_1 cells, continuously being replenished from the thymic cortex, preferentially homing to spleens, and expressing suppressor cell activity there. Perhaps increased mobilization of T_1 cells from thymus to spleen may take place during pregnancy resulting in the enrichment of suppressor cells in the pregnant spleen. This could be one of the explanations for the concomitant manifestation of pregnancy-associated thymic atrophy and spleen enlargement. As pointed out by Anderson (28), thymic atrophy and spleen enlargement similar to those seen during pregnancy also occur in tumor-bearing animals in which spleen-seeking thymocytes have been postulated to operate in the immune suppression associated with certain tumors.

We have demonstrated that at least two cell populations are involved in the decreased PFC responses of pregnant spleen cells. One is T cells and the other is a nylon-wool-adherent cell present in the B-cell-enriched macrophage-depleted fraction. Suppression by T cells is abolished by treatment with anti-Thy 1.2 serum plus complement while such treatment does not alter suppression by the nylon-wool-adherent cells. This suggests that the nylon-wool-adherent suppressor cells are different from the nylon-wool-adherent T suppressor cells described by Tada (29). The nylon-wool-adherent cells of pregnant mice are still suppressive after macrophages contained in this population are depleted. Thus, the nature of the suppressor cell(s) in the nylon-adherent preparations has not been defined, although because B cells represent the majority of cells remaining in the macrophage-depleted Thy 1.2-negative nylon-wool-

adherent fraction, suppression by pregnant spleen cells may be in part caused by the B-cell population.

The mechanisms by which the two cell populations of pregnant spleens exert their suppressive activity are unknown. It is possible that these cells act via interaction with each other and/or other types of cells. For example, the nylon-wool-adherent suppressor cells of pregnant mice could affect the T helper cells in a T-B interaction similar to that described in GVH mice (30). Alternatively, pregnant T cells may secrete soluble factors which in turn inhibit the response of B cells. Our preliminary data suggests that the suppressed PFC responses of normal spleen cells after the addition of pregnant cells may be mediated via a soluble factor(s) released from pregnant spleen cells. However, neither the cells producing the factor nor the target cells producing the factor(s) have as yet been defined. Although most of the suppressive factors contained in pregnant sera have been reported to be the products of the fetus or the placenta, our data suggest the possibility that a suppressive serum factor(s) could be elaborated by suppressor cells in pregnant spleens.

Previous studies have yielded conflicting results regarding mitogen-induced blastogenesis of maternal lymphocytes. The data presented here on the responsiveness of pregnant lymphocytes to mitogens is similar to that by Anderson (28) who has demonstrated that there was little difference between pregnant and control mice. Similarly, antigen-induced proliferation of lymph node cells, including those draining the uterus, are not depressed in pregnant mice. However, some investigators have claimed a significantly decreased response to mitogens in pregnant mice (6). These discrepancies could result from strain difference of mice, time of pregnancy at which the assay was performed, and the in vitro culture conditions. We believe that a major problem in analyzing the effect of mouse sera on immune reactions is the significant variation in suppressive potency of various pools of normal and pregnant sera. It is essential, therefore, to compare multiple pools of pregnant sera with several pools of age-matched nonpregnant female controls. We also emphasize that the present data has been obtained at one point during pregnancy (~15 d) and that studies at different times are necessary. However, we have observed suppression in the primary and secondary splenic PFC response between 10 and 20 d of pregnancy (7), suggesting that similar factors may operate during this time span.

Summary

The primary IgM antibody response to sheep erythrocytes in vivo as well as in vitro is markedly decreased in the spleen cells of pregnant mice, compared to age-matched female controls. Decreased antibody synthesis appears to be mediated by nonspecific suppressor cells, because the addition of pregnant spleen cells to the normal spleen cell cultures causes a significant suppression of plaque-forming-cell responses of the normal spleen cells. Suppressor cell activity was not observed in lymph nodes of pregnant mice. At least two populations of pregnant spleen cells were shown to exert a suppressor cell activity; one is T lymphocytes and the other a nylon-adherent cell present in the B-cell-enriched macrophage-depleted fraction. Pregnant spleen cells cultured in vitro were shown to secrete a soluble suppressive factor(s) into the supernatant medium.

We gratefully acknowledge the gift of FITC anti-mouse kappa antibody from Dr. R. McCarthy and the excellent technical assistance of Mr. Geoffry Curran.

Received for publication 27 June 1979.

References

1. Beer, A. E., and R. E. Billingham. 1971. Immunobiology of mammalian reproduction. *Adv. Immunol.* **14:**1.
2. Han, T. 1975. Human chorionic gonadotropin. Its inhibitory effect on cell-mediated immunity in vivo and in vitro. *Immunology.* **29:**509.
3. Rembiesa, R., W. Ptak, and M. Bubak. 1974. The immunosuppressive effects of mouse placental steroid. *Experientia (Basel).* **30:**82.
4. Hellström, K. E., I. Hellström, and J. Brown. 1969. Abrogation of cellular immunity to antigenically foreign mouse embryonic cells by a serum factor. *Nature (Lond.).* **224:**914.
5. Than, G. N., I. F. Csaba, N. J. Karg, D. G. Szabo, M. Ambrus, and G. Bajtai. 1975. Immunosuppressive effect of pregnancy-associated α_2-glycoprotein. *Lancet.* **II:**515.
6. R. A. Murgita. 1976. The immunosuppressive role of α-fetoprotein during pregnancy. *Scand. J. Immunol.* **5:**1003.
7. Tomasi, T. B., R. A. Murgita, R. L. Thompson, N. J. Calvanico, and R. J. Dattwyler. 1977. Alpha-fetoprotein and the immune response during murine pregnancy and neonatal development. *In* Development of Host Defenses. M. D. Cooper and D. H. Dayton, editors. Raven Press, New York. 273.
8. Smith, G. 1978. Inhibition of cell-mediated microcytotoxicity and stimulation of mixed lymphocyte reactivity by mouse pregnancy serum. *Transplantation (Baltimore).* **26:**78.
9. Hamilton, M. S., and I. Hellström. 1977. Altered immune responses in pregnant mice. *Transplantation (Baltimore).* **23:**123.
10. Kasakura, S. 1971. A factor in maternal plasma during pregnancy that surpresses the reactivity of mixed leukocyte cultures. *J. Immunol.* **107:**1296.
11. Purtillo, D. T., H. Hallgren, and E. J. Yunis. 1972. Repressed maternal lymphocyte response to phytohemagglutinin in human pregnancy. *Lancet.* **I:**769.
12. Stimson, W. H., K. S. Bridge, and J. C. Blackstock. 1974. The suppression of antigen induced inhibition of leukocyte migration by human pregnancy serum. *IRCS (Int. Res. Commun. Syst.) Med. Sci.-Lib. Compend.* **2:**1207.
13. Anderson, R. H., and C. W. Monroe. 1962. Experimental study of the behavior of adult human skin homografts during pregnancy: a preliminary report. *Amer. J. Obstet. Gynecol.* **84:** 1096.
14. Farber, P. A., and L. A. Glasgow. 1968. Factors modifying host resistance to virus infection. II. Enhanced susceptibility of mice to encephalomyocarditis virus infections during pregnancy. *Amer. J. Pathol.* **53:**463.
15. Meritt, K., and M. Galton. 1969. Antibody formation during pregnancy. *Transplantation (Baltimore).* **7:**572.
16. Cunningham, A. J., and A. Szenberg. 1968. Further improvements in the plaque technique for detecting single antibody forming cells. *Immunology.* **14:**599.
17. Alkan, S. S. 1977. Antigen-induced proliferation assay for mouse T lymphocytes: Response to a monovalent antigen. *Eur. J. Immunol.* **8:**112.
18. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus derived murine lymphocytes. *Eur. J. Immunol.* **3:**645.
19. Handwerger, B. S., and R. H. Schwartz. 1974. Separation of murine lymphoid cells using nylon wool columns: Recovery of the B cell-enriched population. *Transplantation (Baltimore).* **18:**544.

20. Clark, D. A., and M. R. MacDermott. 1978. Impairment of host vs. graft reaction in pregnant mice. I. Suppression of cytotoxic T cell generation in lymph nodes draining the uterus. *J. Immunol.* **121:**1389.
21. Smith, R. N., and A. E. Powell. 1977. The adoptive transfer of pregnancy-associated unresponsiveness to male skin grafts with thymus-dependent cells. *J. Exp. Med.* **146:**899.
22. McMichael, A. J., and T. Sasazuki. 1977. A suppressor T cell in the human mixed lymphocyte reaction. *J. Exp. Med.* **146:**368.
23. Elgert, K. D., and W. L. Farrar. 1978. Suppressor cell activity in tumor-bearing mice. I. Dualistic inhibition by suppressor T lymphocytes and macrophages. *J. Immunol.* **120:**1345.
24. Pope, B. L., R. B. Whitney, J. G. Levy, and D. G. Kilburn. 1976. Suppressor cells in the spleens of tumor-bearing mice: Enrichment by centrifugation on hypaque-ficoll and characterization of the suppressor cell population. *J. Immunol.* **116:**1342.
25. Gershon, R. K., E. M. Lance, and K. Kondo. 1974. Immunoregulatory role of spleen localizing thymocytes. *J. Immunol.* **112:**546.
26. Bullock, W. E., E. M. Carlson, and R. K. Gershon. 1978. The evolution of immunosuppressive cell populations in experimental mycobacterial infections. *J. Immunol.* **120:**1709.
27. Whisler, R. L., and J. D. Stobo. 1976. Heterogeneity of murine regulatory T cells. I. Subpopulation of amplifier and suppressor T cells. *J. Exp. Med.* **144:**398.
28. Anderson, D. J. 1978. The responsiveness of various maternal mouse lymphocyte populations to mitogenic stimulation in vitro. *Cell. Immunol.* **41:**150.
29. Tada, T. 1977. *In* Regulation of Antibody Responses by T-Cell Products Determined by Different I Subregions. Proceedings of the Sixth Annual ICN-UCLA Symposia on Molecular and Cellular Biology. E. Sercarz, L. Herzenberg, and C. F. Fox, editors. Academic Press, Inc., New York. 345.
30. Elie, R., and W. S. Lapp. 1977. Graft versus host-induced immunosuppression: mechanism of depressed T cell helper function in vitro. *Cell. Immunol.* **34:**38.