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Cell Mediated Immunity in Healthy Women Taking Oral Contraceptives[1]

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Cell-mediated immune function was assessed by both *in vitro* and *in vivo* techniques in 20 healthy women on continuous oral contraceptive medication for periods varying from 6 mo to 6 yr. Contrary to previous reports no disturbance of cell-mediated immunity was detected.

Recent reports[1,2] have suggested that oral contraceptive medication can suppress the lymphocyte response to stimulation *in vitro* by phytohemagglutination (PHA). As this test is widely used to assess the competence of the cell-mediated immune (CMI) system, such reports raise doubts about the integrity of this axis of the immune response in women using estrogen-progestegin combinations. Further, it has been postulated that the responsible mechanisms may be similar to those depressing cell-mediated immune function during the third trimester of pregnancy[3,4]. Factors which suppress the function of the thymus-derived lymphocyte (T cell) which mediates CMI are important clinically because the sequelae include a higher incidence of cancer[5], and a less adequate response to fungal and viral infection[6]. In preparing to study CMI in women with chronic vaginal candidiasis (many of whom are taking oral contraceptives) we studied cell-mediated immune responses in 20 apparently healthy women receiving either high- or low-dose estrogen combination preparations and found no defect in their immunological capacity.

MATERIALS AND METHODS

Patients

Twenty women ranging in age from 18 to 37 yr were studied. All were in good health and had been taking contraceptives for at least 6 mo (mean 2.8 yr). The preparations used were combinations of progestegin with either 50 μg, or 80 μg of estrogen. The responses observed in this group were compared to those of 20 age- and sex-matched control subjects.

Immunological Methods

All subjects were skin tested for delayed hypersensitivity responses and had blood drawn for analysis *in vitro* of lymphocyte function. Intradermal injections of 0.1 ml of the following antigens were used. A 1/100 dilution of *Candida albicans* (Hollister-Stier, Yeadon, PA), mumps skin test antigen, and a 1/10 dilution of tetanus toxoid fluid USP (Eli Lilly, Indianapolis, IND), and a 1/200 dilution of PHA-P (Difco Laboratories, Detroit, MI). Reactions to PHA were read at 24 hr, and to the antigens at 48 hr. A response of 10 mm of induration was considered positive.

Blood was drawn into heparinized syringes and the lymphocytes so obtained were

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examined for the percentage of thymus-derived (T) lymphocytes and bone marrow-derived (B) lymphocytes in the sample[7]. Lymphocytes were stimulated in tissue culture for 3 days by the mitogens PHA, concanavalin A (Con-A), and pokeweed (PWM)[7], and for 7 days in *Candida albicans* and irradiated pooled allogeneic lymphocytes (mixed lymphocyte reaction -MLR,)[8] DNA synthesis, as detected by the incorporation of tritiated thymidine, was used as a marker of lymphocyte stimulation. Microcultures utilizing a multiple automated sample harvester were used throughout. Responses are reported as stimulation indices (cpm from stimulated cells/cpm from cultured but unstimulated cells). Dose-dependent responses were sought with the mitogens. Thus, to 0.1 ml of culture media containing cells was added: 0.1 ml of a 1/500 or 1/10,000 dilution of PHA-P; 0.1 ml of concanavalin A at a concentration of 200 or 20 μg/ml, and 0.1 ml of pokeweed at a concentration of 10 or 0.5 μg/ml. All of the above cultures were performed with media containing 20% pooled human plasma.

Were a humoral suppressant of cell-mediated immune responses present in the serum of women using contraceptive medication, the thorough washing the above cells received may mask, *in vitro*, the *in vivo* effect of such an agent. Hence, the sera of these subjects were examined for immunosuppressive properties. As we have previously shown that serum from a large number of healthy control subjects when added to cultures of lymphocytes from one subject may produce a two-log scatter of the standard cells response to mitogens and antigens[9], we do not believe that immunosuppressive properties of a given serum can be sought by stimulating normal cells in one serum and comparing the response to that obtained in another. The test system used was to stimulate normal cells with PHA and allogeneic pooled irradiated cells in media already containing optimal amounts of pooled human plasma with or without an added 10% of the serum to be tested. Thus, the response of cells from healthy subjects in a final concentration of 30% plasma (20% pooled human plasma × 10% pooled human plasma or 20% pooled human plasma × 10% test sera) were compared using sera from 20 subjects.

RESULTS

The results are presented in Table 1. Comparison of the mean value and the standard errors of the control group and patient group did not reveal a statistically significant difference for any of the *in vitro* tests although the mean values for the PHA and Con A stimulation were lower in the group taking medication when the smaller dose of mitogen was used in the cell cultures, this difference is not significant when the data are analyzed by Student's T test. A comparison of skin reactivity between the control group and contraceptive users did not suggest a diminished capacity to produce delayed hypersensitivity reactions in the latter group. A comparison of the results in individual subjects with the mean values and standard deviation of the control group did not identify any individual receiving contraceptive medication as immunosuppressed.

The sera from the contraceptive users were not immunosuppressive. The results (Mean Stimulation Index ± S E) follow: Stimulation of normal cells with high-dose PHA in pooled human plasma (30%) 56 ± 3.8; The same cells in pooled human plasma (20%) + test plasma (10%), 61 ± 5.4. With the lower dose of PHA, the indices were 31 ± 4.7 and 33 ± 5.9, respectively. For the mixed lymphocyte reaction the results were 26 ± 3.5 in pooled plasma alone and 21 ± 4.7 in pooled plasma and plasma from the contraceptive users.

TABLE 1

Studies of Cell-Mediated Immunity in Women Using Estrogen-Progestegin Contraceptives

Subject	Dose of oestrogen (μg)	Years on preparation	% B cells	% T cells	Mitogen background (cpm)	Stimulation index PHA H[a]	PHA L[a]	CON-A H[a]	CON-A L[a]	PWM H[a]	PWM L[a]	Antigen background (cpm)	Stimulation index *Candida albicans*	Skin testing[c] MLR	CAN	MU	TET	PHA
1	50	5	21	54	501	17	7	20	9	7	2	ND	ND	ND	+	–	+	+
2	50	0.5	32	55	529	37	14	22	12	18	4	ND	ND	ND	–	–	+	+
3	50	3	41	52	445	27	4	18	8	7	2	ND	ND	ND	+	+	–	+
4	50	2	26	47	442	28	8	19	11	9	4	180	1	15	–	–	+	+
5	50	0.5	32	55	205	68	22	71	5	21	6	170	4	24	+	–	+	+
6	50	2.5	25	53	218	63	14	52	7	28	7	300	2	11	–	+	–	+
7	50	3	17	64	391	29	8	31	3	8	1	1100	2	26	+	+	+	+
8	50	3	19	69	546	32	7	27	8	14	7	1000	10	17	+	+	+	+
9	50	6	31	57	135	80	14	102	11	18	10	1500	2	21	+	–	+	+
10	50	5	42	35	263	62	17	59	14	15	5	1000	3	35	+	–	–	–
11	50	4	36	56	232	56	21	57	13	13	2	1200	1	29	–	+	+	–
12	80	2	26	73	132	49	10	38	18	16	6	850	12	25	+	–	–	+
13	50	3	29	66	227	34	14	22	12	30	2	1400	2	24	–	+	+	–
14	80	0.5	36	52	163	60	22	56	17	12	6	3100	6	8	+	–	–	+
15	80	2	34	71	200	39	11	30	13	16	2	1500	7	13	+	+	+	+
16	50	3	31	55	154	65	22	39	11	23	5	1600	3	16	+	+	–	+
17	50	6	37	59	891	48	13	26	7	13	6	1700	3	12	+	+	+	+
18	50	1	37	69	900	44	11	33	14	11	3	2300	4	8	+	–	+	+
19	50	1	35	70	704	86	12	87	18	50	16	1800	5	8	+	+	–	+
20	50	4	21	55	1701	16	4	40	13	16	4	1400	13	15	+	+	+	+
Mean values			30.4	58		47	12.9	42.4	11.2	17.2	5.0		5.0	17.8				
± SE subjects			±1.6	±2.1		±4.4	±1.2	±5.2	±.91	±2.2	±.76		±.84	±2.1	15/20	11/20	13/20	17/20
Mean values			28	53		50	20	40	19	21	7.5		5.6	12.6				
± SE controls			±2.3	±2.5		±4.9	±5	±6	±6	±4.2	±2.5		±1.12	±2.5	16/20	12/20	13/20	19/20

[a]See text for high (H) and low (L) doses of mitogen. [b]Absolute counts from MLR/$\frac{1}{2}$ × background for irradiated cells + responder cells. [c]CAN = Candida, MU = Mumps, TET = Tetanus toxoid, PHA = Phytohemagglutinin.

DISCUSSION

The assessment of cell-mediated immune (CMI) responses in humans remains difficult and it is being increasingly recognized that no one test is adequate. The approach taken in this study has been to investigate a number of correlates of CMI and to utilize dose-dependent stimulation in assaying mitogen stimulation[10]. This allows detection of an abnormal responsiveness that may be missed with maximal stimulation. The problem of looking for a circulating immunosuppressive factor is also difficult. As has been shown in studies using sera from mice, human sera or plasma from one subject varies greatly in its ability to support in tissue cultures cells from another subject[9]. Thus, if lymphocytes from one control subject are stimulated by PHA in the presence of 50 different samples of sera or plasma from healthy subjects, a wide variation in the response of the cells is noted. It would be easy to assume that those sera associated with the lowest responses contained an immunosuppressive factor. However, that would imply active suppression when it really may be lack of some nutritive factor. In any case, as such a result can be obtained when normal sera are screened it makes it difficult to screen sera from diseased subjects for immunosuppressive factors. Our approach was to supply normal cells with pooled human plasma in optimal amounts and then add the plasma to be tested. A resulting decrease in response to stimulation would be more likely to be biologically significant and to be an active rather than a passive phenomenon. We were interested in the possible immunosuppressive properties of sera from women on oral contraceptives as a number of reports suggest that in pregnancy there is a circulating plasma factor than can suppess CMI. No such factor was found in the plasma of our subjects. Thus, despite the problems inherent in tests whose normal values include wide standard deviations, it seems clear that the commonly used oral contraceptives which feature combinations of estrogen and progestegin do not effect CMI. It is thus unlikely that the changes in immunological function seen in pregnancy are related to the development of a hormonal balance similar to that induced by oral contraceptives. Previous reports suggesting that oral contraceptives affect cell-mediated immunity have been limited in the number of patients studied and the breadth of their assessment of CMI, and insufficient information was provided to establish the similarity of the populations variously studied. Impaired CMI may predispose to cancer formation but there have been no suggestions of an increased incidence of cervical cancer among oral contraceptive users[11], and a lower incidence of breast cancer[12] has been reported. These facts, which suggest indirectly that immunological surveillance for Cancer is not diminished by oral contraceptive preparations, are supported by the current direct evaluation of the CMI system in contraceptive users.

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