SEX HORMONE REGULATION OF IN VITRO IMMUNE RESPONSE

Estradiol Enhances Human B Cell Maturation via Inhibition of Suppressor T Cells in Pokeweed Mitogen-stimulated Cultures*

By TIMO PAAVONEN, LEIF C. ANDERSSON,‡ AND HERMAN ADLERCREUTZ

From the Transplantation Laboratory, Department of Pathology, and Department of Clinical Chemistry, University of Helsinki, Helsinki, Finland

Higher serum immunoglobulin (Ig) levels are usually observed in females more than in males of several mammalian species, including man. Females also show a quantitatively and qualitatively enhanced capacity to produce antibodies after immunization (1–7). Moreover, during their fertile period, women have remarkably higher incidences of autoimmune diseases (8, 9). On the other hand, reports exist (10) indicating that males have stronger cell-mediated responses than females. These sex-dependent differences in the mode and magnitude of immune reactivities are largely abolished by castration (11). A regulatory influence on the immune system has therefore been attributed to the main sex hormones (reviewed in 12).

The cellular mechanisms involved in the sex hormone-induced immunomodulation are yet to be clarified. There are reports (13, 14) indicating that estradiol displays its immunoregulatory effect through the thymus, but the relevant cellular target sites for sex hormone activity in the immune system are not known.

We studied the effect of estradiol and testosterone on human blood lymphocytes in pokeweed mitogen (PWM)[1]-stimulated cultures. We found that addition of physiological concentrations of estradiol to PWM cultures enhanced the B cell differentiation without affecting the proliferative response. This effect was not obtained with testosterone. Cell fractionation studies revealed that estradiol apparently displays its effect by inhibiting the activity of a suppressor T cell population.

Materials and Methods

Human Blood Lymphocytes. Human blood mononuclear leukocytes were purified from the buffy coats of 400-ml blood units obtained from healthy male and female volunteers and were

* Supported by the Finnish Medical Research Council, the Association of Finnish Life Insurance Companies, Lääke Ltd., and the Finnish Medical Foundation, Helsinki, Finland; and by grant 1 R01 CA26294-01A1 from the National Institutes of Health, Bethesda, Maryland.

‡ Address correspondence to Dr. L. C. Andersson, Department of Pathology, University of Helsinki, Haartmaninkatu 3, SF 00290 Helsinki 29, Finland.

[1] *Abbreviations used in this paper:* AET, 2-amino-ethylisothiouronium bromide hydrobromide; ChRBC, chicken erythrocytes; FCS, fetal calf serum; FIP, Ficoll-Isopaque, density 1.077; FITC, fluorescein isothiocyanate; HBSS, Hanks' balanced salt solution; [^{3}H]TdR, tritiated thymidine; IcIg, intracytoplasmic Ig; pA, protein A from *Staphylococcus aureus*; PBS, 0.1 M NaCl–0.01 M sodium phosphate, pH 7.4; PFC, plaque-forming cells; PWM, pokeweed mitogen; SRBC, sheep erythrocytes; Tγ, T lymphocytes with Fc receptors for IgG; Tμ, T lymphocytes with Fc receptors for IgM;

supplied by the Finnish Red Cross Blood Transfusion Service, Helsinki, Finland. The buffy coats were diluted with 2 vol of 0.1 M NaCl-0.01 M sodium phosphate (PBS), pH 7.4, and the mononuclear cells were separated from granulocytes and erythrocytes by one-step Ficoll-Isopaque (FIP; Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Uppsala, Sweden; density, 1.077) gradient centrifugation at 400 *g* for 40 min at +22°C (15). Contaminating erythrocytes were lysed by treatment with a Tris-HCl buffered 0.84% aqueous solution of NH_4Cl, pH 7.6 (Tris-NH_4Cl).

Fractionation of Lymphocyte Subpopulations. The separation of T and B cells and the T lymphocytes carrying Fc-receptors for IgG (Tγ) and IgM (Tμ) was modified from Moretta et al. (16). FIP-isolated blood leukocytes, depleted from phagocytes by iron powder plus magnet treatment, were rosetted with 2-amino-ethylisothiouronium bromide hydrobromide (AET)-treated (17) sheep erythrocytes (SRBC), and the rosetting T cells were separated from the non-rosetting B cells by FIP density gradient centrifugation. The B cell-enriched interphase fraction contained 75–85% lymphocytes that stained positively for surface Ig with fluorescein isothiocyanate (FITC)-conjugated polyvalent rabbit anti-human Ig (Dako-immunoglobulins, Copenhagen, Denmark). Less than 3% surface Ig-positive cells were found in the T cell-enriched pellet fraction. After lysis of the AET-SRBC with Tris-NH_4Cl, the T cells were further enriched for Tγ and Tμ subpopulations by rosetting with rabbit IgG-coated human O erythrocytes. After another centrifugation over FIP, the Tγ-depleted (Tμ-enriched) fraction was recovered from the interphase, while the bottom fraction was enriched for Tγ cells.

Lymphocyte Cultures and Quantitation of the Response. 3×10^5 lymphocytes in 200 μl of RPMI 1640 tissue culture medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) supplemented with 5% fetal calf serum (FCS; Gibco Laboratories, Grand Island Biological Co.) were cultivated in Falcon Microtest II culture tissue plates (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) for the indicated times in a humidified atmosphere of 5% CO_2 in air. The proliferative responses in mitogen-stimulated cultures were quantitated by addition of 0.4 μCi of tritiated thymidine ([^{3}H]TdR, New England Nuclear, Boston, Mass.; specific activity, 6.7 Ci/mmol) per well for 6 h before terminating the cultures on the days indicated. To quantitate the different cell types, 10^4 glutaraldehyde-fixed chicken erythrocytes (ChRBC) were added to each culture well before preparation of the cytocentrifuged smears (18). Smears from parallel cultures were stained with May-Grünwald giemsa (MGG) to assess the cellular morphology in each culture. To quantitate the number of plasmablasts containing intracytoplasmic immunoglobulin (IcIg), the smears were fixed for 10 min in 3% paraformaldehyde at room temperature, washed, and kept in cold (−20°C) acetone for 5 min. After rinsing in PBS, the smears were stained with FITC-conjugated 1:30 diluted polyvalent anti-human Ig. The number of IcIg-containing cells was quantitated per 100 autofluorescent ChRBC.

To quantitate the number of Ig-secreting cells, 5×10^6 lymphocytes in 2 ml 5% FCS containing RPMI 1640 medium per well were cultivated in Nunclon macroplates (S. Nunc, Roskilde, Denmark), and the response was measured by the reversed protein-A (pA; Pharmacia Fine Chemicals) plaque assay according to Gronowitz et al. (19). Sheep erythrocytes were coupled with pA as follows: one part of packed SRBC and one part of pA (0.5 mg/ml) were added to ten parts of 2.5×10^4 M chromium chloride in 0.9% NaCl. The mixture was incubated at 30°C for 1 h and washed once with PBS and twice with Hanks' balanced salt solution (HBSS). For the plaque assay, 25 μl of 30% pA-coupled SRBC and 25 μl of 1:30 diluted rabbit anti-human Ig were incubated at 37°C for 30 min. After incubation, 25 μl preadsorbed guinea pig complement, 100 μl lymphoid cell suspension (0.25×10^6 to 0.5×10^6 cells/ml), and 25 μl of HBSS were added. After mixing, Cunningham chambers (20) were filled with the cell suspension, incubated at 37°C for 2 h, and the plaque-forming cells (PFC) were counted in an indirect light microscope.

X-ray Irradiations. Lymphoid cells were irradiated with a Stabilipan (Siemens AG, Erlangen, Federal Republic of Germany) x-ray machine at a dose rate of 711 rad/min.

Mitogens and Chemicals. PWM (Gibco Laboratories, Grand Island Biological Co.) was used at a final dilution of 1:300. *Staphylococcus aureus* Cowan I bacteria were used 60×10^6 organisms/ml. AET was obtained from Sigma Chemical Co., St. Louis, Mo. Stock solutions were prepared by dissolving estradiol [1,3,4(10)-estratriene-3-17β-diol] (Makog Chemicals Ltd., Jerusalem,

Israel) and testosterone (17β-hydroxy-4-androsten-3-one) (Steraloids Inc., Wilton, N. H.) in absolute ethanol. For all experiments, the stock solutions were diluted 10^{-2}–10^{-4}, which correspond to 26,000–260 pmol/liter estradiol and 2,000–20 nmol/liter testosterone, respectively. Physiological estradiol values in fertile women are 100–2,000 pmol/liter, and physiological testosterone values in men are 14–38 nmol/liter.

Expression of the Results. Results from the various individual experiments are expressed as the percent of response of otherwise identical parallel control cultures without added hormone but containing the same final concentration (0.3%) of ethanol. This concentration of ethanol was nontoxic. When compared with parallel PWM cultures without added ethanol, 0.3% ethanol sometimes induced slight but nonsignificant increase in the proliferative response (<5%) and in the generation of Ig-producing cells (<8%).

Results

Effect of Testosterone and Estradiol on T and B Cell Responses in PWM Cultures. When FIP-isolated blood mononuclear leukocytes from men or women were stimulated with PWM in the presence of indicated dilutions of added estradiol or testosterone, significantly increased accumulation of plasmablasts (IcIg) was recorded on days 4–6 in cultures containing 3×10^{-4} ($P < 0.05$; Student's t test) and 10^{-3} ($P < 0.005$) diluted estradiol. The corresponding concentration range of testosterone did not significantly increase or decrease the B cell differentiation. The highest hormone concentrations inhibited the response slightly (Fig. 1 A).

When cultures were set up in macroplates and the frequency of Ig-secreting cells recorded by the reversed pA-plaque assay on day 6 of culture, an increased number of PFC was seen in estradiol-treated cultures when compared with control cultures without added hormones. The peak response was again obtained with hormone

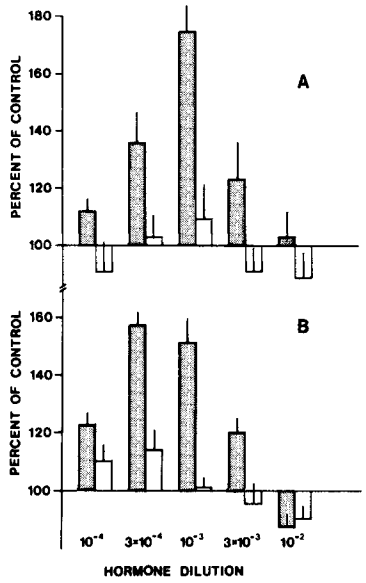

FIG. 1. IcIg-containing cells (A) and Ig-secreting cells (PFC) (B) on day 6 in PWM-stimulated cultures treated with various concentrations of estradiol (shaded columns) or testosterone (open columns). Bars indicate SEM of eight different experiments. The control level (100%) represents results from PWM cultures without added hormone that gave 30×10^3 to 45×10^3 IcIg-containing cells and 1×10^3 to 2×10^3 PFC/10^6 Ficoll-Isopaque-purified blood mononuclear cells.

dilutions of 3×10^{-4} and 10^{-3} ($P < 0.005$). Again, no significant effect on the PFC generation was seen with corresponding dilutions of testosterone (Fig. 1 B).

To test whether the responsiveness in PWM cultures to added sex hormones was differently expressed in male and female leukocytes, parallel cultures with lymphocytes from male and female donors were established, and the IcIg and PFC were measured as above. Table I summarizes the results. Both male and female lymphocytes showed the same degree of enhanced B cell differentiation in the presence of added estradiol in PWM cultures, whereas testosterone in the dose range used neither stimulated nor inhibited male or female lymphocytes, as compared with control cultures without added hormones.

The increased generation of plasmablasts in estradiol-treated PWM cultures could not be explained by an overall stimulated proliferative response. When the DNA synthesis was measured by [^{3}H]TdR incorporation on day 3 in PWM cultures, no significant differences were seen between estradiol- and testosterone-treated cultures and control cultures without added hormone (Table II). The addition of estradiol or testosterone in nontoxic concentration did not change the kinetics of the proliferative response measured by incorporation of [^{3}H]TdR for 6 h on the subsequent culture days (days 3–7) (data not shown). The estradiol-induced increase in the accumulation of IcIg and PFC in PWM cultures was not due to an altered kinetics of the response pattern. When hormone-treated cultures and control cultures were harvested on

TABLE I

Effect of Estradiol on IcIg-containing Cells on Day 6 in PWM-stimulated Cultures of Male (M) or Female (F) Blood Lymphocytes

Estradiol dilution	Percent of control							
	PFC				IcIg			
	F_1	F_2	M_1	M_2	F_1	F_2	M_1	M_2
10^{-2}	95	84	107	85	90	88	100	106
3×10^{-3}	102	92	133	130	151	88	157	143
10^{-3}	183	121	183	138	125	184	200	126
3×10^{-4}	165	153	200	163	223	131	129	88
10^{-4}	130	122	133	94	91	94	114	129
Control	100	100	100	100	100	100	100	100

TABLE II

Effect of Sex Hormones on DNA Synthesis on Day 3 in PWM-stimulated Cultures

Hormone dilution	[^{3}H]Thymidine incorporation*	
	Estradiol	Testosterone
10^{-2}	101 ± 3‡	103 ± 3
3×10^{-3}	100 ± 2	101 ± 2
10^{-3}	102 ± 2	98 ± 2
3×10^{-4}	101 ± 3	97 ± 2
10^{-4}	96 ± 3	102 ± 3

* Results are expressed as percent of control cultures without added hormone.
‡ Mean ± SE from ten different experiments.

subsequent days and the number of PFC was recorded, the estradiol-treated cultures displayed the peak responses on days 5–6, as did cultures without added hormone (data not shown).

Hormone Effects on B Cells Producing the Main Ig Classes. The overall increased B cell maturation was recorded with rabbit anti-human polyvalent sera both in the plaque assay and in the fluorescence assay. To identify the cell type(s) responsible for the increased B cell maturation in estradiol-treated cultures, the number of cells synthesizing and excreting the main classes of human Ig (IgG, IgM, and IgA) was quantitated in plaque assays in estradiol-treated and control PWM cultures. As shown in Fig. 2, the increased accumulation of differentiated B blasts in estradiol-treated cultures was primarily caused by an increase in the number of IgM synthesizing and secreting cells, with only slight (nonsignificant) increases in the accumulation of IgA and IgG synthesizing cells.

Role of Regulatory T Cells for Estradiol-induced Enhancement of B Cell Maturation. To study whether the estradiol-mediated effect was transferred via subsets of regulatory T cells, suppressor, and/or helper cells (21), T cell populations carrying Fc-receptors for IgG (Tγ) and T cells negative for Fcγ (Tμ) were fractionated from the same buffy coat and recombined with an equal number of syngeneic B cells in PWM cultures. When B cells were recombined with syngeneic T cells enriched for Tγ cells, the typical increased accumulation of IgIg-containing cells was seen after addition of estradiol (Fig. 3 A). The depletion of the Tγ cells resulted in an overall increased level of IcIg, indicating the removal of a suppressor cell function. Only a slight (nonsignificant) increase in the response could be obtained after estradiol treatment of such cultures (Fig. 3 B). These findings suggested that estradiol inhibited the suppressive activity of the Tγ cells.

To further investigate the role of T suppressor cells, the FIP-isolated mononuclear cells were x-ray irradiated with 1,000 rad before culture. Such treatment has previously been shown (21, 22) to predominantly remove the suppressor cell activity. When irradiated cells were stimulated in PWM cultures with or without addition of estradiol, an overall increased frequency of PFC was obtained. No further significant enhancement was, however, obtained by addition of estradiol (Fig. 4). These findings indicate estradiol influence on a regulatory radiosensitive T cell population.

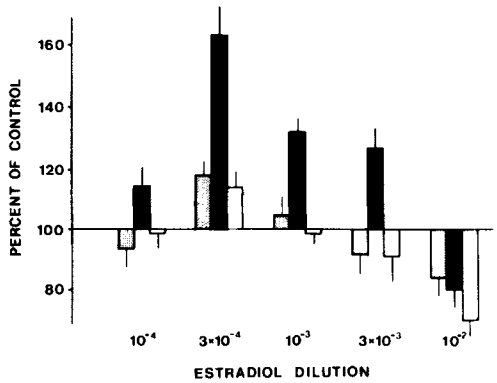

FIG. 2. IgA (shaded columns), IgM (filled columns), and IgG (open columns) secreting PFC on day 6 in PWM cultures treated with estradiol. Bars indicate SEM of five different experiments.

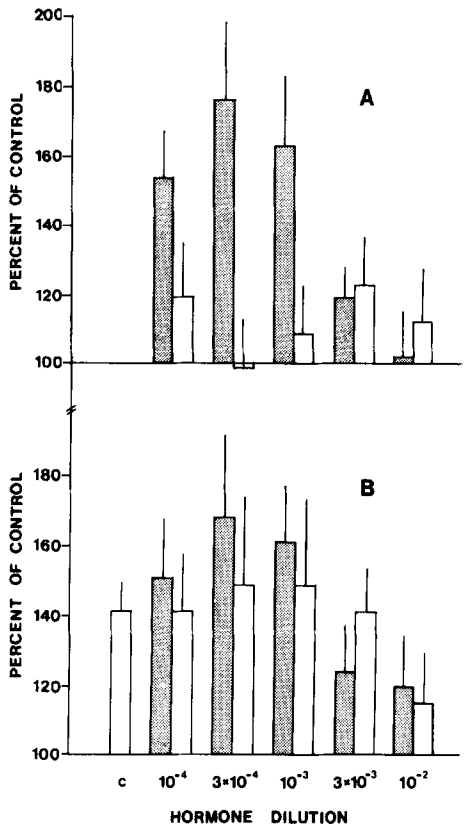

FIG. 3. IcIg-containing cells on day 6 in PWM-stimulated cultures treated with various concentrations of estradiol (shaded columns) or testosterone (open columns). (A) B cells cultivated with syngeneic Tγ-enriched T cells. (B) B cells cultivated with Tγ-depleted syngeneic T cells (Tμ). (c) indicates the increase above the control level (B + Tγ) after depletion of Tγ cells in the absence of added hormone. Bars indicate SEM of three different experiments.

Does Estradiol Display Its Enhancing Effect Directly on B Cells? Because PWM-induced B cell maturation is T cell dependent (22), the direct influence of estradiol on B cells could not be tested with this mitogen. Instead, lymphocytes were stimulated with a T cell-independent mitogen, *S. aureus* Cowan I bacteria (23), in cultures with or without added hormone. Only a slight, nonsignificant increase in the accumulation of PFC could be obtained in *S. aureus*-stimulated cultures treated with estradiol at dilutions of 3×10^{-3} to 3×10^{-4} (Table III).

Discussion

The T cell-dependent B lymphocyte differentiation induced by PWM has been considered a model for the humoral immune response (24). PWM has therefore received a widespread clinical use for the in vitro functional dissection of the immune response to identify subsets of cells responsible for disturbances in the immune system.

We show that the addition of estradiol significantly increased the accumulation of plasmablasts and Ig-segreting cells in PWM cultures of human blood lymphocytes. The concentrations of estradiol giving the peak responses (780–2,600 pmol/liter)

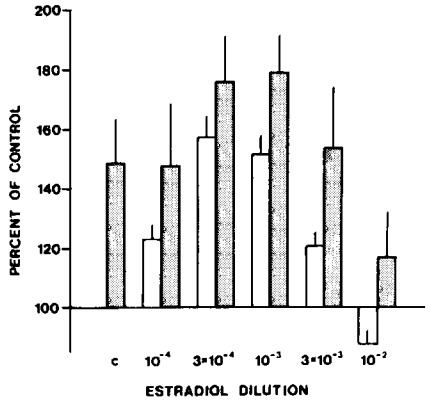

FIG. 4. Frequency of PFC on day 6 in PWM-stimulated cultures treated with various concentrations of estradiol. Shaded columns indicate cells x-ray irradiated with 1,000 rad before culture; open columns indicate control cultures without irradiation. c, effect of irradiation without estradiol treatment. Bars indicate SEM of three different experiments.

TABLE III

Effect of Estradiol on PFC on Day 6 in S. aureus-stimulated Cultures

Estradiol dilution	PFC*
10^{-2}	113 ± 5‡
3×10^{-3}	114 ± 12
10^{-3}	116 ± 2
3×10^{-4}	114 ± 4
10^{-4}	105 ± 3

* Results are expressed as percent of control cultures without added estradiol.
‡ Mean ± SE from three different experiments.

represent physiological levels and are normally present in fertile female serum during late follicular phase before ovulation and in the mid-luted phase (reference values for late follicular phase, 350–2,200; mid-luted phase 300–1,000 pmol/liter; reference 25). Corresponding physiological concentration of testosterone did not significantly change the PWM-induced B cell differentiation.

The FCS used contains detectable amounts of at least seven estrogens. Gas chromatography mass spectrometric (selected ion monitoring) assays revealed a 17β estradiol concentration of about 500–600 pmol/liter. Because the same proportion of FCS (5%) was used in control and testosterone-treated cultures, a major influence by the sex hormone present in the FCS can be excluded.

Higher concentrations of either sex hormone were toxic for both the proliferative response and the B cell differentiation. This is in agreement with earlier reports (26–29), where injection of high doses of estradiol or testosterone to experimental animals showed immunosuppressive and thymolytic effects. High doses of sex hormones have also been shown (30, 31) to suppress the mitogen response of human T cells in vitro. The enhanced B cell maturation seen in the presence of physiological concentrations of estradiol was not accompanied by a significantly increased proliferative response. Because dividing T cells are responsible for most of the thymidine incorporation during the first days of PWM cultures (32), this finding argues against an enhancing mechanism acting through increased helper T cell proliferation. The cell fractionation

studies support this interpretation. When PWM cultures were established by combining B cells with syngeneic T cells depleted for functional suppressor cells either by T rosetting or by irradiation, the overall response measured as PFC increased, but no further significant enhancement was achieved by addition of estradiol. In PWM cultures containing T cells enriched for functional suppressor cells, the estradiol abrogated the suppressive effect. When B cells were directly stimulated by the T independent mitogen *S. aureus*, no significant enhancing effect of the PFC response was obtained by addition of estradiol. Taking together, these findings indicate that estradiol in concentrations of 780–7,850 pmol/liter inhibit the effect of T suppressor cells in PWM cultures. The involvement of T cell-mediated mechanisms suggested here is in agreement with in vivo studies (33, 34) on the NZB/NZWF mice, where Roubinian et al. observed that sex hormones modulate the autoimmunity by acting on thymus-dependent mechanisms. Stimson and Hunter (13) recently reported that serum from estradiol-treated male rats contains an immunoregulatory factor. This factor, which increases the in vitro IgM PFC response of human lymphocytes against SRBC, is not found in the serum of rats receiving estradiol after thymectomy (13). These observations further support the notion that estradiol displays its immunoregulatory activity through the thymus-dependent (T cell) system.

Estradiol-induced enhancement of Ig-synthesis in PWM cultures on human lymphocytes was mainly caused by an increased differentiation of IgM-producing cells. This rather selective effect on the IgM synthesis in vitro is in good agreement with in vivo observations; women of fertile age have higher serum IgM levels than do postmenopausal women or men (3, 35). The early oral contraceptives, which contained high doses of estrogens, have also been found to raise the IgM levels in serum (36). A direct influence on immunocompetent cells by estradiol is further indicated by the finding that similar effects of estradiol were obtained regardless of male vs. female origin of blood leukocytes.

Interestingly, Lahita et al. (37, 38) have recently reported abnormal patterns of estradiol metabolism in both female and male patients with systemic lupus erythematosus. These patients display increased 16α hydroxylation of estrogens, leading to an accumulation of biologically potent 16α metabolites of estradiol. These might well contribute to the pathogenesis of the disease.

The expression of functional receptors for estradiol in various types of immunocompetent cells is still incompletely understood. There are reports (14, 39, 40) on surface receptors for sex hormones on lymphocytes and also on the presence of estradiol-binding receptors in cytosol preparations of rat thymuses. It remains to be established whether there are quantitative or qualitative differences in the sex hormone-binding structures in various subsets of lymphoid cells in man.

Our findings indicate that a direct modulating influence on the immunocompetent cells by the sex hormones contribute to the differences in immune responsiveness between males and females. The enhancing effect of female sex hormone on the B cell maturation might be important for the pathogenesis of various autoimmune disorders.

Summary

The effects of the main male and female sex hormones, testosterones and estradiol, in pokeweed mitogen (PWM)-stimulated cultures of human blood lymphocytes were studied. We found that the addition of physiological concentrations of estradiol (780–

2,600 pmol/liter) to PWM cultures significantly increased the accumulation of immunoglobulin M-containing and -secreting cells detected by immunofluorescence and/or by the reversed protein-A plaque assay. The dose range of estradiol that induced enhanced B cell maturation did not affect the proliferative response. Estradiol displayed the same effect in vitro on lymphocytes from both men and women. Fractionation of lymphocyte subpopulations before culturing revealed that estradiol does not display a direct mitogenic or stimulatory effect on B cells. Instead, estradiol inhibits the suppressive activity of a radio-sensitive (1,000 rad) subset of T lymphocytes bearing Fc-receptors for immunoglobulin G. Nontoxic concentrations of testosterone did not influence the in vitro B cell maturation.

These observations provide a cellular basis for the differences in the immunoreactivities of males and females. The estradiol-induced inhibition of suppressor T cells might be important for the pathogenesis of various autoimmune disorders.

We thank Dr. Sirkka Kontiainen for technical advice, Ms. Monica Schoultz for technical assistance, and Ms. Leena Saraste for secretarial help.

Received for publication 8 July 1981 and in revised form 27 August 1981.

References

1. Von Haam, E., and I. Rosenfeld. 1942. The effect of estrone on antibody production. *J. Immunol.* **43:**109.
2. Stern, K., and I. Davidsohn. 1955. Effect of estrogen and cortisone on immune hemoantibodies in mice of inbred strains. *J. Immunol.* **74:**479.
3. Butterworth, M., B. McClellan, and M. Allansmith. 1967. Influence of sex on immunoglobulin levels. *Nature (Lond.).* **214:**1224.
4. Terres, G., S. L. Morrison, and G. S. Habicht. 1968. A quantitative difference in the immune response between male and female mice. *Proc. Soc. Exp. Biol. Med.* **127:**664.
5. Eidinger, D., and T. J. Garrett. 1972. Studies of the regulatory effects of the sex hormones on antibody formation and stem cell differentiation. *J. Exp. Med.* **136:**1098.
6. Rowley, M. J., and I. R. Mackay. 1969. Measurement of antibody-producing capacity in man. I. The normal response to flagellin from *Salmonella Adelaide. Clin. Exp. Immunol.* **5:**407.
7. Batchelor, J. R. 1968. Hormonal control of antibody formation. *In* Regulation of the Antibody Response. B. Cinander, editor. Charles C. Thomas, Publisher, Springfield, Ill. 276.
8. Dubois, E. L., and D. L. Tuffanelli. 1964. Clinical manifestations of systemic lupus erythrematosus. Computer analysis of 520 cases. *J. Am. Med. Assoc.* **190:**104.
9. Inman, R. D. 1978. Immunologic sex differences and the female predominance in systemic lupus erythematosus. *Arthritis Rheum.* **21:**849.
10. Santoli, D., G. Trinchieri, C. M. Zmijewski, and H. Koprowski. 1976. HLA-related control of spontaneous and antibody-dependent cell-mediated cytotoxic activity in humans. *J. Immunol.* **117:**765.
11. Talal, N. 1976. Disordered immunologic regulation and autoimmunity. *Transplant. Rev.* **31:** 240.
12. Ahlqvist, J. 1976. *In* Endocrine Influence of Lymphatic Organs, Immune Responses, Inflammation and Autoimmunity. Almqvist & Wiksell Int., Stockholm. 33–54.
13. Stimson, W. H., and I. C. Hunter. 1980. Oestrogen-induced immunoregulation mediated through the thymus. *J. Clin. Lab. Immunol.* **4:**27.
14. Brodie, J. Y., I. C. Hunter, W. H. Stimson, and B. Green. 1980. Specific estradiol binding

in cytosols from the thymus glands of normal and hormone-treated male rats. *Thymus.* **1:** 337.

15. Boyum, A. 1968. Separation of leukocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest.* 21. (Suppl.) **97:**1.
16. Moretta, L., M. C. Mingari, A. Moretta, and M. D. Cooper. 1979. Human T lymphocyte subpopulations: studies of the mechanism by which T cells bearing Fc receptors for IgG suppress T-dependent B cell differentiation induced by pokeweed mitogen. *J. Immunol.* **122:** 984.
17. Pellegrino, M. A., S. Ferrone, M. P. Dierich, and R. A. Reisfeld. 1975. Enhancement of sheep red blood cell human lymphocyte rosette formation by the sulfhydryl compound 2-amino ethylisothiouronium bromide. *Clin. Immunol. Immunopathol.* **3:**324.
18. Häyry, P., L. C. Andersson, S. Nordling, and M. Virolainen. 1972. Allograft response in vitro. *Transplant. Rev.* **12:**91.
19. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting Ig of a given type or class. *Eur. J. Immunol.* **6:**588.
20. Cunningham, A. J., and A. Szenberg. 1968. Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology.* **14:**599.
21. Moretta, L., S. R. Webb, C. E. Grossi, P. M. Lydyard, and M. D. Cooper. 1977. Functional analysis of two human T-cell subpopulations: help and suppression of B-cell responses by T cells bearing receptors for IgM or IgG. *J. Exp. Med.* **146:**184.
22. Keightley, R. G., M. D. Cooper, and A. R. Lawton. 1976. The T cell dependence of B cell differentiation induced by pokeweed mitogen. *J. Immunol.* **117:**1538.
23. Forsgren, A., A. Svedjelund, and H.Wigzell. 1976. Lymphocyte stimulation by protein A of *Staphylococcus aureus. Eur. J. Immunol.* **6:**207.
24. Gmelig-Meyling, A. G., C. M. UytdeHaag, and R. E. Ballieux. 1977. Human B-cell activation in vitro. T cell-dependent pokeweed mitogen-induced differentiation of blood B lymphocytes. *Cell. Immunol.* **33:**156.
25. England, B. G., G. D. Niswender, and A. R. Midgley, Jr. 1974. Radioimmunoassay of estradiol-17β without chromatography. *J. Clin. Endocrinol. Metab.* **38:**42.
26. Dougherty, T. F. 1952. Effect of hormones on lymphatic tissue. *Physiol. Rev.* **32:**379.
27. Money, W. L., J. Fager, and R. W. Rawson. 1952. The comparative effects of various steroids on lymphoid tissue of the rat. *Cancer Res.* **12:**206.
28. Reilly, D. W., J. S. Thompson, R. K. Bielsky, and C. D. Severson. 1967. Estradiol-induced wasting syndrome in neonatal mice. *J. Immunol.* **98:**321.
29. Castro, J. E. 1974. The hormonal mechanism of immunopotentiation in mice after orchidectomy. *J. Endocrinol.* **62:**311.
30. Wyle, F. A., and J. R. Kent. 1977. Immunosuppression by sex steroid hormones. I. The effect upon PHA- and PPD-stimulated lymphocytes. *Clin. Exp. Immunol.* **27:**407.
31. Ablin, R. J., G. R. Bruns, P. Guinan, and I. M. bush. 1974. The effect of estrogen on the incorporation of ^{3}H-thymidine by PHA-stimulated human peripheral blood lymphocytes. *J. Immunol.* **113:**705.
32. Chess, L., R. P. MacDermott, and S. F. Schlossman. 1974. Immunologic functions of isolated human lymphocyte subpopulations. I. Quantitative isolation of human T and B cells and response to mitogens. *J. Immunol.* **113:**1113.
33. Roubinian, J. R., R. Papoian, and N. Talal. 1977. Effects of neonatal thymectomy and splenectomy on survival and regulation of autoantibody formation in NZB/NZW F_1 mice. *J. Immunol.* **118:**1524.
34. Roubinian, J. R., N. Talal, J. S. Greenspan, J. R. Goodman, and P. K. Siiteri. 1978. Effect of castration and sex hormone treatment on survival, anti-nucleic acid antibodies, and glomerulonephritis in NZB/NZW F_1 mice. *J. Exp. Med.* **147:**1568.
35. Rhodes, K., A. Scott, R. L. Markham, and M. E. Monk-Jones. 1969. Immunologic sex

differences. A study of patients with rheumatoid arthritis, their relatives, and controls. *Ann. Rheum. Dis.* **28:**104.

36. Bole, G. G., M. H. Friedlaender, and C. K. Smith. 1969. Rheumatic symptoms and serological abnormalities induced by oral contraceptives. *Lancet.* **I:**323.
37. Lahita, R. G., H. L. Bradlow, H. G. Kunkel, and J. Fishman. 1979. Alterations of estrogen metabolism in systemic lupus erythematosus. *Arthritis Rheum.* **22:**1195.
38. Lahita, R. G., H. L. Bradlow, H. G. Kunkel, and J. Fishman. 1981. Increased 16α hydroxylation of estradiol in systemic lupus erythematosus. *J. Clin. Endocrinol. Metab.* In press.
39. Reichman, M. E., and C. A. Villee. 1978. Estradiol binding by rat thymus cytosol. *J. Steroid. Biochem.* **9:**637.
40. McCruden, A. B., and W. H. Stimson. 1980. Androgen and other sex steroids cytosol receptors in the rat thymus. *J. Endocrinol.* **85:**P47.