

Estrogen enhances immunoglobulin production by human PBMCs

Naoko Kanda, MD, PhD, and Kunihiko Tamaki, MD, PhD Tokyo, Japan

Background: It has been suggested that estrogen may enhance humoral immune responses and may be involved in the pathogenesis of autoimmune diseases.

Objective: We studied the *in vitro* effects of 17 β -estradiol (E₂) on spontaneous immunoglobulin production by human PBMCs.

Methods: PBMCs from healthy human volunteers were cultured with E₂. Levels of IgG and IgM and cytokine activity were measured by ELISA. Proliferation was determined by [³H]-thymidine uptake. The cell viability was assessed by a trypan blue exclusion test.

Results: E₂ enhanced IgG and IgM production of PBMCs both from men and women without altering cell viability and proliferation. The stimulatory effect of E₂ was revealed at 10⁻¹⁰ mol/L, increased in a dose-dependent fashion, and was maximized at 10⁻⁸ mol/L. IgG production of PBMCs in the presence of E₂ (10⁻⁸ mol/L) was 220% of control cells, and that of IgM was 211%. Immunoglobulin production of E₂-treated B cells was slightly higher than that of control cells; IgG production was 161% of control cells, and that of IgM was 157%.

Anti-IL-10 antibody partially blocked the E₂ effect on immunoglobulin production of PBMCs; anti-IL-10 reduced IgG production in the presence of E₂ from 206% to 154% of control cells, and that of IgM from 206% to 152%. E₂ increased IL-10 production of monocytes up to 250% of control level, but it did not affect that of T cells or B cells. Exogenous IL-10 (1 U/mL) further enhanced E₂-induced increase of immunoglobulin production by B cells, although this level of IL-10 alone was ineffective for B cells.

Conclusion: These results suggest that E₂ may increase immunoglobulin production of human PBMCs mainly by increasing IL-10 production of monocytes. The results also support that E₂ may act as an important stimulator for human humoral immunity. (*J Allergy Clin Immunol* 1999;103:282-8.)

Key words: Estrogen, immunoglobulin, IL-10, B cell, monocyte

It has been reported that estrogen enhances humoral immune responses.¹⁻⁵ It is thus suggested that this hormone may be involved in the pathogenesis of autoimmune diseases, such as systemic lupus erythematosus (SLE), characterized by abnormally enhanced humoral immuni-

Abbreviations used

E ₂ :	17 β -estradiol
[³ H]-TdR:	[³ H]-thymidine
SLE:	Systemic lupus erythematosus

ty.^{6,7} This hypothesis is supported by the female preponderance of SLE.^{6,7} It has been reported that humoral immune responses are enhanced in pregnant women whose serum estrogen level is highly increased,^{8,9} and the onset and exacerbation of SLE take place more often during pregnancy.^{1,7} In addition, symptoms of SLE have been reported to develop in patients taking estrogen-containing oral contraceptives.⁷ Several studies also reported that estrogen treatment exacerbated the course of autoimmune diseases in mice.^{1,5,10-13} Carlsten et al¹ showed that estrogen administration *in vivo* increased the immunoglobulin production of SLE-prone mice. However, the cellular mechanisms for those estrogen effects have not been precisely defined. Most of these studies used animal immune systems, and there are few studies showing *in vitro* effects of estrogen on human humoral immune responses.¹⁴⁻¹⁶

In this study we examined *in vitro* effects of estrogen on spontaneous immunoglobulin production by human PBMCs to examine its regulatory role in human humoral immunity.

METHODS

Reagents

Endotoxin-free, tissue culture-grade 17 β -estradiol (E₂); 17 α -estradiol; estrone; and estriol were purchased from Sigma Chemical Co (St Louis, Mo). Monoclonal mouse IgG anti-human IL-2, IL-4, and IL-6 antibodies were purchased from Boehringer Mannheim Corp (Indianapolis, Ind). Monoclonal mouse IgG anti-human IL-1 α and IL-1 β antibodies were from Immunotech Inc (Westbrook, Me). Monoclonal mouse IgG anti-human IL-10 antibody and recombinant human IL-10 were from R&D Systems, Inc (Minneapolis, Minn). Monoclonal mouse IgG anti-estradiol antibody 2F9 was purchased from Cymbus Biotechnology, Ltd (Southampton, UK). Control mouse IgG was purchased from DAKO Corp (Carpinteria, Calif).

Preparation of PBMCs, monocytes, B cells, and T cells

Blood was taken from 13 healthy human volunteers (6 men aged 36.7 $\pm$ 7.0 years (mean $\pm$ SD) and 7 women aged 35.7 $\pm$ 9.7 years). All the donors were informed of the objectives and methods of this study and gave consent. PBMCs were isolated by centrifugation over Ficoll-Paque (Pharmacia LKB Biotechnology Inc, Uppsala, Sweden) as described.¹⁷ None of the donors were taking oral con-

From the Department of Dermatology, Faculty of Medicine, University of Tokyo, Tokyo.

Supported in part by a grant from the Japanese Ministry of Education (07770649).

Received for publication July 29, 1998; revised Sept 14, 1998; accepted for publication Sept 14, 1998.

Reprint requests: Naoko Kanda, MD, PhD, Department of Dermatology, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-Ku, Tokyo, 113-0033, Japan.

Copyright © 1999 by Mosby, Inc.

0091-6749/99 \$8.00 + 0 1/1/94555

traceptives or other hormonal drugs. All women were premenopausal and not pregnant, and blood was taken during the follicular phase of their menstrual cycles. Their serum E_2 concentration was measured by using a radioimmunoassay kit for E_2 (ICN Biomedicals, Irvine, Calif) and was $4.0 \pm 2.2 \times 10^{-10}$ mol/L (mean $\pm$ SD), which was the normal level of that phase. The serum E_2 concentration of the men was $1.1 \pm 0.5 \times 10^{-10}$ mol/L, which was the normal male level. PBMCs were allowed to adhere to plastic dishes. From the dish-adherent cells, the $CD3^+$, $CD19^+$, and $CD56^+$ cells were isolated by negative selection with immunomagnetic beads (Dynal, Great Neck, NY) as described¹⁸ and were used as monocytes. This monocyte population was greater than 97% $CD14^+$, and the contamination of $CD3^+$, $CD19^+$, or $CD56^+$ cells was less than 1% as determined by flow cytometry. From the nonadherent cells, $CD56^+$ cells were isolated by immunomagnetic negative selection and were incubated with neuraminidase-treated sheep erythrocytes as described.¹⁹ From the rosette-forming cells, the $CD14^+$ and $CD19^+$ cells were isolated by the immunomagnetic negative selection and were used as T cells. This T-cell population was greater than 98% $CD3^+$, and the contamination of $CD14^+$, $CD19^+$, or $CD56^+$ cells was less than 2%. From the nonrosette-forming cells, the $CD3^+$ and $CD14^+$ cells were isolated by the immunomagnetic negative selection and were used as B cells. This B-cell population was greater than 97% $CD19^+$, and the contamination of $CD3^+$, $CD14^+$, or $CD56^+$ cells was less than 1%.

Production of immunoglobulins

PBMCs or B cells (2×10^5 cells/well) were cultured in triplicate in round-bottom, 96-well, tissue-culture plates with or without E_2 at indicated doses in 200 μ L of mycoplasma, endotoxin, and phenol red-free Hymedim 910 (Kohjin Bio Inc, Tokyo, Japan) at 37°C in an atmosphere of 5% CO_2 in air for 7 days. This culture medium was serum-free, containing human albumin and transferrin. The concentration of E_2 and the other steroid hormones was lower than the detectable level as determined by radioimmunoassay. The culture supernatants were harvested and stored at $-70^\circ C$ until use. The amounts of total IgG and IgM in the supernatants were measured by ELISA as described.²⁰ In parallel experiments, 17α -estradiol, the nonactive metabolite of E_2 , was used as a negative control for E_2 . In each experiment 17α -estradiol was added instead of E_2 at the identical dose, and the result was compared with that of the culture with E_2 . E_2 and 17α -estradiol were dissolved in absolute ethanol and diluted at least 1000-fold to the required concentrations in the final medium. Control cultures without E_2 or 17α -estradiol contained ethanol at the highest concentration used in the experimental cultures, and this level of ethanol was not toxic to PBMCs.

Cell viability

PBMCs or B cells (2×10^5 cells/200 μ L/well) were cultured in triplicate for 7 days with medium alone, with 17α -estradiol, or with E_2 at indicated doses. The viability of these cells was assessed by the trypan blue exclusion test as described.¹⁷

Proliferative responses

PBMCs or B cells were cultured as above for 4 days (unless otherwise indicated) and were pulsed with 1 μ Ci of [^3H]-thymidine (^3H -TdR) for 16 hours. The cells were then harvested, and their ^3H -TdR uptake was counted as described.²¹

Production of cytokines

PBMCs, monocytes, T cells, or B cells (2×10^5 cells/200 μ L/well) were cultured in triplicate for 24 hours with medium alone, with 17α -estradiol, or with E_2 at indicated doses. The activity of cytokines in the culture supernatants was measured by ELISA

kits (Endogen Inc, Cambridge, Mass) according to the manufacturer's instructions. The detection limit for IL-1 α , IL-1 β , IL-2, IL-4, IL-6, and IL-10 were 2, 1, 6, 2, 1, and 3 pg/mL, respectively.

Statistical analyses

The data in Figs 1 and 4 were analyzed by 1-way ANOVA with Dunnett's multiple comparison test. The data in Figs 2 and 3 and Tables I and II were analyzed by 1-way ANOVA with Scheffe's multiple comparison test. A *P* value of less than .05 was considered significant.

RESULTS

Stimulatory effect of E_2 on immunoglobulin production by human PBMCs

First, E_2 was used at various concentrations, and its effect was examined on spontaneous immunoglobulin production by human PBMCs. E_2 enhanced both IgG and IgM production (Fig 1). The stimulatory effect of E_2 was revealed at 10^{-10} mol/L and increased in a dose-dependent fashion. It then reached a plateau at 10^{-8} mol/L in both IgG and IgM production by PBMCs from one man. In contrast, 17α -estradiol and other estrogenic hormones, estrone and estriol, did not affect immunoglobulin production at any concentration examined, indicating the specificity of the E_2 effects. PBMCs from one woman also showed similar dose-response curves (data not shown). Because 10^{-8} mol/L seemed optimal for the stimulatory effect of E_2 , this concentration is used in the following experiments.

In the next experiment E_2 at 10^{-8} mol/L was added to the PBMC culture from 13 healthy donors, and its effect was examined. IgG production by E_2 -treated PBMCs was 220% of control on average (Fig 2, A) and was significantly higher than that of control ($P < .05$ in men; $P < .05$ in women; $P < .01$ in both sexes combined). IgM production by E_2 -treated PBMCs was 211% of control on average (Fig 2, B) and was also significantly higher than control ($P < .05$ in men; $P < .05$ in women; $P < .01$ in both sexes combined). In both IgG and IgM production, no significant difference of the E_2 effect between men and women was observed. In parallel experiments 17α -estradiol at the same concentration did not affect immunoglobulin production by PBMCs from the identical donors (data not shown).

The viability of PBMCs after culture was not changed by E_2 ; viability was $90.4\% \pm 4.1\%$ (mean $\pm$ SD, $n = 13$) of that before culture in E_2 -treated PBMCs compared with $89.3\% \pm 3.9\%$ in control and $90.1\% \pm 4.8\%$ in 17α -estradiol-treated PBMCs. The proliferative response of PBMCs was not altered by E_2 ; ^3H -TdR uptake was 2090 ± 445 cpm (mean $\pm$ SD, $n = 13$) in E_2 -treated PBMCs compared with 2088 ± 441 cpm in control and 2106 ± 461 cpm in 17α -estradiol-treated PBMCs. The results were similar when E_2 concentration was variously changed from 10^{-12} to 10^{-6} mol/L or the culture period with E_2 was changed from 1 to 7 days (data not shown). Flow cytometry revealed that the B-cell proportion in PBMCs was not altered by E_2 ; $CD19^+$ cells in PBMCs were $10.1\% \pm 1.1\%$ in E_2 -treated PBMCs compared with

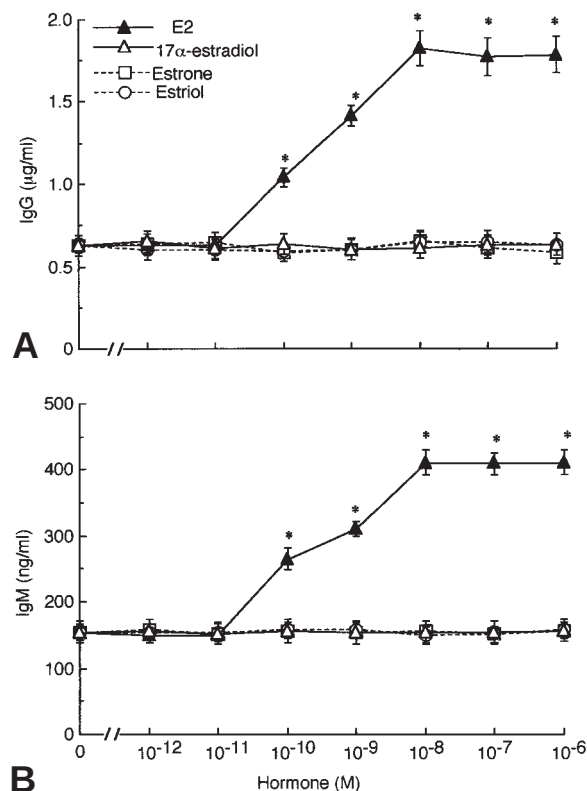


FIG 1. Dose dependency of the stimulatory effect of E₂ on spontaneous immunoglobulin production by human PBMCs. PBMCs (2×10^5 cells/200 μ L/well) were cultured in triplicate for 7 days in the presence or absence of indicated doses of E₂, 17 α -estradiol, estrone, or estriol. Culture supernatants were analyzed for IgG (**A**) and IgM (**B**) by ELISA. Data are means $\pm$ SD of triplicate cultures of PBMCs from one man and represent 6 separate experiments with PBMCs from 6 different donors (3 men and 3 women). * $P < .01$ versus control cultures.

10.2% $\pm$ 1.1% in control and 10.0% $\pm$ 1.5% in 17 α -estradiol-treated PBMCs.

Effect of E₂ on immunoglobulin production by B cells

To examine the direct stimulatory effect of E₂ on immunoglobulin production by B cells, B cells isolated from PBMCs were used. Because it was anticipated that normal B cells in the absence of stimuli may produce very low amounts of immunoglobulins without the help of accessory cells, absolute B-cell number was highly elevated. B cells (1×10^6 /mL) produced 294 $\pm$ 62 ng/mL IgG and 70 $\pm$ 28 ng/mL IgM (mean $\pm$ SD, $n = 13$). These amounts were measurable, although they were still lower than those by the same number of PBMCs, including approximately one tenth the number of B cells (839 $\pm$ 368 ng/mL IgG and 202 $\pm$ 54 ng/mL IgM). B cells treated with E₂ (10^{-8} mol/L) produced 472 $\pm$ 94 ng/mL IgG and 106 $\pm$ 41 ng/mL IgM, which were 161% and 157% of control cells, respectively, and were significantly high-

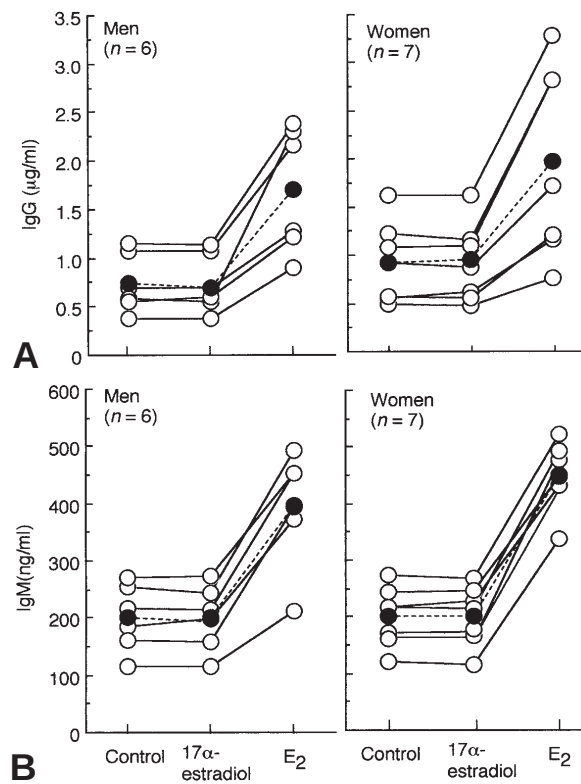


FIG 2. Effect of E₂ on spontaneous IgG (**A**) and IgM (**B**) production by human PBMCs. PBMCs (2×10^5 cells/200 μ L/well) from 6 men and 7 women were cultured for 7 days with medium alone, with 17 α -estradiol, or with E₂ (each 10^{-8} mol/L). Each point (open circles) represents mean of immunoglobulin amounts produced in triplicate cultures from individual donors. SDs of triplicate cultures were less than 10% of means. Corresponding values for each donor are connected by a solid line. Group means are also displayed (filled circles), and corresponding values are connected by a broken line.

er than controls and the amounts in 17 α -estradiol-treated B cells (289 $\pm$ 70 ng/mL IgG and 71 $\pm$ 29 ng/mL IgM) (all $P < .01$ by 1-way ANOVA with Scheffe's multiple comparison test). However, the differences from control were only marginal. B-cell viability was not changed by E₂; the viability was 63.2% $\pm$ 3.2% (mean $\pm$ SD, $n = 13$) of that before culture in E₂-treated B cells compared with 63.5% $\pm$ 2.8% in control and 63.6% $\pm$ 3.1% in 17 α -estradiol-treated B cells. The proliferation of B cells was not changed by E₂; ³H-TdR uptake was 1227 $\pm$ 235 cpm (mean $\pm$ SD, $n = 13$) in E₂-treated B cells compared with 1228 $\pm$ 270 cpm in controls and 1232 $\pm$ 273 cpm in 17 α -estradiol-treated B cells. Thus immunoglobulin production of B cells seemed to be increased by E₂ without altering the cell number. However, the stimulatory effect on B cells was much weaker than that on PBMCs (Fig 2). This indicates that E₂ may mainly act on non-B cells, such as monocytes or T cells, and enhance their function to help immunoglobulin production of B cells through releasing cytokines.

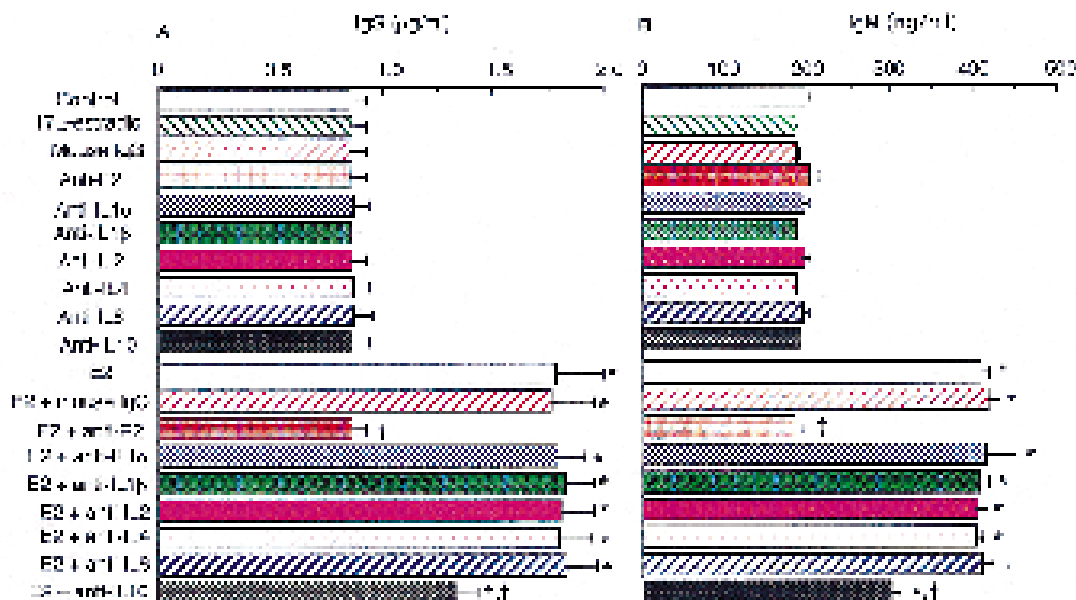


FIG 3. Inhibition by anticytokine antibodies of E₂ induced increase in immunoglobulin production by human PBMCs. PBMCs (2×10^5 cells/200 μ L/well) were cultured in triplicate for 7 days with medium alone, with 17 α -estradiol, or with E₂ (each 10^{-8} mol/L) in the presence or absence of various antibodies (each 10 μ g/mL). IgG (A) and IgM (B) production were measured in each culture as described in the legend for Fig 1. Concentration of antibodies was determined as recommended by the manufacturers. Data are means $\pm$ SD (n = 13, 6 men and 7 women). SDs of triplicate cultures from individual donors were less than 10% of the means. *P < .0001 versus control cultures with medium alone. #P < .0001 versus cultures with E₂ alone.

Inhibition by anticytokine antibodies of the E₂ effect on human PBMCs

To examine the involvement of cytokines in the stimulatory effect of E₂, antibodies against cytokines, which seemed relevant to immunoglobulin production, were added to the PBMC culture together with E₂, and their influence was tested on the E₂ effect. As shown in Fig 3, these antibodies were used at 10 μ g/mL, which did not influence the immunoglobulin production without E₂. E₂-induced enhancement of IgG and IgM production was completely blocked by monoclonal antiestradiol antibody 2F9, indicating the specificity of the E₂ effect. Among various anticytokine antibodies, only anti-IL-10 antibody reduced E₂-induced increase in both IgG and IgM production by PBMCs; anti-IL-10 reduced IgG production in the presence of E₂ from 206% to 154% of control and that of IgM from 206% to 152% of control, respectively. The results were similar when the concentration of each antibody was elevated up to 50 μ g/mL or the antibodies from another source (Endogen, Inc, Cambridge, Mass) were used (data not shown). These results suggest that IL-10 may be involved in the stimulatory effect of E₂ on immunoglobulin production by PBMCs. It is also indicated that E₂ may increase IL-10 production of PBMCs. However, the inhibition by anti-IL-10 antibody of the E₂ effect was incomplete. This indicates the variability of the assay and/or the possibility that another mechanism without involving IL-10 may function in the E₂ effect.

Effect of E₂ on cytokine production by human PBMCs

To examine the effect of E₂ on cytokine production by PBMCs, the supernatants of culture with or without E₂ were assayed for the activity of various cytokines. E₂ treatment significantly increased IL-10 production by PBMCs (Table I). On the other hand, the production of IL-1 α , IL-1 β , IL-2, IL-4, and IL-6 was not significantly changed by E₂. PBMCs were then subfractionated into monocytes, T cells, and B cells, and E₂ effects on IL-10 production by each fraction was further examined. Among 3 fractions, E₂ dose dependently increased IL-10 production of monocytes; however, it did not affect that of T cells or B cells (Fig 4). IL-10 production by monocytes in the presence of E₂ 10^{-8} mol/L was 250% of control. The dose-response curve for the E₂ effect on IL-10 production by monocytes was parallel to that for its effect revealed on immunoglobulin production (Fig 1). Similarly to whole PBMCs, E₂ did not affect the production of IL-1 α , IL-1 β , IL-2, IL-4, and IL-6 by any fraction (data not shown). These results suggest that E₂ may specifically increase IL-10 production of monocytes, and the increase of the endogenous IL-10 may enhance the immunoglobulin production of B cells.

Effect of IL-10 on immunoglobulin production of B cells treated with E₂

We finally examined whether the addition of IL-10 augments immunoglobulin production of B cells treated

TABLE I. Effect of E₂ on cytokine production by PBMCs

Conditions	Cytokine production (pg/mL)					
	IL-1 α	IL-1 β	IL-2	IL-4	IL-6	IL-10
Control	23 $\pm$ 10	22 $\pm$ 13	20 $\pm$ 12	15 $\pm$ 10	112 $\pm$ 29	193 $\pm$ 35
17 α -estradiol	22 $\pm$ 12	20 $\pm$ 14	22 $\pm$ 12	13 $\pm$ 12	114 $\pm$ 31	191 $\pm$ 36
E ₂	25 $\pm$ 11	22 $\pm$ 13	20 $\pm$ 13	15 $\pm$ 11	113 $\pm$ 30	283 $\pm$ 44*

PBMCs (2×10^5 cells/200 μ L/well) were cultured with medium alone, with 17 α -estradiol, or with E₂ (each 10^{-8} mol/L) for 24 hours, and the culture supernatants were analyzed for cytokines by ELISA. Values are means $\pm$ SD (n = 13, 6 men and 7 women). SDs of triplicate cultures from individual donors were less than 10% of the means. *P < .01 versus control cultures.

TABLE II. Effect of IL-10 on E₂-induced increase in immunoglobulin production by B cells

Culture conditions	Immunoglobulin production (ng/mL)	
	IgG	IgM
Control	296 $\pm$ 10	79 $\pm$ 5
17 α -estradiol (10^{-8} mol/L)	293 $\pm$ 12 (99)	78 $\pm$ 6 (99)
E ₂ (10^{-8} mol/L)	480 $\pm$ 19 (162)*	121 $\pm$ 7 (153)*
IL-10 (1 U/mL)	302 $\pm$ 11 (102)	82 $\pm$ 6 (103)
E ₂ + IL-10	641 $\pm$ 27 (217)*†	162 $\pm$ 10 (205)*†

B cells (2×10^5 cells/200 μ L/well) were cultured in triplicate under the indicated conditions for 7 days. Culture supernatants were analyzed for immunoglobulins by ELISA. Data are means $\pm$ SD (n = 13, 6 men and 7 women). Values in parentheses are percentage of immunoglobulin amounts compared with control cultures. *P < .0001 versus control cultures. †P < .0001 versus cultures with E₂ alone.

with E₂. Although a low concentration of IL-10 (1 U/mL) alone was ineffective for B cells in the absence of E₂, it enhanced the immunoglobulin production of B cells treated with E₂ (Table II); IgG production by E₂ plus IL-10-treated B cells was 217% of control and that of IgM was 205% of control. Thus the magnitude of E₂ plus IL-10-induced effect on B cells was comparable to that of the E₂ effect on PBMCs (Fig 2). These results with exogenous IL-10 suggest that monocyte-derived IL-10, which is induced by E₂, may also enhance immunoglobulin production of B cells in whole PBMCs.

B-cell viability was not significantly increased by the treatment with E₂ plus IL-10; the viability was 63.4% $\pm$ 2.8% (mean $\pm$ SD, n = 13) of that before culture in E₂ plus IL-10-treated B cells compared with 63.5% $\pm$ 2.8% in control. The proliferation of B cells was not altered by E₂ plus IL-10; ³H-TdR uptake was 1241 $\pm$ 230 cpm (mean $\pm$ SD, n = 13) in E₂ plus IL-10-treated B cells compared with 1227 $\pm$ 270 cpm in control.

DISCUSSION

In this study we demonstrated that E₂ enhanced spontaneous immunoglobulin production by human PBMCs in vitro. The stimulatory effect of E₂ was mediated mainly by upregulating the production of IL-10, a B-cell differentiation factor,²² in monocytes. Although the immunoglobulin production of B cells seemed to be increased by E₂, the increase was only marginal. It is possible that the observed E₂ effect on B cells may be mediated by the slightly contaminated monocytes in the B-cell population we used; E₂ may increase IL-10 production of contaminated monocytes, which may result in

the increase of immunoglobulin production of the B-cell population. The monocyte contamination was less than 1% as determined by flow cytometry, and thus the E₂-induced IL-10 increase of the B-cell population was less than the level detectable by ELISA (Fig 4). However, even such a small number of monocytes may be sufficient for the significant stimulatory effect on immunoglobulin production of the B-cell population. The B-cell population treated with E₂ showed increased responsiveness to exogenous IL-10 (Table II). This may be because the contaminated monocyte-derived IL-10, which was induced by E₂, bound to IL-10 receptors on B cells and further activated the receptors, thereby increasing their responsiveness. Because E₂ receptors have not been definitively identified in normal peripheral blood B cells, E₂ may not act on B cells but may interact with other subpopulations of PBMCs. Because the E₂ plus exogenous IL-10-induced effect on B cells was comparable with that of E₂ alone on PBMCs, the possibility of an indirect mechanism mediated by other cytokines may be unlikely but should be investigated. To date, several studies have reported varying effects of E₂ on IL-1, TNF- α , and/or IL-6 production. E₂ enhanced IL-1, IL-6, and TNF- α production in murine uterus in vivo,²³ whereas E₂ inhibited IL-6 production of murine bone marrow-derived stromal cells and osteoblasts²⁴ and human endometrial stromal cells in vitro.²⁵ Another study showed that E₂ at low concentrations (10^{-10} to 10^{-9} mol/L) enhanced in vitro IL-1 production of human monocytes, whereas at higher concentrations ($\geq 10^{-7}$ mol/L) it inhibited the IL-1 production.²⁶ Thus the effects of E₂ on the cytokine production may vary with the target cells, species, and/or E₂ concentrations. In this study E₂ did not affect the in vitro production of these

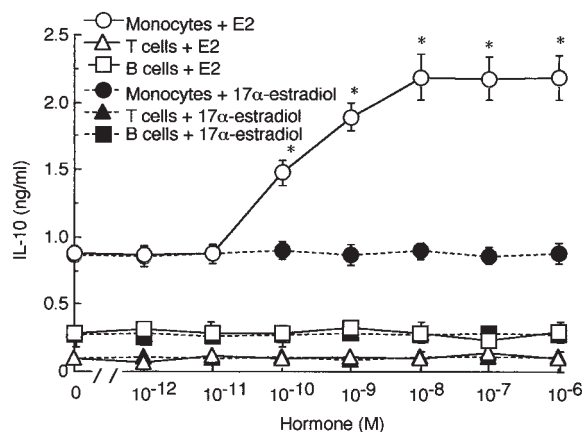


FIG 4. Effect of E_2 on IL-10 production by human monocytes, T cells, and B cells. Monocytes, T cells, and B cells (2×10^5 cells/200 μ L/well) were cultured in triplicate for 24 hours in the presence or absence of indicated doses of E_2 or 17 α -estradiol, and the culture supernatants were analyzed for IL-10 by ELISA. Data are means $\pm$ SD of triplicate cultures of PBMC fractions from one man and represent 4 separate experiments with PBMC fractions from 4 different donors (2 men and 2 women). * $P < .001$ versus control cultures.

cytokines, including TNF- α (data not shown), by human PBMCs.

IL-10 also acts as a B-cell growth factor.²² However, the concentration of endogenously produced IL-10 in the presence of E_2 seems insufficient for growth-promoting effect. Previous studies showed that less than 500 pg/mL of IL-10 did not enhance the proliferation of anti-CD40-activated B cells.^{22,27} This study also revealed that 1 U/mL of IL-10, approximately 200 pg/mL, did not affect B-cell proliferation even in the presence of E_2 .

Several reports support the involvement of E_2 in IL-10 production; a large amount of IL-10 is produced by the placenta,⁸ which contains a high level of E_2 (0.66 to 3.67×10^{-7} mol/kg wet weight tissue).²⁸ IL-10 production is highly increased during pregnancy when serum E_2 level is elevated.^{8,9} However, E_2 effects on IL-10 production of monocytes were not examined in these studies. It is also unknown how E_2 may augment IL-10 production of monocytes. It is hypothesized that E_2/E_2 -receptor complexes may enhance the transcription of IL-10 genes by binding to estrogen-responsive elements²⁹ close to IL-10 promoters or by binding to some transcription factors that directly bind those elements.³⁰ It is also possible that E_2 may promote the expression of genes for such transcription factors. Steroid hormones are reported to regulate the expression of several cytokine genes by these mechanisms.^{31,32} Alternatively, E_2 may enhance the secretion of IL-10 proteins constitutively synthesized in monocytes. These possibilities should further be investigated.

In this study the E_2 effect in vitro was revealed at 10^{-10} mol/L (normal serum level of men, 0.7 to 2×10^{-10} mol/L; nonpregnant women, 0.3 to 14×10^{-10} mol/L³³) and increased in a dose-dependent fashion. This suggests

that immunoglobulin production in vivo may be sustained by E_2 in both genders. Especially in gynecologic organs, such as the ovary or uterus, the serum E_2 level of which is 20 to 30 times that in peripheral blood,³⁴ local immunoglobulin production may be increased. Serum E_2 level in pregnant women (1.8×10^{-10} to 1.47×10^{-7} mol/L)³⁵ often reaches 100 to 1000 times that in nonpregnant women and exceeds its optimal in vitro concentration (10^{-8} mol/L), which produced about 2-fold increase of immunoglobulin production in this study. This suggests that immunoglobulin production in vivo may be further enhanced by E_2 during pregnancy. On the other hand, testosterone has been reported to suppress humoral immune responses.^{5,6,15,36} We also found that testosterone decreased spontaneous immunoglobulin production by human PBMCs in vitro at doses similar to its serum level,³⁷ indicating that testosterone may reverse the immunostimulatory effect of E_2 . Collectively, it is suggested that human humoral immune responses in vivo may be controlled by the balance of female and male hormone levels. If either hormone level is abnormally increased, humoral immune responses may be aberrantly increased or decreased. This possibility should be investigated further. Furthermore, it is possible that E_2 may not only enhance the production of polyclonal immunoglobulins but also of autoantibodies relevant to autoimmune diseases. Studies are underway to determine the E_2 effects on anti-DNA antibody production by PBMCs from patients with SLE.

REFERENCES

- Carlsten H, Tarkowski A, Holmdahl R, Nilsson LA. Oestrogen is a potent disease accelerator in SLE-prone MRL lpr/lpr mice. *Clin Exp Immunol* 1990;80:467-73.
- Ansar Ahmed S, Dauphinee MJ, Montoya AI, Talal N. Estrogen induces normal murine CD5⁺ B cells to produce autoantibodies. *J Immunol* 1989;142:2647-53.
- Ansar Ahmed S, Dauphinee MJ, Montoya AI, Talal N. Estrogen acts directly on B cells to augment autoantibodies [abstract]. *FASEB J* 1988;2:A894.
- Blank M, Mendlovic S, Fricke H, Mozes E, Talal N, Shoenfeld Y. Sex hormone involvement in the induction of experimental systemic lupus erythematosus by a pathogenic anti-DNA idiomorph in naive mice. *J Rheumatol* 1990;17:311-7.
- Steinberg AD, Melez KA, Raveche ES, Reeves JP, Boegel WA, Smathers PA, et al. Approach to the study of the role of sex hormones in autoimmunity. *Arthritis Rheum* 1979;22:1170-6.
- Miller MH, Urowitz MB, Gladman DD, Klinger DW. Systemic lupus erythematosus in males. *Medicine* 1983;62:327-34.
- Ansar Ahmed S, Talal N. Importance of sex hormones in systemic lupus erythematosus. In: Wallace DJ, Hahn BH, editors. *Dubois' lupus erythematosus*. 3rd ed. Philadelphia: Lea & Febiger; 1993. p. 148-56.
- Lin H, Mosman TR, Guilbert L, Tuntipopipat S, Wegmann TG. Synthesis of T helper 2-type cytokines at the maternal-fetal interface. *J Immunol* 1993;151:4562-73.
- Formby B. Immunologic response in pregnancy. Its role in endocrine disorders of pregnancy and influence on the course of maternal autoimmune diseases. *Endocrinol Metab Clin North Am* 1995;24:187-205.
- Roubinian JR, Talal N, Siiteri PK, Sadakian JA. Sex hormone modulation of autoimmunity in NZB/NZW mice. *Arthritis Rheum* 1979;22:1162-9.
- Roubinian JR, Talal N, Greenspan JS, Goodman JR, Siiteri PK. Effect of castration and sex hormone treatment on survival, anti-nucleic antibodies and glomerulonephritis in NZB/NZW F1 mice. *J Exp Med* 1978;147:1568-83.

12. Raveche ES, Klassen LW, Steinberg AD. Sex differences in formation of anti-T cell antibodies. *Nature* 1976;263:415-6.
13. Steinberg AD, Smathers PA, Boegel WA. Effect of sex hormones on autoantibody production by NZB mice and modification by environmental factors. *Clin Immunol Immunopathol* 1980;17:562-72.
14. Paavonen T, Andersson LC, Adlercreutz H. 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. *J Exp Med* 1981;154:1935-45.
15. Sthoeger ZM, Chiorazzi N, Lahita RG. Regulation of the immune response by sex hormones. I. In vitro effects of estradiol and testosterone on pokeweed mitogen-induced human B cell differentiation. *J Immunol* 1988;141:91-8.
16. Mori H, Sawairi M, Itoh N, Hanabayashi T, Tamaya T. Effects of sex steroids on immunoglobulin M production by Epstein-Barr virus-transformed B-cell line SKW6-CL4. *J In Vitro Fert Embryo Transfer* 1991;8:329-35.
17. Boyum A. Separation of leukocytes from peripheral blood and bone marrow. *Scand J Clin Lab Invest* 1968;21(Suppl 97):77-89.
18. Gee AP, Lee C, Sleasman JW, Madden M, Ugelstad J, Barret DJ. T lymphocyte deletion of human peripheral blood and bone marrow using monoclonal antibodies and magnetic microspheres. *Bone Marrow Transplant* 1987;2:155-63.
19. Farrant J, Bryant A, Lever AML, Edwards AJ, Knight SC, Webster ADB. Defective low-density cells of dendritic morphology from the blood of patients with common variable hypogammaglobulinaemia: low immunoglobulin production on stimulation of normal B cells. *Clin Exp Immunol* 1985;61:189-94.
20. Linker-Israeli M, Quismorio FP, Horwitz DA. CD8⁺ lymphocytes from patients with systemic lupus erythematosus sustain, rather than suppress, spontaneous polyclonal IgG production and synergize with CD4⁺ cells to support autoantibody synthesis. *Arthritis Rheum* 1990;33:1216-25.
21. Kimata H, Yoshida A, Ishioka C, Masuda S, Sasaki R, Mikawa H. Human recombinant erythropoietin directly stimulates B cell immunoglobulin production and proliferation in serum-free medium. *Clin Exp Immunol* 1991;85:151-6.
22. Rousset F, Garcia E, DeFrance T, Peronne C, Hsu DH, Kastelein R, et al. Interleukin-10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc Natl Acad Sci U S A* 1992;89:1890-3.
23. De M, Sanford TR, Wood GW. Interleukin-1, interleukin-6, and tumor necrosis factor α are produced in the mouse uterus during the estrus cycle and are induced by estrogen and progesterone. *Dev Biol* 1992;151:297-305.
24. Girasole G, Jilka RL, Passeri G, Boswell S, Boder G, Williams DC, et al. 17 β -estradiol inhibits interleukin-6 production by bone marrow-derived stromal cells and osteoblasts in vitro: a potential mechanism for the antiosteoporotic effect of estrogens. *J Clin Invest* 1992;89:883-91.
25. Tabibzadeh SS, Santhanam U, Sehgal PB, May LT. Cytokine-induced production of IFN- β_2 /IL-6 by freshly explanted human endometrial stromal cells: modulation by estradiol-17 β . *J Immunol* 1989;142:3134-9.
26. Polan ML, Daniele A, Kuo A. Gonadal steroids modulate human monocyte interleukin-1 (IL-1) activity. *Fertil Steril* 1988;49:964-8.
27. Burdin N, Peronne C, Banchereau J, Rousset F. Epstein-Barr virus transformation induces B lymphocytes to produce human interleukin 10. *J Exp Med* 1993;177:295-304.
28. Ferre F, Breuiller M, Tanguy G, Janssens Y, Cedard L. Steroid concentrations and Δ^5 , 3 β -hydroxysteroid dehydrogenase activity in human placenta. *Am J Obstet Gynecol* 1980;138:500-3.
29. Walker P, Germond JE, Brown-Luedi M, Givel F, Wahli W. Sequence homologies in the region preceding the transcription initiation site of the liver estrogen-responsive vitellogenin and apoVLDLII genes. *Nucleic Acids Res* 1984;12:8611-25.
30. Gaub M, Bellard M, Sheuer I, Chambon P, Sassone-Corsi P. Activation of the ovalbumin gene by the estrogen receptor involves the Fos-Jun complex. *Cell* 1990;63:1267-76.
31. Fox HS, Bond BL, Parslow TG. Estrogen regulates the IFN- γ promoter. *J Immunol* 1991;146:4362-7.
32. Beato M. Gene regulation by steroid hormones. *Cell* 1989;56:335-44.
33. Mishell DR Jr, Nakamura RM, Crosignani PG, Stone S, Khanna K, Nagata Y, et al. Serum gonadotropin and steroid patterns during the normal menstrual cycle. *Am J Obstet Gynecol* 1971;111:60-5.
34. Abraham GE. Solid-phase radioimmunoassay of estradiol-17 β . *J Clin Endocrinol Metab* 1969;29:866-70.
35. Batra S, Bengtsson LP. 17 β -estradiol and progesterone concentrations in myometrium of pregnancy and their relationships to concentrations in peripheral plasma. *J Clin Endocrinol Metab* 1978;46:622-6.
36. Ariga H, Edwards J, Sullivan DA. Androgen control of autoimmune expression in lacrimal gland of MRL/MP-lpr/lpr mice. *Clin Immunol Immunopathol* 1989;53:499-508.
37. Kanda N, Tsuchida T, Tamaki T. Testosterone inhibits immunoglobulin production by human peripheral blood mononuclear cells. *Clin Exp Immunol* 1996;106:410-5.